The Egyptian Journal of Hospital Medicine (October 2020) Vol. 81 (3), Page 1609-1613

Evaluation of Serum RANTES as a Biomarker in the Diagnosis of Early-Onset Neonatal Infections

Mahmoud Mohammed Abd-Elsamea*[1], Azza I. El-dousoky[1], Hadeel M. Abd El-Rahman[1], Randa Hussiny Mohammad[2]

Departments of [1]Pediatrics and [2] Medical Biochemistry, Faculty of Medicine, Zagazig University, Sharkia, Egypt

***Corresponding author:** Mahmoud Mohammed Abd-Elsamea. Contact: +201550903974

Email: mahmod.mohmed222@gmail.com

ABSTRACT

Background: Globally, sepsis is still one of the major causes of morbidity and mortality in neonates, despite recent advances in health care units.

Objective: To assess the accuracy of serum chemokine RANTES as a biomarker for early-onset neonatal infection as early diagnosis and to assess its relationship with gestational age, birth weight, sex, mode of delivery, and value of some hematological and biochemical parameters facilitating early good treatment.

Patients and Methods: This case-control study was carried out on neonates with early-onset neonatal infections that were admitted to the Neonatal Intensive Care Unit of Zagazig General Hospital. This study included 45 matched age and sex neonates divided into three groups.

Results: Significantly higher levels of RANTES were found in neonates with EONS compared with non-infected cases. RANTES levels were significantly higher in infected term &preterm neonates compared to healthy controls. Infected full-term neonates had higher RANTES levels than in infected preterm neonates without a statistically significant difference. A positive correlation between serum RANTES concentration and CRP value was found in both infected neonates groups. On the other hand, no correlation between RANTES and the number of white blood cells, platelets, or hemoglobin was noted.

Conclusion: The significant increase of serum RANTES concentration in early-onset infections in neonates, regardless of their gestational age, birth weight, and Apgar score, not only proves the presence of an active immunological process but also may be a useful biomarker for diagnosis of early-onset severe neonatal infections.

Keywords: RANTES, Biomarker, Early, Onset Neonatal Infections.

INTRODUCTION

Globally, sepsis is still one of the major causes of morbidity and mortality in neonates, despite recent advances in health care units [(1)].

The mortality rate as a consequence of irreversible septic shock ranges from 20 to 50%. The most frequent clinical forms of severe early-onset infections are sepsis, pneumonia, meningitis, and urinary tract infections [(2)].

On the other hand, the survivors of neonatal sepsis are vulnerable to short- and long-term neurodevelopmental morbidity [(3)].

Diagnosis of sepsis is a formidable challenge for NICU because clinical signs of sepsis are nonspecific, subtle, and are observed in other non-infectious conditions. Furthermore, bacterial cultures are time-consuming, frequently false negative, especially in neonates, due to low or intermittent bacteremia, small blood inoculation volumes, or intrapartum antibiotics use. Despite its limitations, blood cultures remain a gold standard [(4)].

The early diagnosis of neonatal infection, especially sepsis in preterm neonates, before clinical signs, is important to start antimicrobial therapy and prevent unfavorable complications, such as intraventricular hemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia, and long-term consequences, mainly cerebral palsy [(5)].

A particular subject of interest of researchers is chemotactic cytokines, one of whose representatives is RANTES (regulation on activation normal T-cell expressed and secreted). It is produced by macrophages, epithelial cells, platelets, megakaryoblasts, T lymphocytes, and eosinophils. It acts through CCR1, CCR3, and CCR5 receptors. It has an effect on chemotaxis of monocytes, T lymphocytes (including memory cells), NK cells, eosinophils, dendritic cells, and mast cells including adhesion molecules VCAM-1 and ICAM-1. Moreover, it activates histamine secretion by mast cells, stimulates lymphocyte proliferation and IgE and IgG production, increases the expression of CD80 on antigen-presenting cells, and activates the cytotoxicity of T lymphocytes and NK cells [(6)].

This study aimed to assess the accuracy of serum chemokine RANTES as a biomarker for early-onset neonatal infection as early diagnosis and to assess its relationship with gestational age, birth weight, sex , mode of delivery, and value of some hematological and biochemical parameters facilitating early good treatment.

MATERIALS AND METHODS

This Case-control study carried out on neonates with early-onset neonatal infections that were

Received:30 /6 /2020
Accepted:29 /8 /2020

admitted to the Neonatal Intensive Care Unit of Zagazig General Hospital in the period from February 2018 to February 2019.

Patients:

This study included 45 matched age and sex neonates divided into three groups:

- **Infected term** :(n 15) Diagnosed according to HSS
- **Infected preterm** : (n 15) Diagnosed according to HSS
- **Control group**: includes 15 healthy neonates delivered spontaneously without maternal infection & risk factors or any clinical and laboratory suspicion of sepsis.

Inclusion criteria: Neonates with birth WT >1000g. Gestational age >28w. Age 1 day – 7 days. Positive blood culture in the infected group. HSS is ≥ 5 in the infected group.

Exclusion criteria: Birth Weight <1000g. Gestational age <28w. Age >7days. Congenital anomalies. Genetic disorders. Hyperbilirubinemia

Ethical Clearance

Written Informed consent was taken from the patient's parents to participate in the study.

Approval for performing the study was obtained from Pediatrics and Medical Biochemistry Departments, Zagazig University Hospitals after taking Institutional Review Board (IRB) approval.

All neonates included in the study were subjected to:

Thorough history taking and clinical examination

1) **History:** Complete history taking regarding prenatal, perinatal, gestational age, weight at birth, mode of delivery, sex, and Apgar score.
2) **Physical examination:** Full examination for neonates admitted to NICU for assessment of them including :
   - **General examination:** vital signs, abnormal color, weight , any congenital anomaly, and Apgar score
   - **Systemic examination:** neurological, cardiovascular, GIT, and respiratory
3) **Laboratory investigations:**
   a) **Routine investigations:** The routine laboratory investigations were done according to the Biochemistry Department and Laboratories of Zagazig University Hospitals protocol and include:
   - **Complete blood count (CBC):** measured by automated blood counter.
   - **C-reactive protein (CRP)** using Immunoturbidimetric assay for the quantitative determination of CRP in human serum.
   - **Blood culture.**
   - **ESR.**

   b) **The hematological scoring system (HSS):**
   - All neonates included in the infected group of this study have HSS ≥ 5.
   - All neonates included in the control group of this study have HSS ≤ 2.

   c) Special investigations:

Serum RANTES level was measured using enzyme-linked immune sorbent assay (ELISA).

Statistical Analysis

All data were collected, tabulated, and statistically analyzed using SPSS 20.0 for windows (SPSS Inc. Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi 2$) and Fisher exact were used to calculate the difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation). One way ANOVA test supplied with post hoc was used to compare between more than two dependent groups of normally distributed variables while Friedman's test ranks test was used for non-normally distributed variables. Pearson's correlation tests were used for correlating normal and non-parametric variables respectively.

The receiver operating characteristic (ROC) curve was constructed. All statistical comparisons were two-tailed with a significance level of P-value ≤ 0.05 indicates significant.

RESULTS

There is a highly significant difference between the three studied groups as regard gestational age and birth weight **(Table 1).**

There is a significant difference between the two infected groups as regard to the microorganism. The most common organism in the infected group was klebsiella (33.3%). The most common organism in the infected preterm group was klebsiella (40%). The most common organism in the infected term group was GBS (30%) **(Table 2).**

There is a significant difference between the three groups regarding RANTES **(Table 3)**.

There is no significant difference regarding RANTES between patients in the infected group according to the site of infection **(Table 4).**

There is a positive significant correlation between RANTES and GA in the infected preterm group and a positive correlation between RANTES and CRP in both infected groups **(Table 5)**.

The best cut-off value of RANTES biomarker for early-onset neonatal infection was ≥707.17 with sensitivity 93.3%, specificity 73.3%, and accuracy 54% **(Table 6)**.

Table (1): Age, GA , weight and sex distribution of the three studied groups

Variable		**Healthy Controls** (n=15)	**Infected Preterm** (n=15)	**Infected Term** (n=15)	**F / χ^2**	**P-value**
Age (days) Mean ± SD		1.8 ± .941	1.2 ± .414	1.9 ± .994	3.044	.060
GA (weeks) Mean ± SD		35.4 ± 2.165	32.07 ± 2.12	39.87 ± 1.13	**65.974**	**.000**
Birth weight (kg) Mean ± SD		2.34 ± .609	2.21 ± .721	3.27 ± .586	**12.306**	**.000**
Sex	Male	7 (46.7%)	9 (60%)	12 (80%)	3.592	.166
	Female	8 (53.3%)	6 (40%)	3 (20%)		

Table (2): Microorganism of the two infected groups according to blood culture results

	Infected group (n=30)	**Infected Preterm** (n=15)	**Infected Term** (n=15)	**χ^2**	**P-vaue**
Staph heamolyticus	8 (26.7%)	4 (26.7%)	4 (26.7%)	**6.451**	**.009**
Staph epidermidies	4 (13.3%)	3 (20%)	1 (6.6%)		
Klebsilla	10 (33.3%)	6 (40%)	4 (26.7%)		
Staph hominis	1 (3.3%)	0	1 (6.7%)		
Group B streptococcus	7 (23.4)	2 (13.3%)	5 (30%)		

Table (3): RANTES of the three studied groups

Variable	**Healthy Controls** (n=15)	**Infected Preterm** (n=15)	**Infected Term** (n=15)	**F**	**P-value**
RANTES Mean ± SD	949.85 ± 503.8	1037.61 ± 317.4	1250.7 ± 207.5	**4.709**	**.048**

Post hoc test of RANTES of the three studied groups

(I) G	**(J) G**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	Preterm	87.759267*	132.944183	.031	-356.05149	180.53296
	Term	300.888333*	132.944183	.029	-569.18056	-32.59611
Preterm	Term	213.129067	132.944183	.116	-481.42129	55.16316

Table (4): RANTES level according to site of infection in infected groups

Variable	**Pneumonia (n=13)**	**UTI (n=4)**	**Meningitis (n=4)**	**Sepsis (n=9)**	**F**	**P-value**
RANTES Mean ± SD	1086.6 ± 333.1	1203.3 ± 188.4	1125.3 ± 190.5	1209.4 ± 298.1	.371	.774

Table (5): Correlation of RANTES with other parameters in the two infected groups

Variable		Preterm group	Term group
GA	r	**.561**	-.089
	p	**.030**	.754
Birth weight	r	.108	.361
	P	.702	.186
Apgar 1	r	-.050	.391
	p	.884	.299
Apgar 5	r	-.193	-.332
	P	.570	.382
Hemoglobin	r	-.278	-.127
	p	.316	.653
Platelets	r	.498	.224
	p	.059	.422
TLC	r	-.073	.361
	p	.797	.186
CRP	r	**.397**	**.412**
	p	**.017**	**.009**

Table (6): RANTES as an early biomarker for early-onset neonatal infections

AUC	S.E	Sig.	95% Confidence Interval		Cut-off	Sensitivity	Specificity
			Lower Bound	Upper Bound			
.706	.098	**.026**	.514	.897	707.17	93.3%	73.3%

DISCUSSION

In this study, a novel marker RANTES was measured in neonates with EONS and non-infected newborns for their potential use in the diagnosis of early-onset neonatal infections.

This study included 30 infected neonates 15 term and 15 preterm and a control group of 15 neonates 17 of them were females and 28 males.

In our study, we found a significant difference between the groups in gestational age and birth weight. These results were in line with **Stojewska *et al.*** [7]**.**

Similar to our findings, **El-Lahony *et al.*** [8] found a significant decrease in weight in the septic neonates.

In the present study, we found that the prevalence of micro-organisms among the infected group was 33.3% klebsiella, 26.7% staph hemolyticus , 23.4% GBS,13.3% staph epidermidies, and 3.3% staph hominis. While the prevalence among the infected preterm group was 40% klebsiella,26.7% staph heamolyticus,20% staph epidemidies, and 13.3% for GBS, and among the infected full-term group was 30% GBS,26.7% klebsiella,26.7% staph heamolyticus ,6.6% staph epidemidies, and 6.7% staph hominis.

Also, **Stojewska *et al.*** [7] found that the prevalence of micro-organisms among the infected group was 4.5% klebsiella, 3.3% staph aureus, 24.7% staph epidermidies, 4.5% E coli, and 1.1% serratia marcescens. This difference referred to the difference in the geographical area of research and common organisms.

In the current preliminary study, significantly higher levels of RANTES were found in neonates with EONS compared with non-infected cases. These results are following the recent study by **Stojewska *et al.*** [7]**.**

In this study, we found that RANTES levels were significantly higher in infected term & preterm neonates compared to healthy controls. Infected full-term neonates had higher RANTES levels than in infected preterm neonates without a statistical significant difference.

Similar to our study, **Stojewska *et al.*** [7] documented that levels of RANTES in infected neonates were significantly higher than in healthy neonates.

On the contrary, **Manoura *et al.*** [9]**;** reported that infected neonates had a significantly lower

RANTES concentration compared with healthy controls.

In our study, we found that there was no significant difference in RANTES in the infected group either term or preterm regarding the type of infection. this finding was in accordance with **Stojewska *et al.*** [(7)], who found no significant difference in RANTES regarding the type of infection or organism in the infected group.

In our study, a positive correlation between serum RANTES concentration and CRP value was found in both infected neonates groups. On the other hand, no correlation between RANTES and the number of white blood cells, platelets, or hemoglobin was noted.

Also, there was a positive correlation between RANTES and gestational age in the preterm group only and no correlation between RANTES and birth weight, Apgar at one minute, and Apgar at five minutes in both infected groups.

Similar to our findings, **Carrol *et al.*** [(10)] found a positive correlation between RANTES and CRP in infected neonates.

Carrol *et al.* [(10)] **have** reported an association of poor recovery in sepsis and meningitis with lower RANTES concentration. They hypothesized a correlation between low RANTES concentration and disseminated intravascular coagulation (DIC) as a result of a low number of platelets, which secrete RANTES.

Ng *et al.* [(11)], suggested that preterm neonates are capable of eliciting chemotactic and pro- and anti-inflammatory responses to invading pathogens.

Sato *et al.* [(12)] claimed that differences between the lymphocytes are the effect of a stronger chemotactic response of RANTES to CD45RO than to CD45RA in the peripheral blood. They also emphasized that on the surface of peripheral blood cells there are CCR1, CCR2, CCR5, and CCR6 receptors, while on the surface of cord blood cells there are only CXR4 receptors, which may reflect the differences in chemokine activity regulation between the cord and peripheral blood.

In our study, the ROC curve showed that the best cutoff value of RANTES as an early biomarker for early-onset neonatal infections was ≥ 707.17 with a sensitivity 93.3%, specificity 73.3% while in **Shouman and Badr** [(13)] study sensitivity of RANTES as an early biomarker for early-onset neonatal infections was 89% and specificity was 30%.

Conflicts of interest: The authors declare that they have no competing interests.

CONCLUSION

The significant increase of serum RANTES concentration in early-onset infections in neonates, regardless of their gestational age, birth weight, and Apgar score, not only proves the presence of an active immunological process but also may be a useful biomarker for diagnosis of early-onset severe neonatal infections.

REFERENCES:

1. **Wu J, Chen C, Tsao P *et al.* (2009):** Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. Pediatrics and Neonatology, 50(3): 88–95.
2. **Del Vecchio A, Stronati M, Franco C *et al.* (2014):** Bi-directional activation of inflammation and coagulation in septic neonates. Early Hum. Dev., 90(1): 22-25.
3. **Ferreira R, Mello R, Silva K (2014):** Neonatal sepsis as a risk factor for neurodevelopmental changes in preterm infants with very low birth weight. Journal de Pediatria, 90: 293–299.
4. **Chiesa C, Kemp A, Avis A (2004):** Procalcitonin, CRP, IL-6, and immature to the total neutrophil ratio in the diagnosis of early neonatal sepsis. Clin Intens Care, 7: 14-18.
5. **Wang Z, Yu J (2013):** Recent progress in the diagnosis of neonatal septicemia. Zhongguo Dang Dai Er Ke Za Zhi., 15: 236-241.
6. **Bose C, Laughon M, Allred E *et al.* (2013):** Elgan Study Investigators: Systemic inflammation associated with mechanical ventilation among extremely preterm infants. Cytokine, 61: 315-322.
7. **Stojewska M, Wąsek-Buko M, Jakub B *et al.* (2016):** Evaluation of serum chemokine RANTES concentration as a biomarker in the diagnosis of early-onset severe infections in neonates. Postepy Hig Med Dosw., 70: 272-9.
8. **El-Lahony D, El-Sayed H, El-Hawy M *et al.* (2018):** L-carnitine serum level in healthy and septic neonates. Kasr Al Ainy Med J., 24: 26-31.
9. **Manoura A, Gourgiotis D, Galanakis E *et al.* (2010):** Circulating concentrations of alpha- and beta-chemokines in neonatal sepsis. Int J Infect Dis., 14:806–809.
10. **Carrol E, Thomson A, Mobbs K *et al.* (2000):** The role of RANTES in meningococcal disease. J Infect Dis., 182: 363-366.
11. **Ng P, Li K, Leung T *et al.* (2006):** Early prediction of sepsis-induced disseminated intravascular coagulation with interleukin-10, interleukin-6, and RANTES in preterm infants. Clin Chem., 52: 1181–9.
12. **Sato K, Kawasaki H, Nagayama H *et al.* (2001):** Chemokine receptor expressions and responsiveness of cord blood T cells. J Immunol, 166: 1659-1666.
13. **Shouman B, Badr R (2010):** Regulated on activation, normal T cell expressed and secreted, and tumor necrosis factor-α in septic neonates. J Perinatol., 30: 192-196.