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Plasma Protein Z Level in Neonates with Respiratory Distress Syndrome Compared with Healthy Neonates

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Abstract

Background: Data on Protein Z (PZ) level may be useful in neonatal respiratory distress syndrome (RDS) as in RDS the defect in both coagulation and fibrinolytic systems may play a role in the pathogenesis and progression of the disease. **Aim:** To evaluate the plasma protein Z levels in neonatal respiratory distress syndrome. **Methods:** This study was performed on 40`neonates having respiratory distress syndrome and 40 age and sex matched apparently healthy neonates as controls. Plasma Protein Z levels were assessed in plasma of all neonates by ELISA. **Results:** There was statistically significant decrease in plasma protein Z level among patients than controls. In addition, there was a significant decrease in PZ among died than survived patients. A significant negative correlation was observed between PZ and severity (down score) while a significant positive correlation was observed between PZ level and gestational age. **Conclusion:** Plasma Protein Z. level is decreased in neonatal respiratory distress syndrome and it may be a good biomarker for detection of the severity and prognosis of the disease.

Key words: Plasma Protein Z; Respiratory Distress Syndrome; Severity

Introduction:

Neonatal respiratory distress syndrome (RDS) is a condition of pulmonary insufficiency that in its natural course commences at or shortly after birth and increases in severity over the first 2 days of life [(1)]. It is characterized by leakage of plasma proteins into the air space, leading to interstitial and intra-alveolar thrombin generation and fibrin deposition as well as systemic activation of clotting, complement and polymorphnuclear lymphocytes [(2)]. Abnormalities in the

coagulation and fibrinolytic systems may play a role in the pathogenesis of RDS and contribute to the progression of the disease [(3)].

Protein Z (PZ) is a single chain, vitamin K-dependent glycoprotein that was purified from human plasma. Analogous with the majority of the coagulation proteins, protein Z is synthesized in the liver [(4)]. Based on amino acid sequence homology, the domain structure is similar to that of other vitamin K-dependent zymogens, which include; factor VII, factor IX, factor X, and protein C **[(5)].** Human protein Z can be prepared from fresh frozen plasma. The purified protein Z is supplied in 50% (vol/vol) glycerol/H_2O and should be stored at -20^oC. **[(6)]** It is 62 kDa large and 396 amino acids long. The Protein-Z (PROZ) gene has been linked to the thirteenth chromosome (13q34) **[(7)]**.

The mean plasma concentration of Protein-Z in adults was found to be between 1500 and 3000 ng/dl, while its level in babies and younger children range below 1500 ng/dl **[(8)]**. There are some previous reports in healthy term newborns, compared with older children and adults.

The physiological function of protein Z is still rather ill-defined. Functionally protein Z has been shown to be a direct requirement for the binding of thrombin to endothelial phospholipids **[(6)]**. The cofactor action of PZ is manifested after its binding to phospholipids and presumably involves the proper localization of protein Z (PZ)–dependent inhibitor (ZPI-PZ) complex on the phospholipids surface, for interaction with the Xa**[(9)]**.

The half-life time of protein Z in plasma is about 2.5 days. There are also some indications that only a fraction of the Protein-Z pool existing in plasma; higher concentrations seem to be found in proliferating vascular endothelium **[(10)]**. Like other coagulation proteins and inhibitors, protein Z is consumed during disseminated intravascular coagulation (DIC) **[(11)]**. Reduction of protein Z in RDS may contribute to this prothrombotic condition **[(12)]**.

Aim of the study:

This work aimed to study the level of plasma protein Z in healthy newborns and newborns with respiratory distress syndrome.

Subjects and Methods

This case-control study was carried out on 80 neonates recruited from Benha University Hospital during the period from October 2020 to October 2021. An informed consent was obtained from all the patient's and control's guardians before participation of their neonates in the study. The study was approved by Benha Faculty of Medicine Research Ethic Committee at Benha University and conducted according to the principles of the Declaration of Helsinki.

The neonates included in the study were divided into two groups:

Group I: included 40 preterm neonates suffering from respiratory distress syndrome as a patient group. Their diagnosis was based on clinical examination and radiological grading of severity of RDS [(13).]

Neonates with sepsis, hemorrhagic disorders or congenital anomalies were excluded from the study.

Group II: included 40 apparently healthy preterm neonates who match group I in age and sex as a control group.

All the neonates received 1mg i.m. vitamin k1 at birth.

The neonates included in the study were subjected to detailed history taking with emphasis on sex, age, mode of delivery, gestational age, birth weight, Apgar score at 1 and 5 min and history of maternal diseases and medications as well as, thorough clinical examination including assessment of the degree of RDS using down score. Chest X-rays of the patients were used to sub-classify them into subgroups according to their grades of RDS [(13).]

Laboratory Investigations were performed for all the enrolled subjects.

Sampling:

Four milliliters of venous whole blood were obtained from the patients and controls in the first day of life under complete aseptic conditions and was divided to:

- One ml of blood was collected in a test tube containing EDTA (1.2 mg/ml) for CBC.

- Also, 1.6ml of blood was collected in a tube containing 0.4cm (0.11 M sodium

citrates) then centrifuged at 4°C for 15 mins at 1000 × g within 30 mins of collection. The separated plasma was collected and assayed promptly for PT and PTT then the rest of the sample was aliquoted and stored at -80°C for Protein Z level detection by ELISA.

- The rest of blood was collected in a plain test tube, then allowed to clot then centrifuged at 1500 rpm for 30 minutes for serum preparation for CRP

Laboratory tests:

1- Complete blood count was performed using automated hematology system (Sysmex XE 5000).
2- C-reactive protein (CRP) estimation was detected by nephelometry methodology using MISPA-i2 kit supplied by AGAPPE Diagnostics, Kerala , Indea.
3- Arterial blood gases (A.B.G) when indicated.
4- Prothrombin time, partial thromboplastin time (PT, PTT) using automated coagulometer (Sysmex CA-600) using Thromborel S kit: Amelung GmbH, Lierne, Germany .
5- Plasma Protein Z. level assayed by ELISA technique using (Bioassay Technology Laboratory kit, Cat.No.E1177Hu) according to the manufacturer's recommendations.

III. Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company).Categorical data were presented as number and percentages. Chi square test (X^2), or Fisher's exact test (FET) were used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilkstest, assuming normality at P>0.05, they were expressed as mean ± standard deviation, median, IQRand range. Student "t" test was used to analyze normally distributed variables among 2 independent groups. While non-parametric variables were analyzed using Man Whitney U test. Kruskal Wallis test (KW) was used for analyzing non parametric variables among 3 independent groups. Significant KW test was followed by Bonferroni adjusted Mann Whitney to detect the significant pairs. Spearman's correlation coefficient (rho) was used to assess liner association between variables . The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

Results:

The characteristics of the patients and control groups were shown in **(Table 1)**. There were no statistically significant differences in terms of gestational age, birth weight, sex, mode of delivery and history of antenatal steroids between RDS patients and the control group (P>0.05).

Our study included 40 preterm neonates with RDS, 20 (50%) males and 20 (50%) females. Their mean gestational age was 30.20 ± 3.94 weeks. 40 healthy neonates, 20 (50%) males and 20 (50%) females were served as a control group. Their mean gestational age was 31.35± 2.63 weeks **(Table 1).**

In this study, 8 patients (20%) received servanta, 10 patients (25%) were on continuous positive airway pressure (CPAP), 22 patients (55%) were on mechanical ventilation (MV) and 8 patients (20%) were on nasal pronge. Regarding the outcome, 12 patients (30%) died and 28 patients (70%) were discharged. Regarding the days of O_2 support, the mean of nasal CPAP was 20.000 ± 1.63 days, the mean of MV was 25.50 ± 4.03 days and the mean of nasal pronge was 13.37± 2.50 days as shown in **(Table 2).**

In our study, there were no statistically significant differences between the patients group and the control group regarding the CBC findings, C-reactive protein (CRP) levels, prothrombin time, and partial thromboplastin time. **(Table 3)**

Regarding plasma protein Z level in the enrolled subjects, there was statistically significant decrease in plasma protein Z. level among patients than controls (p value .000) as shown in **Table 3 and Figure 1.** In addition, there was a statistically significant decrease in Plasma Protein Z with increasing grades (severity) of RDS. As there was a significant decrease in plasma protein Z in grade 4 in comparison with other grades (1&2&3) (p value=.000 .039 .049), significant decrease of plasma protein Z in grade 3 in comparison with grade2&1 (p value=.047 .002), and significant decrease of plasma protein Z in grade 2 in comparison with grade 1(p value=.013), **(Table 4 and Figure 1).**

As demonstrated in **Table 5 and Figure 1,** there was statistically significant decrease

in Plasma Protein Z. level among died than discharged patients**.**

Table (6) shows that there was statistically significant positive correlation between Plasma Protein Z. level and Gestational age, and significant negative correlation between Plasma Protein Z. level and (severity, down score), while there was no statistically significant correlation between Plasma Protein Z. level and other numerical data.

The ROC curve showed that the cutoff level of Plasma Protein Z. for detection of severity of RDS was 26.6 with sensitivity 80%, and specificity 50%, PPV was 34.8% and NPV was 88.2%, accuracy 57.5% as shown in **table 7 and Figure 2.**

Table (1): Comparison of some demographic and clinical data in the two studied groups:

Demographic & clinical data			Patients group	Controls group	t.test	P. value
Gestational age (wks)	**Mean ± SD**		30.20± 3.94	31.35± 2.63	-4.200	.092
Birth weight (gm)	**Mean ± SD**		1408.25 ± 405.54	1589.13 ± 451.31	3.555	0.063
Sex	**female**	**No.**	20	20	X^2 0	1
		%	50.0%	50.0%		
	male	**No.**	20	20		
		%	50.0%	50.0%		
Mode of delivery	**CS**	**No.**	28	30	X^2 .251	0.617
		%	70.0%	75.0%		
	VD	**No.**	12	10		
		%	30.0%	25.0%		
History of antenatal steroids	**NO**	**No.**	11	5	X^2 2.813	.094
		%	27.5%	12.5%		
	YES	**No.**	29	35		
		%	72.5%	87.5%		

Table (2): Clinical characteristics of the patient's group (n=40)

Clinical characteristics		No.	%
Received servant	**no**	**32**	**80.0**
	yes	**8**	**20.0**
O_2 support	**CPAP**	**10**	**25.0**
	MV	**22**	**55.0**
	nasal	**8**	**20.0**
Outcome	**died**	**12**	**30.0**
	discharge	**28**	**70.0**
		Mean ± SD	
Days of O_2 support	**CPAP**	**20.000± 1.63**	
	MV	**25.50± 4.03**	
	nasal prong	**13.37± 2.50**	

Table (3): Comparison between patient group and control group regarding laboratory investigations

laboratory investigations		Patients group	Controls group	t.test	P. value
Hb (mg/dl)	**Mean ± SD**	12.91 ± 1.44	12.65 ± 1.00	0.930	0.355
TLC (10^3)	**Mean ± SD**	8.95± 1.45	8.25± .854	2.617	0.091
Platelets (cell/cmm)	**Mean ± SD**	291.45 ± 73.05	288.75 ± 56.52	0.185	0.854
C reactive protein (CRP)	**Negative**	40 (100%)	40 (100%)	0	1
	Positive	0 (0.0%)	0 (0.0%)		
Prothrombin time (seconds)	**Mean ± SD**	12.23 ± 0.81	12.17 ± 0.52	0.155	0.694
partial thromboplastin time (seconds)	**Mean ± SD**	33.66 ± 1.57	34.01 ± 1.45	1.073	0.304
Plasma Protein Z. level (ng/L)	**Mean ± SD**	26.04± 5.25	35.85± 11.22	-5.009	.000

Table (4): Relation between the radiological grading of RDS in the Patients group and Plasma Protein Z. level.

Plasma Protein Z. level (ng/L)		Mean ± SD	F test	p. value	LSD
Radiological grading of RDS in Patients group	**grade1**	30.15± 5.18	2.74	0.001	P1=.013
	grade2	24.2± 3.56			P2=.002
	grade3	21.4± 4.92			P3=.000
	grade4	18.5± 3.61			P4=.047
					P5=.039
					P6=.049

P1 ---→ between grade1 and grade2P2---→between grade1 and grade3

P3---→between grade1 and grade4P4---→ between grade2 and grade3

P5---→between grade2 and grade4P6---→ between grade3 and grade4

Table (5): Relation between plasma protein Z. level and the outcome of the patients.

	Plasma Protein Z. level (ng/L)		t.test	P. value
	Died(N=12)	Discharged (N=28)		
OutcomeMean ± SD	22.60± 4.86	27.51± 4.76	-2.961	0.005

Table (6): Correlation between Plasma Protein Z. level with some clinical and laboratory variables.

Correlations	Pearson's correlation	
	r	p
Gestational age * Plasma Protein Z. level	0.518	**0.001**
birth weight gm * Plasma Protein Z. level	.253	0.115
Apgar score * Plasma Protein Z. level	.61	0.13
HB * Plasma Protein Z. level	0.199	0.077
TLC * Plasma Protein Z. level	-0.45	0.077
Platelets * Plasma Protein Z. level	-0.132	0.242
Prothrombintime * Plasma Protein Z. level	0.203	0.071
partial thromboplastin time * Plasma Protein Z. level	0.384	0.080
Severity * Plasma Protein Z. level	-0.40	0.**010**
Down score * Plasma Protein Z. level	-0.49	0.**001**

Table (7): Diagnostic accuracy of plasma protein Z. level in detection of severity in the patients group.

	Cut off value	AUC	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy
Plasma Protein Z. (ng/L)	26.6	0.76	80%	50%	34.8%	88.2%	57.5%

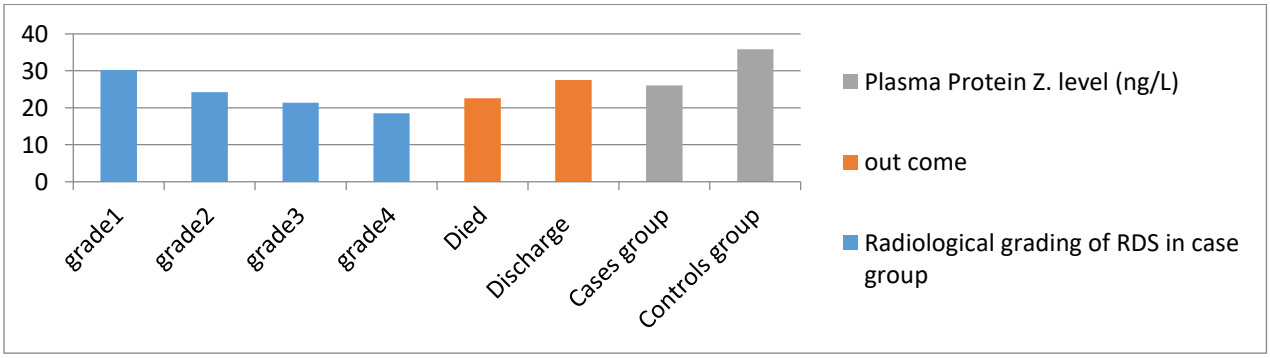

Figure1. Disruption of studied groups regarding their Protein Z level

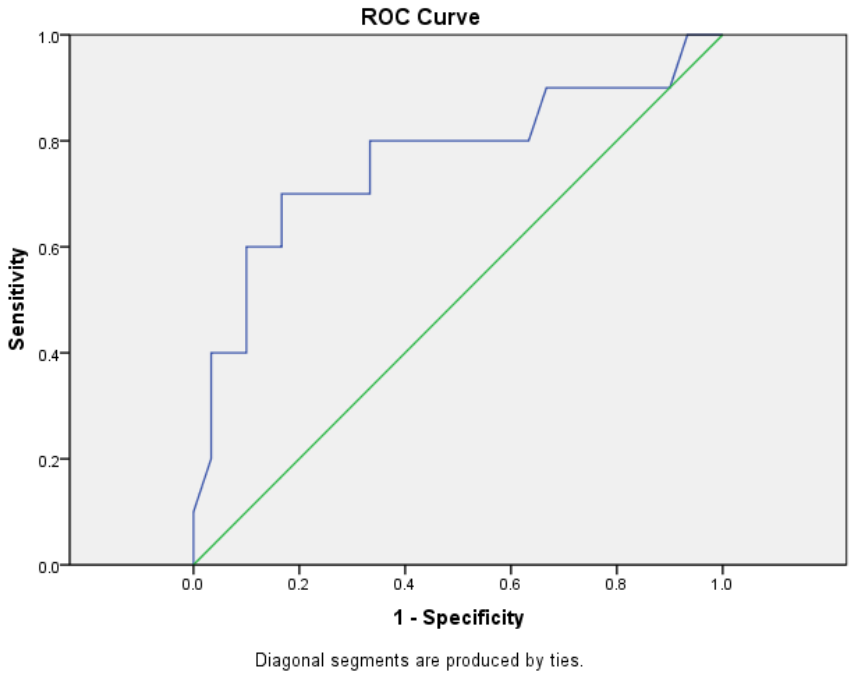

Figure (2): ROC curve for plasma protein Z. level in detection of severity in patients group.

Discussion:

Respiratory distress syndrome (RDS) is one of the most important diseases in preterm infants and a major cause of morbidity and mortality in neonatal ICU. It presents within 4–6 h's of life and is characterized by tachypnea, respiratory distress with chest retractions, grunting, and cyanosis **(14)**.

Fibrin deposition has been demonstrated in pulmonary microcirculation and in small airways in neonatal RDS. (15) Protein Z which is a vitamin K dependent protein was proved to play a pivotal role in the prevention of coagulation (8). Accordingly, the presence of fibrin deposition in neonatal RDS has been explained by the activation of the coagulation system and the reduction of PZ in RDS may contribute to this prothrombotic condition (15).

In the current study we found a significantly lower level of plasma protein Z in RDS preterm infants when compared with the control group. These results are in consistent with other researchers **(5)** who aimed to evaluate the importance of measuring plasma protein Z (PZ) levels in healthy and high risk newborns. They

revealed that PZ deficiency occurs in newborns affected by RDS. They suggested that the reduction of PZ in RDS may contribute to the prothrombotic condition. Our results also were in agreement with the results of a study done by other researchers [16] who found lower levels of protein Z (PZ) in newborns suffering from RDS than in healthy newborns.

Previous studies [15 & 17] related the difference in PZ levels between RDS patients and preterm controls to the coagulation theory, as there is activation of the coagulation mechanism in RDS patients. Protein Z acts as an essential cofactor for PZ-dependent protease inhibitor (ZIP), which in turn is a potent down-regulator for coagulation factor X. Lower levels of PZ in RDS patients result in activation of coagulation with intra-alveolar fibrin deposition which would significantly impair the surfactant's function.

Regarding the outcome, the present study showed that 30% of RDS patients died. This was in agreement with other studies [18] who documented that the mortality rate varying from 23% to 37%. In addition, previous studies [19 & 20] revealed also high mortality rates among studied neonates with respiratory distress syndrome. It was 58.8% and 43.61% respectively.

The results of our study revealed a significant positive correlation between plasma protein Z and gestational age while a significant negative correlation with severity of RDS (Down score). There was no significant correlation between plasma protein Z level and weight, APGAR score, hemoglobin %, TLC and platelets count.

This was in agreement with other researchers [16] who showed no effect of weight, APGAR score, hemoglobin %, TLCs on PTZ levels. Also, our results were in agreement with other researchers [21], who noted no effect of weight on PTZ levels. On the contrary, other researchers [17] stated that the hypercoagulable state in RDS patients is responsible for the increase in platelets levels. They related the abnormalities in fibrinolytic system in these patients to lung damage and local platelet activation which lead to coagulation abnormalities other than DIC.

In the present study, there was no statistically significant correlation between plasma protein Z. level and PT

and PTT. These results were in accordance with other researchers [17] who reported that the activation of clotting is not prominent in the early stages of RDS. On the contrary a previous study [22], found a decrease in activated protein C (APC) levels in the broncho-alveolar lavage fluid in patients with moderate to severe RDS when compared to normal controls, they attributed this finding to activation of coagulation in their patient's group. In addition**,** other researchers [23] stated that in response to hypoxia in RDS, an inflammatory cascade is initiated and micro-vascular injury ensues. Specifically, within 10 min, leukocyte adherence to the endothelium begins and leukocyte emigration and vascular leak soon follow.

Conclusions

Plasma Protein Z. level is decreased in neonates with respiratory distress syndrome. It is significantly decreases with increasing the severity (radiological grades) of RDS. It is significantly lower in died patients than in the survived patients. It may be a good biomarker for diagnosing the severity of RDS patients and their prognosis.

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