

## Plasma protein Z levels in healthy and high-risk newborn infants

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**Abstract:** *Objectives:* To evaluate plasma protein Z (PZ) levels in healthy and high-risk newborn infants. *Background:* Protein Z (PZ) is a vitamin K-dependent plasma protein, As is the case with other coagulation proteins and inhibitors, protein Z is consumed during disseminated intravascular coagulation (DIC), Functionally protein Z has been shown to be a direct requirement for the binding of thrombin to endothelial phospholipids, Protein Z also serves as a cofactor for the inhibition of coagulation factor Xa by a plasma serine called protein Z-dependent protease inhibitor (ZPI), The inhibitory function is exerted by the Protein Z- dependent protease inhibitor (ZPI), which circulates in the human plasma in a complex with PZ, The physiological function of protein Z is still rather ill-defined and may play role in high risk newborn. *Methods:* This study was conducted on 85 newborns divided in 4 groups, (group I newborns affected by respiratory distress syndrome (RDS), group II newborns from mothers with pre-eclampsia, group III newborns small for gestational age (SGA) and group IV healthy term and preterm newborns normal for gestational age. Newborns with sepsis, congenital malformation or hemorrhagic disorders were excluded, Plasma PZ levels was measured. *Results:* In the neonates of the study groups, protein z level was significant lower in patient group than control group, in group I ( $0.79 \pm 0.32$ ), group II ( $0.70 \pm 0.30$ ), group III ( $0.78 \pm 0.32$ ) and group IV ( $1.44 \pm 0.43$ ) (p value < 0.001). *Conclusion:* PZ deficiency occurs in newborns affected by severe RDS, in newborns from preeclamptic mothers and in SGA newborns, probably owing to activated coagulation in the first two conditions and to reduced PZ synthesis in the last one.

**Keywords:** Hemostasis, Healthy Newborns, Newborns from Mothers Affected by Pre-Eclampsia and SGA Newborns, High-Risk Newborns, Protein Z, Respiratory Distress Syndrome

## 1. Introduction

Protein Z (PZ) is a single chain, vitamin K- dependent glycoprotein that was purified from human plasma in 1984(7). Analogous with the majority of the coagulation proteins, protein Z is synthesized in the liver(10) With a molecular weight of 62 000 Da (11).

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 (10).

Human protein Z is prepared from fresh frozen plasma similar to the procedure described by Broze and Miletich.

The purified protein Z is supplied in 50% (vol/vol) glycerol/H<sub>2</sub>O and should be stored at -20°C. Purity is assessed by SDS-PAGE analysis (10).

It is 62 kDa large and 396 amino acids long. The PROZ gene has been linked to the thirteenth chromosome (13q34) (13).

The mean plasma concentration of Protein-Z of adults was found to be between 1500 and 3000 ng/dl, the levels of babies and younger children range below 1500 ng/dl (6).

The half-life time in plasma is about 2.5 days (11). 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 (11).

The physiological function of protein Z is still rather ill

defined. As is the case with other coagulation proteins and inhibitors, protein Z is consumed during disseminated intravascular coagulation (DIC) (10).

Functionally protein Z has been shown to be a direct requirement for the binding of thrombin to endothelial phospholipids (10).

The cofactor action of PZ is manifested after its binding to phospholipids and presumably involves the proper localization of ZPI-PZ complex on the phospholipid surface, for interaction with the Xa (1).

Few data are currently available with regard to plasma PZ concentration in newborns. There are some reports in healthy term newborns, compared with older children and adults (11), and in newborns with early respiratory distress syndrome (RDS) (12), without definitive statements, whereas no data have been published for newborns with intrauterine growth retardation (IUGR) or newborns from mothers with preeclampsia.

This study evaluated PZ in different groups of high-risk newborns, to evaluate differences related to intrauterine growth, the presence of RDS and a maternal history of pre-eclampsia.

## 2. Patients and Methods

This study was conducted on 85 neonate admitted to the pediatric Department of Monoufia University neonatal intensive care unit from January 2013 to 30 July 2013. On the day of birth, all information on gestational age, based on last menstrual period and Dubovitz assessment, head circumference, weight, Apgar score at 1 and 5 min, clinical data and medications was recorded. Additional information was collected on maternal history, maternal diseases, medications and mode of delivery.

All newborns received 1 mg i.m. vitamin K1 at birth.

Inclusion criteria. Healthy term and preterm newborns normal for gestational age (NGA) and newborns belonging to one of the following groups: newborns small for gestational age (SGA); newborns affected by RDS, diagnosed on lung X-ray findings, together with the beginning of respiratory

symptoms before 6 h of age and the need for any type of ventilatory support; and newborns from mothers affected by pre-eclampsia.

Exclusion criteria. Newborns with sepsis, congenital malformations or hemorrhagic disorders.

### 2.1. Groups

Newborns were divided into the following groups in accordance with the inclusion criteria: Group I, newborns affected by RDS 20 (10 males and 10 females). Group II, newborns from mothers affected by pre-eclampsia 18 (10 males and 8 females), Group III, newborns small for gestational age (SGA) 17 (9 males and 8 females), (group IV) Healthy term and preterm neonate 30 (13 males and 17 females).

Investigations:- Chest X-ray when indicated, Complete blood count (C.B.C), C-reactive protein (C.R.B) to exclude sepsis, Arterial blood gases (A.B.G) when Indicated, Prothrombin time , partial thromboplastin time (PT , PTT) and Protein Z by Immunoenzymatic method (ELISA).

### 2.2. Protein Z Method

Blood samples (1 ml) were collected in tubes containing 0.11M sodium citrate. The blood was centrifuged within 1 h at 2500 g for 10 min. Platelet-poor plasma was fractioned and frozen ( $-80^{\circ}\text{C}$ ), and thawed before.

Assay within 3 mo. of storage. PZ was measured by a quantitative immune enzymatic assay.

### 2.3. Statistical Analysis

Results were statistically analyzed by statistical package SPSS version 16.

One a way ANOVA (F test) Kruskal -Wallis test: Post hoc test: Chi-Squared Fisher's exact test: Mann-Whitney test: Pearson's Correlation analysis: P value significant difference if  $P < 0.05$  highly significant difference if  $P < 0.001$ .

## 3. Results

Table 1. Distribution of the studied groups regarding their characteristics

	I (n=20)		II (n=17)		III (n=18)		IV (n=30)		Test	P value
	no	%	no	%	no	%	no	%		
HC									F=47.95	P1=0.236(NS)
Mean $\pm$ SD	33.65 $\pm$ 1.42		34.52 $\pm$ 0.71		30.27 $\pm$ 1.52		30.06 $\pm$ 1.04		P<0.001	P2=0.211(NS)
									(HS)	P3<0.001(HS)
Weight									F=82.90	P1=0.901(NS)
Mean $\pm$ SD	2911.0 $\pm$ 357.54		3019.4 $\pm$ 265.43		1449.4 $\pm$ 135.84		3019.3 $\pm$ 481.65		P<0.001	P2=0.999(NS)
									(HS)	P3<0.001(HS)
Post natal age									$\chi^2$	
1d	8	40.0	13	76.5	7	38.9	17	56.7	5.20	P1=0.157(NS)
2d	6	30.0	3	17.6	6	33.3	11	36.7	1.99	P2=0.368(NS)
3d	5	25.0	1	25.0	4	22.2	2	6.7	4.59	P3=0.204(NS)
4d	1	5.0	0	5.0	1	5.6	0	0.0		
Sex									$\chi^2$	
Male	10	50.0	8	47.1	8	44.4	16	53.3	0.05	P1=0.817(NS)
Female	10	50.0	9	52.9	10	55.6	14	46.7	0.0	P2=0.979(NS)
									0.02	P3=0.881(NS)

P1=1#4 P2= 2#4 P3=3#4

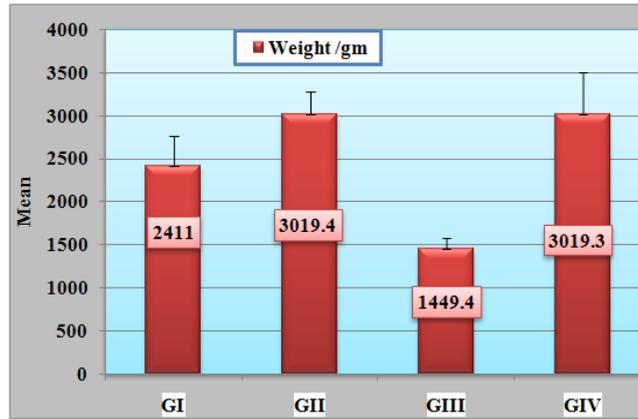


Fig 1. Distribution of the studied groups regarding their weight

Table 2. Distribution of the studied groups regarding their Protein Z

	I (n=20) Mean ±SD	II (n=17) Mean ±SD	III (n=18) Mean ±SD	IV (n=30) Mean ±SD	Kruskal-Wallis Test	P value
Protein Z	0.79 ±0.32	0.70± 0.30	0.78 ±0.32	1.44 ±0.43	36.58 P<0.001	P1<0.001(HS) P2<0.001(HS) P3<0.001(HS)

P1=1#4 P2= 2#4 P3=3#4

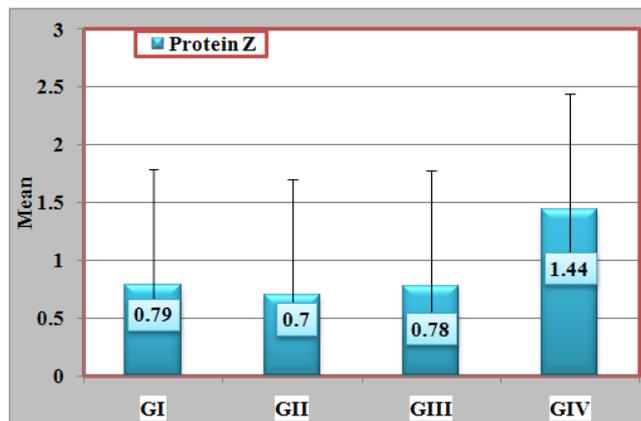


Fig 2. Distribution of the studied groups regarding their protein Z

Table 3. Distribution of the studied groups regarding their lab investigations

	I (n=20) Mean ±SD	II (n=17) Mean ±SD	III (n=18) Mean ±SD	F test	P value
Hb	13.31± 2.92	12.58 ±2.53	11.72 ±2.99	1.47 P=0.273	I vs. II=0.440(NS) I vs. III =0.092(NS) II vs. III =0.376(NS)
WBCs	10.13± 2.33	9.02 ±2.45	8.78 ±1.80	2.03 P=0.141	I vs. II =0.134(NS) I vs. III =0.066(NS) II vs. III =0.749(NS)
Platelets	229.05± 61.20	265.65 ±86.12	217.22 ±68.94	2.13 P=0.128	I vs. II =0.130(NS) I vs. III =0.616(NS) II vs. III =0.052(NS)
PT	13.23± 1.13	13.27 ±1.06	13.11 ±1.15	0.09 P=0.908	I vs. II =0.913(NS) I vs. III =0.764(NS) II vs. III =0.767(NS)
PTT	36.65± 4.13	35.64 ±2.69	35.94 ±3.29	0.41 P=0.663	I vs. II =0.385(NS) I vs. III =0.534(NS) II vs. III =0.801(NS)
CRP					
+ve	0 (0%)	0 (0%)	0 (0%)		
-ve	20 (100%)	17 (100)	18 (100%)		

## 4. Discussion

Data on PZ levels in newborns could be useful, particularly in diseases such as RDS in which fibrin deposition has been demonstrated in the pulmonary microcirculation and in small airways (12).

Neonatal RDSs are characterized by leakage of plasma proteins of varying sizes into the airspace, which leads to interstitial and intra-alveolar thrombin generation with subsequent fibrin deposition (5), systemic activation of clotting, complement and polymorph nuclear lymphocytes (14). PTZ, which is a vitamin K dependent protein, proved to play a role in the prevention of coagulation (6).

Accordingly, the presence of fibrin deposition has been explained by the activation of the coagulation system (12). Reduction of PZ in RDS may contribute to this prothrombotic condition.

In this study, we aimed to evaluate importance of measuring plasma protein Z (PZ) levels in healthy and high-risk newborn.

Regarding the demographic data of the studied neonates, there was no significant difference in age (days), sex, mode of delivery, maternal history and these is agree with (Schettini, et al., 2004).

In the present study, the clinical data shows no significant difference between the studied groups, as regard, gestational age (weeks), length, head circumference and systems examinations (with exclusion of cases with RDS). This helped us a lot to exclude other causes in the studied neonates that might cause protein z deficiency.

These results are in accordance with (Aycicek et al., 2008) and (Schettini, et al., 2004), who found that there was no significant difference in the demographic or clinical data of his studied neonates.

The current study showed no effect of either gestational age, Apgar score, hemoglobin percentage, TLCs, pH or PCO<sub>2</sub> on PTZ levels in all groups included in the study. This was in agreement with Corral et al, (2007), who noted no effect of either the gestational age or weight on PTZ levels.

The present study confirm low PZ levels were found in the newborns with RDS, These results are in accordance with (Schettini, et al., 2004) who found that there was significant difference in protein z level in the newborns with RDS.

This in contrast to Yurdakok et al. (2002), which found similar PZ levels in newborns with RDS and in healthy preterm newborns. This discrepancy could be the result of a different degree of severity of RDS.

Previous studies (Schettini, et al., 2004) found low levels of antithrombin and high levels of thrombin-antithrombin complex (TAT) in newborns with severe RDS, and therefore low levels of PZ could be a consequence of this prothrombotic condition.

Low levels of PZ in the RDS newborns can be related neither to prematurity, because no difference was found in PZ levels between term and preterm newborns, nor to IUGR, because no newborn was SGA among RDS newborns, nor to

vitamin K deficiency as all newborns taken vitamin k injection at brith.

In newborns born to mothers affected by pre-eclampsia PZ deficiency could be a consequence of the prothrombotic effect of proinflammatory cytokines released via the placenta (Grignani, et al., 2002).

Normal pregnancy is characterized by an increased plasma concentration of protein Z (9) which has been proposed to be part of a compensatory mechanism for the increased concentration of factor X and perhaps for the increased thrombin generation. Preeclampsia is associated with an exaggerated hypercoagulable state and excessive thrombin generation, as determined by higher maternal plasma concentrations of TAT complexes (8) and lower antithrombin III concentrations than patients with a normal pregnancies. Moreover, patients with PE who delivered preterm have a higher rate of thrombotic lesions in the decidua and in the placental villi than normotensive patients with indicated or spontaneous preterm delivery. Therefore, it is possible that an exaggerated procoagulant state will account for the lower plasma concentration of protein Z among women with preeclampsia (2).

The finding that women with fetal demise have a higher rate of protein Z deficiency than women with normal pregnancy. Thus, the physiologic hypercoagulable state that accompanies pregnancy may facilitate the occurrence of thrombotic events of the placenta and adverse pregnancy outcome (i.e. fetal demise) in potentially thrombophilic patients that were clinically "silent" in the non-pregnant state. In addition, Gris et al reported that six out of eight patients with protein Z deficiency had one parent who is also protein Z deficient; thus, the possibility that in some cases protein Z deficiency may be inherited cannot be ruled out (14).

The current study confirm low PZ levels in the newborns of mothers with pre-eclampsia than control group, This was in agreement with, Yurdakok et al. (2002) and Schettini et al. (2004).

In SGA newborns PZ deficiency might be explained by a reduced synthesis of this protein, as already known for other plasma proteins (11).

The current study show significant decrease in protein z level in SGA newborns than control group, And This was in agreement with Schettini et al., (2004).

## 5. Conclusion

Newborns affected by severe RDS, newborns born to pre-eclamptic mothers and in SGA newborns there is a PZ deficiency which is probably related to activated coagulation in the first two conditions and to a reduced synthesis in the last one.

## Recommendations

This study did not investigate parents of newborns with PZ deficiency and did not determine whether this deficiency

persisted after recovery from RDS.

Serial measures of plasma PTZ levels in premature may be of benefit in RDS to follow up their condition. Further studies are recommended to evaluate the role of PTZ on outcome in premature newborns with RDS and to evaluate the relationship between protein PTZ and PTC and other coagulation factors incriminated in the development of RDS.

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