The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: 1476-7058 (Print) 1476-4954 (Online) Journal homepage: www.tandfonline.com/journals/ijmf20

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To cite this article: Emel Eksi Alp, Nuvit Altinkaya, Zeynep Cagman & Fikriye Uras (2018) Plasma growth arrest-specific 6 levels in term and preterm newborns, The Journal of Maternal-Fetal & Neonatal Medicine, 31:9, 1151-1155, DOI: 10.1080/14767058.2017.1311311

To link to this article: https://doi.org/10.1080/14767058.2017.1311311

Published online: 11 Apr 2017.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE, 2018
VOL. 31, NO. 9, 1151–1155
http://dx.doi.org/10.1080/14767058.2017.1311311

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ORIGINAL ARTICLE

Plasma growth arrest-specific 6 levels in term and preterm newborns

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ABSTRACT

Objective: Growth Arrest-Specific 6 (GAS6) is a vitamin K-dependent protein. Despite a similar structure to Protein S, it has no anticoagulant activity. An association between GAS6 and some diseases for adults has been reported. In the absence of prospective clinical studies of GAS6 in neonates, so far, the objective of this study is to obtain, for the first time, plasma GAS6 levels before and after vitamin K1 prophylaxis in full-term and pre-term newborns.

Methods: 80 newborns (40 term and 40 preterm) were recruited for this study. Cord blood samples and peripheral blood samples 48 h after vitamin K1 injection were collected into EDTA-tubes. GAS6 levels were measured in platelet-poor plasma by ELISA.

Results: Cord blood plasma GAS6 levels in preterm and term newborns were 9.07 ± 5.30 ng/mL and 9.75 ± 4.34 ng/mL, respectively. In response to vitamin K1 injection, GAS6 levels increased in preterm newborns (10.50 ± 5.28 ng/mL) ($p < .05$), but not in term newborns (9.12 ± 3.42 ng/mL, $p > .05$).

Conclusion: This pilot study provided, to the best of our knowledge, the first report that GAS6 levels increased significantly after vitamin K1 prophylaxis in preterm newborns but not in term infants. This study may serve as a first step toward more extensive studies in neonates.

ARTICLE HISTORY
Received 23 September 2016
Accepted 22 March 2017

KEYWORDS
GAS6; vitamin K; newborn; term; preterm; fetal growth retardation

Introduction

Some coagulation factors are well-known vitamin K-dependent (VKD) proteins. However, there are many others such as Growth Arrest-Specific 6 (GAS6) and Matrix Gla Protein (MGP), which are not involved in the coagulation system. Their physiological roles have not been clarified.

GAS6 was described as a gene upregulated by growth arrested fibroblasts [1]. The structure of GAS6 is similar to Protein S and shares the same distinctive structural components. However, unlike VKD-coagulation factors [2,3], GAS6 is not mainly synthesized in the liver [4,5]. It is expressed in peripheral tissues including vascular smooth muscle cells [4], endothelial cells [1], and platelets [5]. As a ligand, GAS6 has different affinities to TAM receptors (Tyro3, Axl and Mer) from the tyrosine kinase family [6].

The warfarin embryopathy has been known for decades indicating the role of vitamin K-dependent proteins during embryonic development. The reason of bone and neurologic abnormalities were considered to be developmental problems from hemorrhage and subsequent calcification within these areas [7]. However, it was recognized that this was unlikely, since clotting factors were known to be absent during the first trimester of pregnancy [8]. The incomplete carboxylation of GAS6 has been reported in the fetus [9]. This may be responsible for teratogenic effects, at least part of the pathology, of maternal warfarin therapy. Expression of GAS6 and the TAM receptor is tissue-specific and developmental pattern.

So far, a cell survival factor [1], a growth factor [4], as well as an essential bridge between apoptotic cells and macrophages during phagositosis have been reported for GAS6 [10]. *GAS6* knockout mice showed that they were resistant to venous and arterial thrombosis [11]. It was suggested that GAS6 increases the tendency to thrombosis by leading to platelet plaque stabilization. The role of GAS6 on platelets is not clear.

GAS6 or TAM receptors knockout mice exhibited autoimmunity [12] due to a problem in the clearance of apoptotic cells. In homeostasis and the resolution of inflammation, the removal of cells is critical. TAM receptors are expressed on the surface of phagocytes. GAS6 makes a bridge by binding to TAM receptors on the phagocytes and the phosphatidylserine on the surface of the apoptotic cells. TAM receptors are also

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expressed on osteoclasts in bone and natural killer cells. GAS6 and its receptors are important for regulating tissue homeostasis via anti-inflammatory effects.

Our current knowledge on GAS6 is mainly gained from experimental studies and recent clinical studies for adults. Some publications for adults have reported that there are associations between GAS6 and some diseases or their complications including sepsis [13], albuminuria in patients with type 2 diabetes [14], systemic lupus erythematosus [15], psoriasis [16], preeclampsia [17], and idiopathic recurrent pregnancy loss [18]. Only a few publications have focused on clinical studies in adolescents regarding relationship of GAS6 and diseases such as thyroid cancer [19], obesity, and insulin resistance [20]. Conditions, such as sepsis, occur in both adults and neonates. However, it is inappropriate to apply to neonates results obtained from clinical studies conducted in adults. Recently in a retrospective study, Lindqvist et al. [21] have reported GAS6 levels in cord blood obtained from pregnant women. In the absence of any prospective clinical study of GAS6 related to neonates, so far, it would be a starting point to ask what the plasma GAS6 concentrations are in term and preterm neonates. Accordingly, we hypothesized that plasma GAS6 levels of neonatals would be increased after vitamin K1 prophylaxis. The objective of this study is to obtain, for the first time, plasma GAS6 concentrations in newborns before and after vitamin K1 prophylaxis.

Material and methods

Study population and sample collection

Eighty newborns (40 term and 40 preterm) were enrolled in this study in the Hospital of Maltepe University Medical School, Istanbul. The study protocol followed the ethical guidelines of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent forms were signed by parents before birth. Newborns with sepsis, congenital anomalies, and suspected metabolic disease were not included in the study. Neonates were excluded if there had been maternal treatment during pregnancy with certain anticonvulsants such as phenytoin, carbamazepine, or phenobarbital; antibiotics such as carbamazepine; antitubercular drugs such as rifampin and isoniazid. Babies' cord blood samples were taken immediately after birth. Peripheral blood samples were collected after 48 h from vitamin K prophylaxis of intramuscular administration of vitamin K1 (1 mg) to the vastus lateralis muscle immediately after birth.

Samples from umbilical cord blood and peripheral venous blood were taken into EDTA-tubes. They were centrifuged at 3000 *g* for 10 min. Platelet-poor plasma was stored at −80 °C until analysis.

ELISA for plasma GAS6

The human GAS6 sandwich ELISA development kit and Substrate Reagent Pack were from R&D Systems Inc., Minneapolis, MN. ELISA was performed in line with our previous publications [14,16,18].

Statistical analysis

Statistical analyses were performed using Statistical Packages for Social Sciences (SPSS) 17.0 statistical package for Windows. Descriptive statistical methods to evaluate the data (mean, standard deviation), as well as to compare differences between two groups Mann–Whitney U-test was used. A value of $p < .05$ was considered statistically significant.

Results

Eighty newborns (40 term and 40 preterm) were enrolled in this study. The term group consisted of 25 girls and 15 boys; and the preterm group of 22 girls and 18 boys. A total of 5 newborn infants (1 term and 4 preterm) had intrauterine (fetal) growth retardation (IUGR). The birthweight of term and preterm newborns, who had no IUGR, was between 2580 g–3850 g (mean 3255 ± 325 g) and 1550 g–2400 g (mean 2063 ± 230 g), respectively. Three newborns (1 preterm and 2 term) were delivered vaginally and 77 newborns were delivered cesarean section.

Cord blood plasma GAS6 levels were not significantly different between preterm (9.07 ± 5.30 ng/mL) and term newborns (9.75 ± 4.34 ng/mL) ($p > .05$) (Figure 1). Forty-eight hours after vitamin K1 injection, plasma GAS6 levels were 10.5 ± 5.28 ng/mL in preterm infants, and 9.12 ± 3.42 ng/mL in term infants indicating no significant difference between two groups ($p > .05$). In preterm newborns, plasma GAS6 levels increased significantly after vitamin K1 injection (9.07 ± 5.3 and 10.5 ± 5.28 ng/mL, respectively) ($p < .05$). Mean cord blood GAS6 level of 5 newborns with IUGR was 3.70 ± 4.32 ng/mL despite 9.76 ± 4.71 ng/mL in newborns without IUGR, indicating a significant difference between two groups ($p < .05$). In this study, there were 18 infants of diabetic mothers (IDMs) in 80 newborns (22.5%). Cord plasma GAS6 levels were 8.27 ± 3.83 ng/mL for IDMs, and 9.74 ± 5.06 ng/mL for infants of non-diabetic mothers. There was no

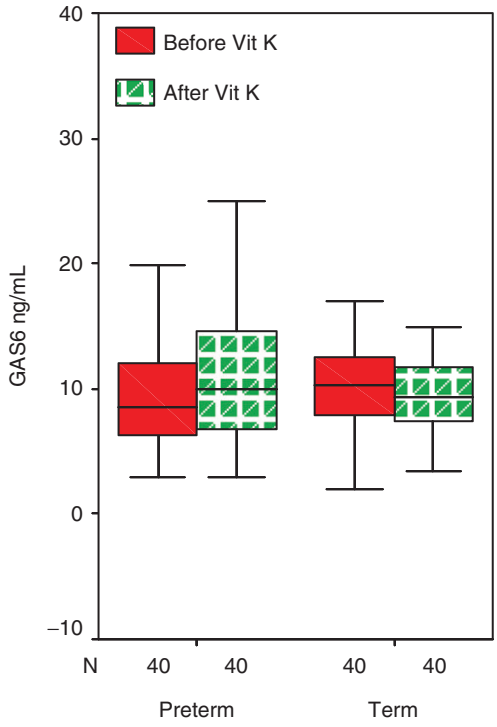

Figure 1. Box-and-Whisker plots of plasma GAS6 concentrations of the newborn.

significant difference between infants of diabetic mothers and non-diabetic mothers ($p > .05$). The performance characteristics of GAS6 ELISA were given in our earlier publication [16].

Discussion

In this study, plasma GAS6 levels were measured in cord blood and 48 h after vitamin K1 injection in peripheral blood. All the newborns had no sepsis, congenital anomalies, or suspected metabolic diseases. In preterm newborns, plasma GAS6 levels were increased significantly after vitamin K1 injection ($p < .05$). However, there were no differences for term infants ($p > .05$).

Recently, some publications have reported that plasma GAS6 concentrations increase during sepsis in adults [13,22]. Ekman et al. [22] found increased levels of GAS6 in patients who had severe sepsis, sepsis and systemic inflammatory response syndrome, with or without infection. The increase was proportional to the severity of the disease. They also showed that levels of GAS6 and its soluble receptor Axl were correlated with the concentration of procalcitonin and Interleukin-6. It has been suggested that GAS6 may regulate the interaction between platelets and leukocytes with endothelium as well as reducing cytokine synthesis with its anti-inflammatory feature [23]. Neonates, particularly premature babies, can be more susceptible to developing sepsis. Since it has been a big challenge to detect sepsis accurately and timely, there is a substantial need for new biomarkers. Current studies in adults urge further studies in neonates and children, which will assess whether GAS6 may be a promising candidate to recognize infection and sepsis earlier and differentiate between infectious and non-infectious inflammation.

According to the World Health Organization, infants born alive before 37 weeks' gestation are called preterm [24]. Mostly, pathogenesis of preterm labor remains unknown. Various factors cause premature birth, such as stress experienced during pregnancy, first birth, inadequate or non-existent prenatal care, chronic infections, hypertension, and diabetes [25]. Intrauterine infection has been linked with preterm delivery. An inflammatory response occurs due to intrauterine infections and this process triggers premature rupture of membranes or delivery. It is still unclear how these infectious agents enter into the uterine cavity, and how different inflammatory responses exist according to the different types of infectious agents [26].

The mean GAS6 level of term infants seems to be comparable to our previously published [27] reference intervals for adults (157 female, 151 male) with medians for males [7.8 (5.8–10.7) ng/mL] and females [9.9 (7.1–13.5) ng/mL]. Age- and gender-specific reference interval estimation are needed not only for neonates but also for children taking into account growth, development, and nutritional status.

Preterm birth rate has increased from 7% to 10–12% in recent years [28]. Preterm infants' survival rates have increased as compared to previous years, probably due to the administration of antenatal steroid to pregnant women to accelerate the baby's lung maturation in preterm labor, protection of baby from hypothermia after birth, early surfactant therapy, and reduction in the postnatal use of dexamethasone [29].

Premature rupture of membranes occurs in 1–5% of pregnancies, which is associated with 30–40% premature deliveries, leading to an increased risk of infection and inflammation [30]. Postnatally, longer hospitalized preterm newborns are exposed to umbilical venous catheterization and invasive procedures such as peripheral venous blood samples taken more frequently than full-term babies. GAS6 may be released from endothelium to circulation at higher rates due to the disruption of endothelial integrity as a result of invasive procedures and with the inflammatory process during preterm rupture of the placenta. Taking into

account GAS6/TAM signaling, future studies will provide evidence for actual mechanism.

In this study, there were 18 infants of diabetic mothers (IDMs) in 80 newborns (22.5%). Cord plasma GAS6 levels were 8.27 ± 3.83 ng/mL for IDMs, and 9.74 ± 5.06 ng/mL for infants of non-diabetic mothers. No significant difference was found between the two groups ($p > .05$). Hsiao et al. [20] have been reported for overweight and obese adolescents that GAS6 levels are associated with inflammation, adiposity, and insulin resistance. Homeostatic parameters of IDMs are particularly affected by various factors of prenatal, natal, and postnatal stages. Gestational diabetes affects 2% of pregnant women and insulin-dependent diabetes is emerging in ~1/1000 of pregnant women [24]. Hormonal profile of IDMs, who are plethoric and macrosomic, includes low glucagon, high insulin, and low adrenaline in IDMs in contrast.

The role of the GAS6/TAM pathway in neonatal obesity and DM is unknown and needs to be clarified. Further studies in this area are needed.

Intrauterine (fetal) growth retardation is defined as being below 2 standard deviation from mean value in weight growth curve, which reflects gestational age and birthweight of babies [24]. In this study, the lowest GAS6 levels in cord blood plasma were observed in the subgroup of IUGR. Mean GAS6 level in infants with IUGR (3.70 ± 4.32 ng/mL) was increased after vitamin K1 prophylaxis (9.76 ± 4.71 ng/mL). The number of infants with IUGR ($n = 5$) were too small compared to the number of cases without IUGR ($n = 75$) to permit meaningful statistical analysis for comparison. However, this observation may be a starting point to plan further studies on neonates with IUGR. A recent study of Lindqvist et al. [21] supported our observation. They have compared GAS6 levels in the cord blood of pregnant women with small-for-gestational age (SGA) or appropriate-for-gestational age (AGA) cases. That was a retrospective study on 25 women with fetuses with SGA and 36 AGA fetuses. In plasma GAS6 levels of cord blood, there was no significant difference between SGA and AGA pregnancies. However, GAS6 concentrations were higher in cases with abnormal umbilical cord Doppler tracings (19.5 ± 5.3 ng/mL vs. 15.0 ± 3.2 ng/mL). The authors reported insignificant associations between cord plasma GAS6 levels and birthweight, the degree of birthweight deviation, or gestational age. The authors speculated that GAS6 might have a role in the fetal adaptation to placental insufficiency. Comprehensive studies for comparing GAS6 levels in term and preterm newborns with special features such as IDMs and IUGR with healthy newborns are needed.

Vitamin K is essential for post-translational carboxylation of glutamic acid resides of the vitamin K-dependent proteins. In vitamin K deficiency, functionally defective des-carboxylated proteins are formed. They cannot adopt its natural conformation to bind calcium and phospholipids. Des-carboxylated (or under-carboxylated) proteins are called "protein-induced by vitamin K absence" (PIVKA). There has not been any publication of a method to measure PIVKA-GAS6 concentrations in plasma or serum. If such an assay is developed, it will provide measurement of biologically active GAS6. This will make possible early detection of vitamin K status in term and preterm infants as well as adults. In addition, measurement of plasma PIVKA-GAS6 levels may aid the development of improved prophylactic regimens. Further studies in this area are needed.

Although we obtained first results of plasma GAS6 levels before and after vitamin K1 prophylaxis in full-term and pre-term newborns, the major limitation of this study is the small sample size. More extensive studies are required to confirm these associations, possibly including monitoring, across selected time periods, especially for the first months of life.

In conclusion, to the best of our knowledge, this study provides the first report on plasma concentration of GAS6 before and after vitamin K1 prophylaxis in full-term and pre-term newborns. In preterm newborns, plasma GAS6 levels were increased significantly after vitamin K1 injection, but not in term infants. The lowest GAS6 levels in cord blood plasma were observed in the subgroup of IUGR, whose mean GAS6 level was increased in response to vitamin K prophylaxis. This pilot study may serve as a first step toward larger and more comprehensive many clinical studies in neonates involving estimation of reference intervals of GAS6, vitamin K status, method development for PIVKA-GAS6, and focusing on changes in diseases/conditions such as sepsis, infection, inflammation, autoimmunity, obesity, diabetes, IUGR, and cancer.

Acknowledgements

We appreciate Brian Parker for reviewing this manuscript for its English content as a native speaker.

Disclosure statement

The authors report no conflicts of interest.

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