Thrombosis Research 133 (2014) 281–284

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Evaluation of indirect microparticle activity and parameters of thrombin generation test in healthy infants

Filiz Simsek Orhon [a,*], Yonca Egin [b], Betul Ulukol [a], Sevgi Baskan [a], Nejat Akar [b]

[a] Ankara University School of Medicine, Department of Pediatrics, Division of Social Pediatrics
[b] Ankara University School of Medicine, Department of Pediatrics, Division of Pediatric Molecular Genetics

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form 23 October 2013
Accepted 18 November 2013
Available online 21 November 2013

Keywords:
Healthy infants
microparticle activity
thrombin generation

ABSTRACT

Introduction: Circulating microparticles support thrombin generation. The aim of this study is to determine the indirect microparticle activity and the parameters of thrombin generation in healthy infants.
Materials and methods: A total of 85 infants who were brought to follow-up visits were taken into the study. Blood samples were collected. Thrombin generation parameters and indirect microparticle activity were measured.
Results: The infants were divided into four groups according to the time of follow-up visits. Mean ages were 1.18 ± 0.19 months in Group 1, 6.15 ± 0.16 months in Group 2, 12.38 ± 0.46 months in Group 3 and 24.53 ± 0.39 months in Group 4, respectively. There was no statistical difference among the age-based groups with respect to the indirect microparticle activity. The lag time and the TTP levels in Group 1 were lower than that found in Group 2. The ETP and peak levels were higher in Group 1 than that of Group 2. The ETP and peak levels in Group 2 were found lower than those found in older children, but the TTP level was found relatively higher. Statistically correlations were found between indirect microparticle activity and all parameters of thrombin generation.
Conclusions: The absence of a difference in terms of age-based microparticle levels may suggest that the features of microparticles in healthy children of this age group are similar. Age-dependent changes in thrombin generation parameters may suggest a regulation mechanism for the thrombin generation system over the first years of life. The results may provide mean values for indirect microparticle activity and thrombin generation in this healthy group.

Introduction

Thrombin, the central enzyme in blood coagulation, is the end product of the coagulation cascade which is initiated after the exposure of the tissue factor on the subendolthelium [1]. Given the central role of thrombin in blood clotting, the tendency of a plasma sample to generate thrombin might contain useful information about thrombotic or hemorrhagic risk. Previous studies indicated that increased formation of thrombin in plasma induces a risk of venous thrombosis [2,3]. Therefore, measurement of a participant's capacity to generate thrombin is very useful as a reflection of a thrombotic phenotype compared to conventional coagulation tests [4].

Microparticles are submicron vesicles shed from plasma membranes in response to cell activation, injury and/or apoptosis. These elements circulate in the peripheral blood and play active roles in thrombosis, inflammation and vascular reactivity [5,6]. Microparticles are the main carriers of the circulating tissue factor, the principal physiologic initiator of thrombosis. Further, circulating microparticles provide an additional procoagulant phospholipid surface for the accumulation of the enzyme complexes of the coagulation cascade. In other words, microparticles express phospholipids which are procoagulants, and support thrombin generation [7–9]. Elevated levels of circulating microparticles may be a possible independent risk factor for venous thromboembolism [10].

Previous studies showed that microparticles circulating in healthy individuals may stimulate low-grade thrombin generation which is necessary for normal protein C activation [11,12]. Differences in the plasma levels of the assay determinants account for the large interindividual variation in the thrombin generation parameters observed in previous population studies [13,14]. In recent years, microparticle associated thrombin generation in thrombotic or prothrombotic states have been studied; on the other hand, microparticle activity and thrombin generation mediated by microparticles have not been studied in healthy infants extensively. The aim of this study, therefore, is to determine the levels of both indirect microparticle activity and the parameters of thrombin generation in healthy infants.

Materials and Methods

This study was conducted at the Department of Pediatrics, Division of Pediatric Molecular Genetics and Social Pediatrics of the Ankara

* Corresponding author at: Karliova sokak, No: 8/17, 06010, Etlik, Ankara, Turkey. Tel.: +90 312 5957202; fax: +90 312 3191440.
E-mail address: simsekfiliz@hotmail.com (F.S. Orhon).

0049-3848/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.thromres.2013.11.011

University. Ethics approval was obtained from the Ethics Committee of the School of Medicine. A written informed consent was obtained from the parents of subjects who were admitted to the study.

The study population consisted of healthy infants who were admitted to the Department of Social Pediatrics for 1, 6, 12 or 24 month follow-up visits. A total of 85 infants (51 males and 34 females) who were free of any acute or chronic disease and whose parents gave consent were admitted to the study. Samples of these healthy infants were obtained from the blood that was collected for the routine anemia screening in healthy child visits conducted during 6, 12 and 24 months. On the other hand, the samples of the infants admitted for the 1 month visit were obtained from the blood that was collected in case there was any suspicion about the health of the respective children. Blood was collected into tubes containing 1 milliliter 0.109 M trisodyum citrate. The samples were centrifuged at 2500 g for 15 minutes, and plasma samples were stored at -20°C. Frozen samples were thawed in a water bath at 37 °C for 5 minutes and then vortexed.

Indirect Microparticle Activity

The plasma samples were studied using a STA-PROCOAG-PPL Kit (Diagnostica Stago Inc, France). Samples were added to a PPL/MP-free plasma and then incubated. An activated reagent (FXa) was added to the serum samples. The coagulation time was measured by a STart 4 Hemostasis Analyzer (Diagnostica Stago Inc, France).

Thrombin Generation Test

Calibrated automated thrombography (CAT) was used to measure the thrombin generation. This assay follows the *in vitro* thrombin generation in plasma after activation of coagulation with tissue factor, phospholipids and $CACl_2$ [15]. This method employs a low-affinity fluorogenic substrate for thrombin (Z-Gly-Gly-Arg-AMC) to continuously monitor the thrombin activity in clotting plasma. According to the manufacturer's instructions, measurements were conducted on 80 μl full plasma in a total volume of 120 μl and in the presence of 416 μl fluorogenic substrate and 16 mM CaCl2. In order to correct for inner-filter effects and substrate consumption, each thrombin generation measurement was calibrated against the fluorescence curve obtained in the same plasma with a fixed amount of thrombin–α_2-macroglobulin complex (Thrombin calibrator; Thrombinoscope BV, Maastricht, the Netherlands). Thrombin generation curves were calculated using the Thrombinoscope software (Thrombinoscope BV, Maastricht, the Netherlands). The parameters were derived from the thrombin generation curves: lagtime (LT, min), i.e. time to initiation of thrombin generation; endogenous thrombin potential (ETP, nmol/L x min^{-1}), i.e. area under the thrombin generation curve; peak thrombin activity (peak, nmol/L); and time to peak thrombin generated (TTP, min). All times were measured from $t = 0$ min.

Statistical Analysis

The statistical analysis was performed using SPSS 11.5. A descriptive analysis summarizing the parameters of the thrombin generation test and indirect microparticle activity was presented. Data were expressed as the mean ± standard deviation. Since the measurements of microparticle activity, ETP, peak and TTP were normally distributed, parametric tests were conducted to compare these parameters. On the other hand, since the lag time measurement was not normally distributed, nonparametric tests were conducted for comparing this parameter.

Results

Totally eighty-five healthy infants (51 boys and 34 girls) were studied. The mean age of all study group was 12.95 ± 8.48 months (median:12, range 1.0-25.4 months). These infants were divided into four groups according to the time of the follow-up visits. Group 1 consisted of 10 infants (6 boys and 4 girls, mean age 1.18 ± 0.19 months, range 1.0-1.4 months), who were brought to the 1 month follow-up visit. Group 2 consisted of 25 infants (17 boys and 8 girls, mean age 6.15 ± 0.16 months, range 5.9-6.5 months), who were brought to the 6 month follow-up visit. Group 3 consisted of 24 infants (11 boys and 13 girls, mean age 12.38 ± 0.46 months, range 11.33-13.20 months), who were brought to the 12 month follow-up visit. Finally, Group 4 consisted of 26 infants (17 boys and 9 girls, mean age 24.53 ± 0.39, range 23.67-25.40 months), who were brought to the 24 month follow-up visit.

When the results were evaluated according to the gender, there was no statistical difference between boys and girls with respect to the levels of the indirect microparticle activity and the entire parameters of the thrombin generation test ($p > 0.05$ in all parameters).

Table 1 shows the data of the indirect microparticle activity and the parameters of thrombin generation test of the study groups. Among the study groups, there was no statistical difference in terms of the indirect microparticle activity. On the other hand, statistically significant differences were found in all thrombin generation parameters among the study groups.

As shown in Table 2, the data of the study groups were compared among themselves. There was not found a statistical difference in the indirect microparticle activity among the groups. The data showing the statistically significant difference on the parameters of the thrombin generation were evaluated as below.

- It was determined that the lag time in Group 1 was statistically lower than those found in the other groups.
- It was found that the ETP levels in Group 1 were higher than that in Group 2 without any statistically difference. On the other hand, the ETP levels in Group 2 were found significantly lower than those of Group 3 and Group 4.
- The peak levels in Group 1 were found higher than that of Group 2. Further, the peak levels in Group 2 were statistically lower than those of Group 3 and Group 4.
- The TTP levels in Group 1 were found statistically lower than those found in the other groups. Additionally, it was found that the TTP levels in Group 2 were significantly higher than those in Groups 3 and 4.

In the correlation analysis, there was no correlation between the indirect microparticle activity and the age groups, as well as between the indirect microparticle activity and the gender. A weak correlation was found between age and the ETP levels, and between age and the peak parameter ($r = 0.296$, $p = 0.006$ and $r = 0.238$, $p = 0.029$, respectively). Neither of the thrombin generation test parameters were found in correlation to the gender.

As shown in Table 3, there were negative and positive correlations between the indirect microparticle activity and the parameters of thrombin generation. In the parameters of thrombin generation, there were a positive correlation between the lag time and TTP, and between ETP and peak. On the contrary, a negative correlation was found between the lag time and peak, and between peak and TTP.

Discussion

Our study may be regarded as an attempt to define the determinants of thrombin generation parameters and of the indirect microparticle activity, as well as of the age-dependent changes in thrombin generation in a healthy infant population.

Previous studies reported age-dependent changes in the coagulation factors, and all laboratory measurements confirm the concept of developmental hemostasis, indicating that physiologic concentrations of coagulation proteins gradually increase after birth [16]. Microparticles expressing procoagulant phospholipids and supporting the thrombin generation are important elements that act on coagulation [7,8]. As a