In Vitro Stability of Pregnancy-Associated Plasma Protein A

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Abstract

Objective: Pregnancy-associated plasma protein A is produced in high concentration by trophoblasts during pregnancy and maternal serum assessment between 11 and 14 weeks of gestation has significant utility in screening for Down syndrome and other chromosomal anomalies. We aimed to investigate the *in vitro* stability of pregnancy-associated plasma protein A.
Materials and Methods: Blood samples were collected from volunteer pregnant women and divided into two groups. The first and second groups were stored at +2 to +8°C for 7 days and at –20°C for 28 days, respectively. Pregnancy-associated plasma protein A was determined each day in the first group and each week in the second group.
Results: There was no statistically significant difference between measurements in each group (p>0.05).
Conclusion: We concluded that serum pregnancy-associated plasma protein A is stable for at least 7 days at +2 to+8°C and 28 days at –20°C, and could be preserved for up to one month in average laboratory conditions.

Keywords: Down syndrome, *in vitro*, pregnancy-associated plasma protein A, pregnancy, stability

Özet

Gebelikle İlişkili Plazma Protein A'nın *In Vitro* Stabilitesi

Amaç: PAPP-A gebelik süresince trofoblastlardan yüksek konsantrasyonda salınır ve gebeliğin 11.-14. haftalarında Down sendromu ve diğer kromozomal anomalilerin taranmasında kullanılmaktadır. Bu çalışmada PAPP-A'nın *in vitro* stabilitesini araştırdık.
Materyal ve Metot: Kan örnekleri gönüllü gebe kadınlardan alınarak iki gruba ayrıldı. Birinci gruptaki örnekler +2 ilâ +8°C'de 7 gün ve ikinci gruptakiler –20°C'de 28 gün boyunca bekletildi. PAPP-A ilk grupta her gün, ikinci grupta ise haftada bir kez ölçüldü.
Sonuçlar: Gruplarda yapılan ölçümlerde istatistiksel olarak anlamlı bir fark bulunmadı (p>0.05).
Tartışma: Serum PAPP-A'nın +2 ilâ +8°C'de en az 7 gün ve –20°C'de ise en az 28 güne kadar stabilitesini koruduğunu ve normal laboratuvar koşullarında bir aya kadar saklanabileceğini düşünmekteyiz.

Anahtar sözcükler: Down sendromu, *in vitro*, pregnancy-associated plasma protein A, gebelik, stabilite

Introduction

Pregnancy-associated plasma protein A (PAPP-A) was described by Lin et al. (1) in 1974 as a high molecular weight component of serum obtained from pregnant women in late pregnancy.

Originally, circulating PAPP-A was thought to occur as a dimmer (2), but recently it has been shown that PAPP-A is present as a component of a heterotetramer consisting of two subunits disulfide-bonded to two subunits of the proform of eosinophil major basic protein (proMBP) (3). In circulation, PAPP-A exists as a PAPP-A/pro-MBP complex (4). This complex is detectable in pregnancy serum 4 to 6 weeks after conception, progressively increases to a concentration of approximately 50 µg/mL in late pregnancy serum and then rapidly declines postpartum (5,6). Although a low serum level of PAPP-A has been used as an indicator of certain genetic fetal developmental disorders such as Down's syndrome (7) and Cornelia de Lange syndrome (8), the function of PAPP-A except for acting as an insulin-like growth factor binding protein-4 (IGFBP-4) protease (9) is unclear.

In addition to pregnancy, the clinical value of PAPP-A continues to grow as new data become available. PAPP-A is produced by human fibroblasts (9) and osteoblasts that have been implicated in the regulation of local insulin-like growth factor (IGF) bioavailability during bone growth and remodeling (10).

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Moreover, PAPP-A appears to enhance local IGF bioavailability in response to injury and play an important role in wound-healing processes (11) and tissue remodeling *in vivo* (12).

Serum levels of PAPP-A have recently been linked to plaque instability and is increased in acute coronary syndromes (13). Measurement of plasma PAPP-A is a strong independent predictor of ischemic cardiac events in patients who present with suspected myocardial infarction but remain troponin negative (14).

With the increasing clinical demand for fetal abnormalities to be diagnosed in the first rather than in the second trimester of pregnancy, maternal serum PAPP-A is being used in routine trisomy screening with nuchal translucency. Thus, maternal serum assessment between 11 and 14 weeks of gestation has significant utility in screening for Down syndrome and other chromosomal anomalies. Nevertheless, except for its use in detecting possible fetal genetic anomalies in pregnancy, there is still no clinical value associated with PAPP-A. Therefore, we use serum PAPP-A concentration only to evaluate genetic anomalies like Down syndrome in our laboratory. Due to lack of laboratory equipment or insufficient number of patients, it may not always be possible to study serum PAPP-A in pregnancy immediately or it may be necessary to transport the specimens to another laboratory for cost effectiveness. The stability of PAPP-A in this period is important for accurate results. In this study, we aimed to investigate the *in vitro* stability of PAPP-A in pregnant specimens.

Materials and Methods

We use serum PAPP-A concentration only to evaluate genetic anomalies like Down syndrome in our laboratory. Blood samples were collected from 10 pregnant women at a gestational age of 11-14 weeks during nuchal translucency measurement. Each of these 10 blood samples was divided into two aliquots to form two groups. The first and second groups were stored at +2 to +8°C for 7 days and at –20°C for 28 days respectively. Serum PAPP-A was determined by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (monoclonal murine anti-PAPP-A) each day in the first group, and each week in the second group on IMMULITE (DPC, Los Angeles, USA).

Data were expressed as the mean ± SD. The significance of differences among all groups was analyzed using ANOVA for repeated measures (nonparametric variables). Values of $p < 0.05$ were considered statistically significant.

Results

The results of the PAPP-A measurements in the first and second groups are summarized in Figures 1 and 2, respectively. In the first group, the serum PAPP-A concentration was 3.7±1.6 mIU/ml on the first day and 3.5±1.4 mIU/ml on the 7th days. There was no statistically significant difference between measurements in the first group ($p>0.05$).

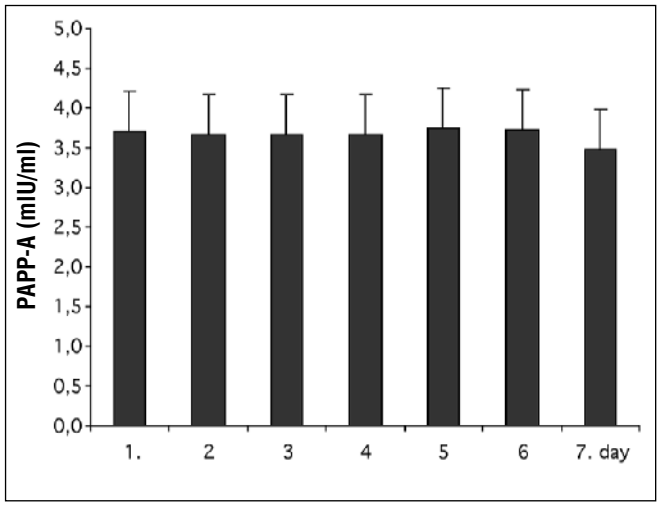

Figure 1. Serum PAPP-A concentration stored at +2 to +8 °C, (n=10).

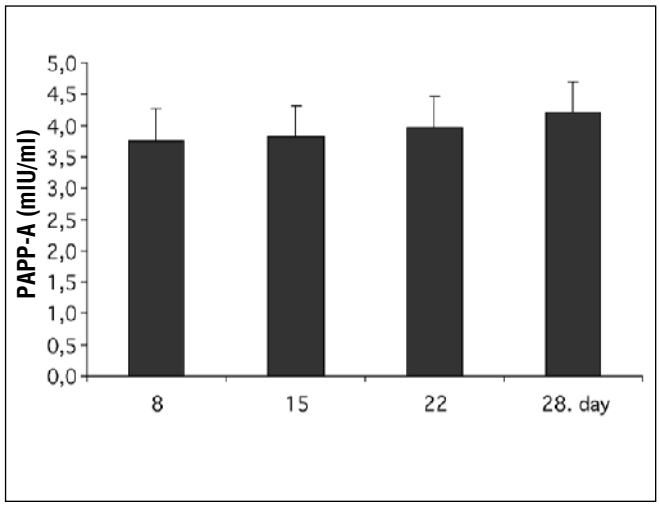

Figure 2. Serum PAPP-A concentration stored at –20 °C, (n=10).

Similarly, in the second group, the serum PAPP-A concentration was 3.8±1.7 mIU/ml on the 7th days and 4.2±1.9 mIU/ml on the 28th days. There was also no statistically significant difference between measurements in this group ($p>0.05$).

Discussion

The data we obtained in this study show that serum PAPP-A is stable for at least 7 days at +2 to +8°C and for 28 days at –20°C. To prolong these periods more than 28 days for serum PAPP-A will gain no clinical benefits, since the triple test is used in screening for Down syndrome and other chromosomal anomalies after the 16th weeks of gestation.

Bersinger and coworkers demonstrated that repeated freezing and thawing did not affect results, at both high and low concentrations of PAPP-A (15). It is also possible to introduce the serum into the test as a dry sample on blotting paper, easily posted in an envelope. A decrease of 21% was observed after such dry storage for three weeks at room temperature, which can be compensated for by the inclusion of a dried control serum, mailed with the sample(s) (15).

PAPP-A seems to be a unique protein, since it shows no global homology to any protein, but contains some motifs, including an elongated zinc binding motif (16). In serum, >99% of PAPP-A is found as a complex with highly and unusually glycosylated proMBP (3). In the placenta, PAPP-A and proMBP are synthesized in different cell types. PAPP-A is synthesized in the syncytiotrophoblast, and proMBP is synthesized in extravillous cytotrophoblasts (17). Therefore, the PAPP-A/proMBP complex must form in the extracellular compartment after secretion. PAPP-A/proMBP complex formation requires a specific interaction between subunits. Interestingly, this interaction is covalent and seems to be unique to this complex. This covalent interaction may increase the stability of PAPP-A and prolong its storage period.

In conclusion, we demonstrate that PAPP-A could be preserved for at least up to one month in average laboratory conditions. Sometimes, it may not always be possible to study serum PAPP-A during pregnancy in short time due to insufficient number of patients or lack of laboratory equipment. Therefore, the stability of PAPP-A in this period plays an important role for accurate results.

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