Development of a well characterized PAPP-A2 chemiluminescence assay to measure PAPP-A2 in maternal biological fluids.

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ABSTRACT

Relevance: Pregnancy-associated plasma protein-A2 (PAPP-A2) is a novel metalloproteinase identified as a homolog of PAPP-A in the matriline superfamily of papains. PAPP-A2 shares 46% sequence identity with PAPP-A, PAPP-A2 is a noncovalently linked dimer of two 220-kDa subunits. It exhibits proteolytic activity against IGFBP-5 and IGFBP-3. PAPP-A2 is expressed in a wide range of tissues and is abundant in placental syncytiotrophoblasts and the pregnant uterus. The physiological importance of PAPP-A2 is not known.

Methodology: We have developed a well characterized two-step sandwich-type enzymatic microparticle CLIA to measure PAPP-A2 levels in the maternal serum and other biological fluids. The assay measures PAPP-A2 in 50 µL of sample (diluted 20 folds in sample diluent) against recombinant PAPP-A2 calibrators (0.25-25 ng/mL). The antibody pair used in the PAPP-A2 ELISA measures PAPP-A2 and does not detect proMBP, dimeric PAPP-A and PAPP-A1-proMBP complex.

Validation: Total imprecision calculated on 4 samples over 12 runs, 4 replicates per run, using CLSI EPI-A guidelines, was 4.64% at 1.016 ng/mL, 3.76% at 1.839 ng/mL, 2.94% at 2.87 ng/mL, and 3.86% at 7.49 ng/mL. The limit of detection calculated using six serum samples in the range of 0.125-1.93 mg/L, over 12 runs is 0.113 mg/mL. The functional sensitivity of the assay at 20% CV was 0.136 mg/mL. Dilution studies showed an average recovery of 98%–110%. The median PAPP-A2 value on second trimester samples (n=65) was 47.06 ng/mL.

Conclusions: A quantitative, robust and fully characterized microparticle PAPP-A2 CLIA has been developed to measure PAPP-A2 in maternal serum. The approximate median PAPP-A2 levels found in first and second trimester maternal serum can be measured with ≤5% CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in a variety of pregnancy-related disorders.

INTRODUCTION

Correlation of circulating PAPP-A and A2 concentrations with various clinical parameters. (A) Serum concentration of PAPP-A (right) and A2 (left) measured by ELISA. Maternal blood samples were obtained either at the time of diagnosis or during surgery. The concentrations were compared between sera from un complicated pregnancies (circles) and from pre eclampsia (triangles). The horizontal bars indicate mean values. (B) Correlation between PAPP-A2 and gestational week or disease severity. (C) Correlation between PAPP-A2 and normalized birth weight. Clear circles indicate control uncomplicated pregnancy, whereas open triangles indicate pre eclampsia. A regression line is shown with correlation coefficients and P-values. (D) Correlation between PAPP-A2 concentration and placental weight. Haruki Nishizawa et al. Molecular Human Reproduction Vol 14, No 10 pp. 595-602, 2008

METHOD

CAPTURE ANTIBODY

SHRP

ANTIGEN BIOTIN Labeled ANTIBODY

RESULTS

Analytical Specificity: The antibody pair used in the PAPP-A2 CLIA measures bioactive PAPP-A2 (full length) and does not detect other variants of PAPP-A (Truncated C-Terminus).

Limit of Detection: The lowest amount of PAPP-A2 in a sample that can be detected with a 95% probability (n=24) is 0.171 ng/mL. The value was determined by processing six serum samples in the range of 0.12 to 1.9 ng/mL. Two assay runs per day were performed over six days with all samples run in duplicate per run.

Limit of Quantitation: The estimated minimum PAPP-A2 dose achieved at 20% total imprecision is 0.184 ng/mL. The value was determined by processing seven samples in the range of 0.19 to 1.9 ng/mL over twelve runs and six days in duplicate (n=24) following CLSI EP17 guidelines.

Imprecision: Reproducibility of the PAPP-A2 CLIA was determined on four serum pools. Pool samples were replicated in four assays/2 runs.

Sample

Mean conc.

Within run

Between run

Total

PAPP-A

1.008

0.065

0.036

0.026

0.045

4.46%

PAPP-A1

1.839

0.056

0.028

0.011

0.134

7.30%

PAPP-A2

2.875

0.039

0.059

0.025

0.085

3.04%

Cross Reactivity:

S. No

Antigen

Conc. (ng/mL)

% Difference to Control

1

PAPP-A

10

ND

2

ProMBP

0.05

ND

3

Hemoglobin

1250

4.98

4

Triglyceride

5000

-0.71

5

Bilirubin

600

0.246

Sample Stability: Fresh drawn samples were compared to frozen specimens.

N

20

RS

0.97

2-failed p
<0.0001

Slope

2.47

Intercept

-0.08

Log PAPP-A2 (ng/mL)

-0.5

0.0

0.5

1.0

1.5

2.0

2.5

3.0

3.5

4.0

4.5

Figure 1

CONCLUSIONS

A sensitive, reliable and easy-to-run microparticle PAPP-A2 assay has been developed. The approximate median PAPP-A2 levels found in first and second trimester pregnancy can be measured with ≤5% CV using this assay. The assay has shown excellent analytical performance and is suitable for studies in the area of pregnancy related complications.

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