Maternal serum pregnancy-associated plasma protein-A levels in hyperemesis gravidarum: a prospective case control study

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Summary

Purpose: To investigate if maternal serum pregnancy-associated plasma protein-A levels are affected in hyperemesis gravidarum (HG). *Materials and Methods:* A prospective case control study was conducted in 169 HG cases who had one or more antepartum hospitilization for HG. The control pregnancies were 132 and were selected randomly among all women who had first trimester prenatal screening in antenatal outpatient clinic between 2011 and 2012. *Results:* Maternal serum pregnancy-associated plasma protein-A levels were significantly higher in hyperemesis gravidarum group compared with control group ($p = 0.002$ $p < 0.05$ 95% CI). Power analysis of independent sample t-test, two-sided, for pregnancy-associated plasma protein-A was 0.88. Maternal serum free β-human chorionic gonadotropin values were not different between two groups ($p > 0.05$). *Conclusion:* Increased pregnancy-associated plasma protein-A levels associated with HG, even after excluding potential cofounders.

Key words: Pregnancy-associated plasma protein-A (PAPP-A); Hyperemesis gravidarum; Human chorionic gonadotropin (hCG).

Introduction

Hyperemesis gravidarum (HG) is a disease characterized with severe nausea, vomiting, and anorexia in early pregnancy and leading to dehydration, weight loss, electrolyte imbalance, and metabolic disturbance. As the etiology remains obscure, the treatment remains supportive and symptomatic. About 50% to 90% of pregnant women experience nausea and vomiting but HG occurs only in 0.3–2% of pregnancies [1, 2].

Although the exact pathogenesis of HG is unknown, it is widely accepted that gestational vomiting results from various metabolic and endocrine factors, many of placental origin. The most implicated factor is human chorionic gonadotropin (hCG). This link between hCG and HG is based largely on the temporal relationship between the peak of HG and the peak of hCG production, both of which occur between 12 and 14 weeks' gestation. In addition, nausea and vomiting are often worse in pregnant women with conditions associated with elevated hCG levels, such as molar pregnancies, multiple gestations, and Down syndrome [3].

PAPP-A was one of four proteins identified in the plasma of pregnant women, and accordingly was given the name 'pregnancy-associated plasma protein-A' [4]. During pregnancy, PAPP-A is produced by placental syncytiotrophoblasts and secreted into the maternal circulation where its concentration increases until term [5]. It has proteolytic activity against IGF binding protein-4 (IGFBP-4) in ovarian follicular fluid and in conditioned medium from fibroblasts, osteoblasts, granulosa cells, lung cells, and smooth-muscle cells [6]. PAPP-A was also found to be ubiquitously expressed with particularly high expression in kidney and bone, clearly indicating a role for PAPP-A outside pregnancy [7].

Pregnancy outcomes of HG is conflicting. Most of the studies have concluded that there are no adverse affects of HG on fetal outcome including gestational age, birth weight, incidence of prematurity, and Apgar scoring [8–10]. However some studies have reported lower birth weight associated with HG [11, 12].

There is only one study that had investigated the relation between maternal serum PAPP-A levels and HG therefore in this study the authors aim to evaluate the relation between PAPP-A, free β-HCG, and HG.

Materials and Methods

The study was conducted in Zekai Tahir Burak Women's Health Hospital in Ankara, Turkey. Ethics approval was obtained from the institutional review board. Informed written consent was obtained from study participants. Study was designed as a prospective case-control study, in which the cases were singleton pregnancies, hospitalized with the diagnosis of HG, between 2011 and 2012 (n = 169). Controls were pregnancies who didn't complain of emesis (n=132) and selected randomly, by using randomization table among all women who had first trimester prenatal screening in antenatal outpatient clinic and same gestational week as in each study case. Gestational age was calculated via combination of first trimester ultrasound findings and the last menstrual period.

Inclusion criteria confirmed singleton pregnancy, clinical HG (with dehydration and ketonuria), and one or more antepartum hospitalization for hyperemesis. Women with diagnostic cofounders such as hyperthyroidism (which diagnosed before), stomach diseases, gastroenteritis were excluded. Venous blood was collected to obtain the levels of hemoglobin, thyroid stimulating hormone (TSH), triiodothyronine (T_3), thyroxine (T_4), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), and albumin as initial laboratory assessment on admission before any treatment. PAPP-A and β-HCG values of the subjects

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Table 1. — *The characteristics of the study groups.*

	HEG (n=169)	Control (n=132)	*p value*
Maternal age*	27.6 ±5.14	25.53 ± 5.11	0.002[a]
PAPP-A (MoM)*	1.19 ± 0.71	0.96 ± 0.54	0.002[a]
Free βHCG (MoM)	0.99(0.71)	0.91(0.55)	0.86[b]
TSH (mIU/ml)*	0.96 ± 0.78	1.45 ± 0.87	0.00[a]
T_4* (ng/dl)	1.31(0.33)	1.18(0.00)	0.001[b]
T_3 (ng/dl)	3.09(0.79)	3.21(0.42)	0.2[b]
ALT (U/l)	14.00(7.00)	13(0.00)	0.071[b]
AST (ng/dl)	17.00(5.5)	17.5(6.00)	0.886[b]
Albumin	4.41(0.55)	4.19(0.53)	0.202[b]

[a] Independent sample t-test; [b] Mann-Whitney U test.
Values are given as median (IQR: interquartile range) or mean ± SD
* $p < 0.05$ is significant.

were obtained from routine first-trimester prenatal screening analysis performed at the authors' hospitals. Upon collection, plasma samples were analyzed within three hours. Free *β*-HCG and PAPP-A kits were utilized. The plasma levels were expressed as gestational age-specific multiples of the median (MoM). In risk evaluation, the prenatal screening program was utilized.

Statistical analysis was performed using SPSS version 17.0 and power analysis was performed using NCSS 2007. The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Because the data of maternal age, PAPP-A, and TSH were normally distributed, parametric test (independent sample t-test) were used for analyses. For the other not normally distributed variables, non-parametric test were used for analyses. Logistic regression analysis was used for variables independently associated with HG.

Results

A total of 169 pregnant women with HG and 132 of healthy pregnants as in control group were included in the study. Characteristics of this study are shown in Table 1. Maternal age was significantly higher in HG group compared with control group ($p = 0{,}002$ $p < 0.05$ 95% CI). Free β-HCG, T3, ALT, AST, and albumin values were not different between two groups ($p > 0.05$). Serum PAPP-A levels were significantly higher in HG group compared with control group ($p = 0.002$ $p < 0.05$ 95% CI). Serum TSH levels significantly lower in HG group compared with control group ($p = 0{,}00$ $p < 0.05$ 95% CI). Although there were no significant differences determined in serum T3 levels between two groups, serum T4 levels were significantly higher in HG group compared with control group ($p = 0.001$ $p < 0.05$ 95% CI).

Power analysis in Table 2 reveals the power analysis of independent sample t-test, two-sided results of PAPP-A.

Table 3. — *Logistic regression analysis for variables independently associated with HG[a].*

	Wald	*p value*	Relative risk	95% confidence limits	
				Lower	Upper
Age	8.67	0.003	1.082	1.027	1.141
PAPP-A	8.82	0.003	1.833	1.229	2.734
Free βHCG	0.00	0.99	0.99	1.01	9.866
T4	7.192	0.007	9.376	1.826	48.131
ALT	4.638	0.031	1.045	1.004	1.087
AST	1.482	0.223	1.029	0.983	1.078

[a] Variable(s) entered on step 1: TSH, T_3, T_4, ALT, AST, albumin, PAPP-A, βHCG.

Logistic regression analysis in Table 3 revealed that: age, PAPP-A, T_4, and ALT values were significantly correlated with risk of HG ($p < 0.05$ 95% CI). There were no significant correlations found with HG risk and serum AST, and free βHCG values.

Discussion

HCG is often considered as the most likely cause of HG [3]. However in this study, there were no significant free βHCG level differences in HG group. The majority of literature, suggests a relationship between HG and high HCG levels. However few studies have not found any significant relation between HCG and HG as in the present study [13-15]. Possible explanation for the inconsistent finding of elevated HCG levels in HG patients is that HG is not simply caused by elevated HCG levels, but that specific isoforms of HCG are causing HG. In a study by Tsuruta *et al*., HG patients had significantly increased levels of the HCG fraction that contains HCG with asialo-carbohydrate chain [14]. Also, Jordan *et al*. had found HG group increased HCG concentrations in the more acidic half (pH < 4) of the chromatofocusing pH range than seen in control subjects [16].

This study showed that elevated PAPP-A levels are independently associated with HG, even after excluding potential cofounders. To the authors' knowledge no other studies than Derbent *et al.* have investigated PAPP-A levels in combination with HG that have also been published to date [17]. In vivo, PAPP-A expression has been shown to be upregulated in response to acute injury in several systems. In healing human skin wounds, PAPP-A expression is induced over time in dermal granulation tissue [18]. The proinflammatory cytokines tumor necrosis factor (TNF)-alfa and interleukin (IL)-1b are the most potent stimulators

Table 2. — *Numeric results for two-sample t-test.*

Power	N1	N2	Ratio	Alpha	Beta	Mean 1	Mean 2	S1	S2
0.88911	169	132	0.781	0.05000	0.11089	1.2	1.0	0.7	0.5

Null hypothesis: mean1=mean2; alternative hypothesis: mean 1< > mean 2. The standard deviations were assumed to be unknown and unequal.

of PAPP-A expression in cultured human dermal fibroblasts and human coronary artery endothelial and smooth-muscle cells. Yet, starvation normally causes suppression of immune functions, Kaplan *et al*. prospectively compared the IL-1, IL-2, IL-6, IL-8 and tumour necrosis factor-alpha (TNF alfa) levels between women with HG, pregnant, and non-pregnant controls and found a significantly higher level of TNF alfa in HG patients [19]. These findings rather support an activated immune system which may stimulate PAPP-a expression in HG.

PAPP-A has primarily local biological effects [19]. There have been several studies, that demonstrated PAPP-A- mediated enhancement of IGF bioactivity in vitro by its degradation of IGFBP-4 [20, 21]. IGFs are necessary for normal fetal growth. Low level of PAPP-A may cause impaired release of IGFs which may cause pregnancy complications. Therefore increased PAPP-A levels may reduce risk of pregnancy complications as Depue *et al.* who have demonstrated a reduction in fetal losses and Bashiri *et al.* showing a reduction in miscarriages in HG [22, 23].

Same TSH and T_4 results has been observed in this study as the literature; however means of TSH and T_4 were in normal ranges. Even though the mechanism of hyperthyroidism is unclear, it has been suggested that the high incidence of hyperthyroidism in HG patients is caused by elevated circulating HCG levels [24].

The findings in this study show significant increase levels of PAPP-A which may be the reason of possible activated immune system in HG. Also this increase in PAPP-A levels may lead to decrease in miscarriages and fetal losses due to PAPP-A's effect on IGF, which is important in normal fetal growth. Further studies are crucial to understand, the role of PAPP-A in physiology and patho-physiology of pregnancy and in the pathogenesis of HG.

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