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First trimester maternal serum PAPP-A and free β-HCG levels in hyperemesis gravidarum

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Objective To evaluate whether hyperemesis gravidarum (HG) affects first-trimester maternal serum PAPP-A and free β-hCG levels.

Method An observational study was conducted in 115 cases of HG and 110 control pregnancies who attended the first-trimester prenatal screening program between January 2006 and July 2010.

Results Maternal serum TSH levels were lower and free T4, and transaminases (ALT, AST) levels were higher in pregnancies complicated with HG compared with controls ($p < 0.05$ for all). In HG cases, median values of maternal serum PAPP-A were significantly higher with respect to normal pregnancies (1.2 vs 1.0 MoM; $p = 0.009$). Similarly, median values of free β-hCG were 1.3 MoM in HG pregnancies and 1.0 MoM in controls ($p = 0.006$). Multivariate analysis revealed that PAPP-A and hCG were independently associated with HG after controlling for TSH, free T4, AST, and ALT.

Conclusion HG is associated with elevated levels of PAPP-A and free β-hCG, and such changes are independent of serum indicators of thyroid and liver function. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: maternal serum screening; PAPP-A; free β-hCG; hyperemesis gravidarum; transaminase; TSH

INTRODUCTION

About 50 to 90% of pregnant women experience nausea and vomiting (Gadsby *et al*., 1993); however, only 0.5 to 2% of all pregnancies are complicated by hyperemesis gravidarum (HG) (Bashiri *et al*., 1995). HG is characterized by intractable nausea and vomiting leading to dehydration, electrolyte and metabolic disturbances, nutritional deficiency, and weight loss.

The etiology of HG remains unknown (Jueckstock *et al*., 2010). Epidemiological studies demonstrated that the syndrome is closely associated with hypersecretion of hCG and hyperstimulation of the thyroid gland. The increase in hCG is thought to be responsible for thyroid stimulation (Rodien *et al*., 2004).

PAPP-A, a protease to insulin-like growth factor binding protein-4 (IGFBP-4), facilitates the breakdown of this protein resulting in a release of free insulin-like growth factor (IGF) (Lawrence *et al*., 1999). IGFs are believed to play an important role in the regulation of trophoblast invasion of the decidua. Impaired release of free IGFs may be a cause for poor placental perfusion, thus affecting fetal growth and the onset of other pregnancy complications (Kirkegaard *et al*., 2010). Indeed a number of studies have found associations between low PAPP-A levels during first-trimester screening and rates of fetal loss, small-for-gestational age (SGA) fetuses, preterm delivery (PTD), or preeclampsia among pregnancies without any chromosomal abnormalities (Smith *et al*., 2006; Spencer *et al*., 2008; Grill *et al*., 2009).

Pregnancies complicated by HG have lower probability of miscarriage and to develop pregnancy-induced hypertension (PIH) and preeclampsia (Dodds *et al*., 2006; Maconochie *et al*., 2007), but higher risk for PTD, SGA, low birth weight (LBW), and low 5-min Apgar scores (Bailit, 2005). The literature suggests that adverse obstetric outcome is linked to the severity of the symptoms, related to malnutrition and lack of vitamins and trace elements, and mostly limited to women with poor weight gain (Oppenraaij *et al*., 2009).

Since no investigations have examined changes in maternal serum PAPP-A in pregnancies complicated with HG so far, our study aims at evaluating the first-trimester biomarkers, PAPP-A and free β-HCG in relation to HG.

METHODS

The study was conducted at the Departments of Obstetrics and Gynecology of Fatih University Hospital and Başkent University Hospital in Ankara, Turkey. It was designed as a case–control study, in which the cases were singleton pregnancies hospitalized with the diagnosis of HG, between January 2006 and July 2010 ($n = 115$). Controls were pregnancies who did not complain of emesis ($n = 110$). One control was randomly selected among all women who had first-trimester prenatal screening at the same gestational week as each study

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case. Gestational age was based on the combination of the last menstrual period and ultrasound findings in the first trimester.

We defined hyperemetic pregnancies as those with one or more antepartum hospitalization for hyperemesis, the first of which had to have occurred before 20 completed weeks of gestation. Women with diagnostic confounders such as overt hyperthyroidism, stomach disease, cholelithiasis, or gastroenteritis and women who had multiple pregnancies were excluded. We recorded the levels of hemoglobin, TSH, free T4, free T3, ALT, AST, and urine ketone test at the initial laboratory assessment on admission before any treatment. PAPP-A and β-HCG values of the subjects were obtained from routine first-trimester prenatal screening analysis performed at our hospitals. Upon collection, plasma samples were analyzed within 3 h using the Siemens Immulite 2000 immunoanalyzer with IMMULITE® Free β-HCG and IMMULITE® PAPP-A kits (Siemens Medical Solutions Diagnostics Limited, UK). The plasma levels were expressed as gestational age-specific multiples of the median (MoM). In risk evaluation, the prenatal screening program (PRISCA 4.0 typolog Software Gmbh, Hamburg) has been utilized.

Statistical analysis was performed using SPSS version 17.0 (SPSS, Chicago, IL USA). The Shapiro–Wilk test was used to evaluate the distribution of variables. Because the data were not normally distributed, nonparametric tests were used for analyses. Categorical variables were compared with the chi-square test or Fisher's exact test when appropriate. The Mann–Whitney test was used to compare continuous variables. A p value of less than 0.05 was regarded as significant. Multiple logistic regression model (backward: likelihood ratio binary logistic regression) was used to assess independent risk factors for HG while controlling for potential confounders. Maternal age and gestational age were used as independent scale variables. Fetal gender, PAPP-A, β-HCG, TSH, free T4, ketonuria, ALT, AST, weight loss, and number of admissions were used as independent categorical variables. Probability of entry and probability of removal were regarded as 0.05 and 0.10, respectively. All variables were included in stepwise multivariate logistic regression analysis using a backward elimination procedure. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using multivariate logistic regression analysis.

RESULTS

Median time of hospitalization for HG was 8.3 weeks, and 94% of our patients were hospitalized before 14.0 weeks of gestation. Median duration of hospitalization in the HG group was 2 days (minimum 1 day, maximum 18 days). Eighty-seven women in the HG group (75.6%) were admitted to hospital only once, whereas 28 cases (24.4%) were admitted more than once (minimum of two times and maximum four times).

Forty-five women in the HG group (39%) received intravenous fluids, Vitamin B1, B6, C, and antiemetics (Dimenhydrinate or Metoclopramide), 68 women (59%) received procalamine combined with these medications, and 2 women received chlorpromazine and prednisolone each.

The characteristics of our study groups are shown in Table 1. Maternal age was significantly lower in the HG group compared with the control group ($z = 3282$; $p = 0.010$). Gravidity, parity, body mass index, and hemoglobin values were not different between the two groups. Pregnancies complicated with HG had 71 (61.7%) female fetuses, as compared with 58 (52.7%) among controls ($p = 0.171$).

Maternal serum TSH levels were lower, and free T4, alanine transaminase (ALT), aspartate transaminase (AST) levels were significantly increased in pregnancies with HG compared to control group ($p < 0.05$ for all). Only five (4.5%) women in the HG group had increased ALT values, ranging from 35 to 137 U/L; all of them had also TSH values lower than normal (range, 0.005–0.280 mU/L). Urine ketone levels were also significantly higher in the HG group.

Among the 115 pregnancies complicated with HG, median PAPP-A MoM value was significantly higher compared with normal pregnancies (1.2 vs 1.0; $p = 0.009$). Similarly, median free β-hCG was 1.3 MoM in HG pregnancies and 1.0 MoM in controls ($p = 0.006$).

On univariate analysis (Table 2), HG appeared to have significant positive association with raised serum-free β-hCG MoM, PAPP-A MoM, freeT4, ALT, and AST levels as well as with ketonuria; besides a significant negative relationship with TSH levels. Elevated ALT levels and ketonuria were significantly related to the duration of hospitalization. Raised PAPP-A MoM levels had significant positive association with free β-hCG MoM (Rho = 0.170, $p = 0.019$) and freeT4 (Rho = 0.308, $p = 0.003$) measurements, there was no significant relationship between PAPP-A MoM levels and elevated liver function tests. There was a positive relationship between serum levels of free thyroxine and ALT (Rho = 0.247, $p = 0.010$).

Multivariate regression analysis revealed that maternal serum TSH, FreeT4, and AST measurements were not significantly correlated with the risk of HG ($p > 0.05$) after taking maternal serum free β-hCG and PAPP-A MoM levels into consideration (Table 3).

DISCUSSION

Our observational study showed that elevated PAPP-A and elevated free β-hCG MoM levels are independently associated with HG, even after adjusting for potential confounders.

Although the pathophysiologic mechanism underlying HG has not yet been identified, hCG is the most likely endocrine factor which is related with the development of HG. This conclusion is based on the reported associations between hypersecretion of hCG and HG such as in molar or in multiple pregnancies (Masson *et al*., 1985; Goodwin *et al*., 1994). Also, the incidence of hyperemesis is highest around 8 to 12 weeks when hCG

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Table 1—Characteristics of the study population

	HG ($n = 115$)	Control ($n = 110$)	Z[a]	p^*
Maternal age	27.7 ± 4.1	29.5 ± 4.9	3.282	**0.010**
Gravidity	1.0 (1.0)	1.0 (1.0)	1.746	0.081
Parity	0.0 (1.0)	0.0 (1.0)	1.144	0.253
BMI	22.0 (3.2)	22.4 (3.8)	1.562	0.118
Gestational age (weeks)	11.6 (1.7)	12.2 (1.5)	1.380	0.176
Fetal sex (%)			$\chi^2 = 1.867$[b]	0.171
Male	44 (38.3)	52 (47.3)		
Female	71 (61.7)	58 (52.7)		
TSH (mU/L)	0.8 (1.2)	1.4 (1.0)	4.989	**<.001**
Free thyroxine (ng/dL)	1.5 (0.40)	1.3 (0.5)	2.061	**0.039**
free β-hCG (MoM)	1.3 (0.8)	1.0 (0.6)	2.742	**0.006**
PAPP-A (MoM)	1.2 (0.5)	1.0 (0.7)	3.171	**0.009**
Both high[c] (%)	58 (50.4)	36 (32.7)	$\chi^2 = 4.529$	**0.033**
Ketonuria	3.0 (3.0)	0.0 (0.5)	9.187	**<.001**
Hemoglobin (g/dL)	12.4 ± 1.3	12.7 ± 0.7	0.307	0.759
Weight loss (kg)	3.0 (2.2)	—		
ALT (U/L)	16.0 (7.0)	14.0 (5.0)	3.798	**<.001**
AST (U/L)	15.0 (8.7)	14.0 (4.0)	3.018	**0.001**
Number of admissions				
1	87 (75.6%)			
2–4	28 (24.4%)			
Hospital stay (days)	2.0 (2.0)			

Values are given as median (IQR: interquartile range) or mean ± SD. Hospital stay: duration of hospitalization.
[a] Mann–Whitney test.
[b] Chi-square test.
[c] Women with PAPP-A >1.0 MoM and free β-hCG >1.0 MoM.
* $p < 0.05$ is significant.

Table 2—Correlation coefficients determined by simple correlation between the HG and other related laboratory and clinical factors

	HG		Hospital stay	
Variables	Rho	p	Rho	p
Maternal age	−0.222	**0.001**	−0.108	0.256
Gravidity	−0.141	**0.037**	0.005	0.961
Parity	−0.112	0.098	−0.076	0.424
BMI	−0.173	**0.036**	−0.097	0.417
Fetal sex	0.087	0.345	0.184	0.174
Free β-hCG	0.198	**0.006**	0.011	0.920
PAPP-A	0.208	**0.002**	0.121	0.274
TSH	−0.338	**<.001**	0.101	0.288
FreeT4	0.198	**0.039**	0.031	0.807
Ketonuria	0.622	**<.001**	0.189	**0.045**
AST	0.217	**0.001**	0.048	0.619
ALT	0.259	**<.001**	0.259	**0.006**

Rho: Spearman correlation coefficient; hospital stay: duration of hospitalization.
$p < 0.05$ is significant.

Table 3—Logistic regression analysis for variables independently associated with hyperemesis gravidarum[a]

				95% Confidence limits	
Parameters	Wald	p	Odds ratio	Lower	Upper
Free β-hCG	4.304	**0.038**	**4.545**	1.086	19.230
TSH	3.191	0.074	0.548	0.057	2.523
FreeT4	3.659	0.056	0.079	0.006	1.063
AST	2.605	0.107	1.154	0.969	1.377
PAPP-A	8.117	**0.004**	**8.064**	1.915	33.333

[a] Variable(s) entered on step 1: Fetal gender, PAPP-A, β-HCG, TSH, freeT4, ketonuria, ALT, AST, weight loss, and number of admissions to hospital.

production reaches its peak during pregnancy. Consistent with the literature, we have found elevated concentrations of hCG in the HG group, together with suppressed TSH and hyperthyroxinemia. Increased production of hCG in these pregnancies leads to thyrotrophic activity (Rodien *et al*., 2004). Suppressed TSH has been reported in most (70%) of the patients with HG (Goodwin *et al*., 1992). Thyroxine levels have also been correlated with hCG concentrations. The role of hyperthyroidism in HG is unclear. Whether it participates in the triggering of vomiting or is a parallel consequence of hypersecretion of hCG is not known.

Elevated levels of ALT and AST were found in our HG group compared to control group. Literature suggests that levels of aminotransferases can rise one to two times above the normal in 50% of patients with HG (Kuscu and Koyuncu, 2002). However, in our HG series only five (4.5%) women had transaminase values above the normal (ranged from 35 to 137 U/L). These patients had lower TSH levels; simple correlation analysis also showed a significant positive association between serum levels of free thyroxine and ALT. Increases of AST and ALT in both Graves disease and painless thyroiditis suggest that elevation of transaminases may be induced by changes in thyroid function (Kubota *et al*., 2008). It

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has been reported that the mechanism of hepatic injury appears to be relative hypoxia in the perivenular regions, due to an increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow (Malik and Hodgson, 2002).

Our findings show that pregnancies with HG have higher maternal serum PAPP-A levels in the first trimester compared with normal pregnancies. To our knowledge, this is a novel finding. It is not clear which factors are responsible for increased maternal serum PAPP-A levels in HG. PAPP-A functions as an insulin-like growth factor binding protein (IGFBP) protease. Boldt and Conover wrote that PAPP-A was shown to be expressed by a variety of cell types, and thus no longer could be considered to be just "pregnancy-associated". PAPP-A gene expression has been documented in both human fibroblasts and in human coronary artery smooth muscle cells when stimulated by pro-inflammatory cytokines involved in injury repair responses, specifically tumor necrosis factor (TNF)-a and interleukin (IL)1-b, and pre-treatment of human fibroblasts with the antioxidant, N-acetyl cysteine (Resch *et al*., *2004*). Moreover, smooth muscle cells exposed to resveratrol inhibited cytokine-induced PAPP-A expression and IGFBP-4 proteolytic activity (Conover *et al*., 2006). Thus, the effects of these pro-inflammatory cytokines may be mediated partially through oxidative stress.

Increased lipid peroxidation and oxidative stress are observed during pregnancy owing to excess free-radical activity and impaired antioxidant defense system. In pregnancies with HG, low antioxidant enzyme activities (Ustun *et al*., 2004; Guney *et al*., 2007) and increased oxidant stress (Aksoy *et al*., 2009) have been reported compared to normal pregnancies. In the light of these findings we speculate that increased oxidative stress and decreased antioxidant activity in HG could be the reason of induced PAPP-A expression.

IGFs are small peptides, similar to insulin, which are usually bound to IGFBPs in the circulation. PAPP-A, a protease to IGFBP-4, facilitates the breakdown of this protein resulting in a release of free IGF (Lawrence *et al*., 1999). IGFs are believed to play an important role in the regulation of trophoblast invasion of the decidua. A low level of PAPP-A in maternal blood may cause impaired release of IGFs and may be complicated with poor placental perfusion, and the onset of other pregnancy complication (Smith *et al*., 2006; Spencer *et al*., 2008; Grill *et al*., 2009). It can similarly be postulated that increased levels of PAPP-A in the maternal serum may play a role in the reduced risk of adverse obstetric outcomes in pregnancies with HG.

CONCLUSION

PAPP-A and free β-hCG levels are elevated in the serum of pregnant women with HG in the first trimester, even after adjusting for potential confounders. PAPP-A is emerging as a critical determinant of growth and development. Further studies are necessary to analyze the role of PAPP-A in physiology and patho-physiology of the pregnancy, which can be valuable in understanding the mechanism of the development of HG.

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