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Salivary gustin and hyperemesis gravidarum

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Abstract
Aim: Hyperemesis gravidarum (HG) is described as unexplained excessive nausea and vomiting during pregnancy. Gustin is a polypeptide hormone present in saliva. It is necessary for normal taste sensation. Inhibition of gustin release causes gustatory buds abnormalities. In this study, levels of salivary gustin were evaluated in pregnant women with HG.
Material and Methods: This prospective case-control study was conducted on 30 women with HG and 30 healthy pregnant women without symptoms of HG. Fasting saliva samples were taken from all subjects for measurement of salivary gustin levels, and the groups were compared with each other for gustin levels.
Results: Salivary gustin levels were slightly lower in the HG group than in the control group. But this difference did not reach statistical significance. Logistic regression analysis revealed that gustin level has a borderline significance in the prediction of HG. High gustin levels decreased HG development risk by 35.6%. Gustin level predicted 36.3% of HG cases in the ROC analysis, which was not statistically significant.
Discussion: Gustin does not play any role in the etiopathogenesis of hyperemesis gravidarum, but due to the excessive number of variables affecting appetite, the role of salivary gustin on the etiopathogenesis of HG must be studied in larger prospective studies, which will include a greater number of variables in this matter.

Keywords
Emesis; Saliva; Hormone; Pregnancy; Gustin; Carbonic anhydrase

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Introduction

Hyperemesis gravidarum (HG) is described as unexplained excessive nausea and vomiting during pregnancy, which causes fluid and electrolyte imbalances, nutritional deficiency and weight loss. It occurs in about 0.5% to 2% of pregnant women and is the most common cause of admission to the hospital in early pregnancy [1-3].

Many etiopathogenic factors have been considered for HG, including endocrine-hormonal factors such as higher levels of hCG, progesterone, and thyroid hormones during early pregnancy, hepatic dysfunction, changes in lipid metabolism, upper gastrointestinal system dysmotility, and psychological factors. However, no specific causative factors have been established [4].

The mechanisms that drive the pregnancy-induced changes in appetite remain largely unknown. Many maternal metabolic adaptations occur during pregnancy. The mother has to establish a negative energy balance during 1st trimester to avoid intake of toxic compounds, and a positive energy balance during the rest of the pregnancy to ensure the growth of the fetus, as well as fetal and maternal wellbeing. Changes in taste sensation and taste threshold are parts of this adaptation and they might be responsible for HG.

Gustin is a 37 KD molecular weight polypeptide hormone present in saliva. It is released by the parotid glands in the human. It contains two zinc atoms. Gustin has six fractions, and fraction II is mostly found in the parotid saliva. Its structure was found to be similar to that of Carbonic anhydrase 6 (CA6) [5]. Gustin is necessary for the normal development of the tastebuds. Inhibition of gustin release causes gustatory buds abnormalities, and it was shown that gustin affects directly gustatory buds and olfactory epithelium, as a growth factor. Gustin is relatively absent in patients with hypogeusia [6].

Nowadays some peripheral hormones secreted from GIS and regulating appetite become popular. Since the salivary gustin hormone is important in taste sensation, its abnormal secretion might be involved in the etiopathogenesis of HG. In this study, we evaluated salivary gustin levels in pregnant women with HG.

Material and Methods

This prospective case-control study was conducted on 60 pregnant women in their first trimester of pregnancy. The women were selected from the outpatient clinic of Maternity Clinic and the in-patient wards of the Hospital of Obstetrics and Gynecology at Turgut Ozal University Hospital between July 2012 and October 2013. The patients were divided into two groups according to the presence of HG. Thirty women with HG and 30 healthy pregnant women without symptoms of HG were taken into the study. HG was defined as persistent nausea and vomiting associated with ketosis and weight loss > 5% of pre-pregnancy weight. Inclusion criteria were as follows: singleton pregnancy with a live embryo, healthy women without any medical disorders, age range between 18 and 35 years, weight within 20% of normal weight for height at the beginning of pregnancy, and gestation between 6-14 weeks. The exclusion criteria were as follows: pregnant smokers or drug users, cases with any systemic disease and/or psychological disorder that can cause vomiting, and multiple gestation. In addition, those women with uncertain dates or early pregnancy loss were also excluded from the study. Approval for this study was obtained from the Local Institutional Review Board of the Faculty of Medicine, Turgut Özal University (No: B-30 2 FTH 0 20 00 00-2492, Date: Dec 12, 2011). Informed consent was obtained from all participants.

All relevant data, including demographic information (age, gravida, parity, body-mass index (BMI), obstetric history, gestational week) were collected for further analysis. The gestational age was calculated according to the modified Naegele's rule. Last menstrual period-derived gestational age was compared with ultrasound-derived gestational age using CRL [7], and if there was a marked discrepancy of 2 weeks or more, the woman was excluded from the study.

Fasting venous blood and saliva samples in the morning were taken from all subjects. Serum TSH (μIU/mL) level was measured by electrochemiluminescence immunoassay (ECLIA); serum FT3 (pg/mL), serum FT4 (ng/dL); serum human chorionic gonadotrophins (serum hCG) were measured quantitatively by the Sandwich principle. Blood urea, serum creatinine, sodium, potassium, glucose, AST, ALT and complete blood count were also measured to detect the severity of emesis. In addition, ketones in a morning urine sample were measured with urine stripes. Saliva samples were collected after rinsing their mouths with water thoroughly. Immediately after collection, saliva samples were centrifuged for 15 min at 4.000 rpm to remove any particles or sediments. The human CA6 concentrations in the samples were measured in saliva using a sandwich enzyme-linked immunosorbent assay (ELISA) with a human CA-6 commercial kit (Cusabio Biotech Co., LTD, Wuhan, China) according to the manufacturer's instructions. Absorbance values were calculated at 450 nm in an automatic ELISA reader. The concentrations were calculated by converting the optical density readings with a standard curve. All samples were calculated in a duplicate manner. Saliva CA-6 levels were determined as ng/ml. The detection range was between 0.63 ng/ml and 40 ng/ml. The intra-assay and inter-assay variations were 8% and 10%, respectively.

Statistical analysis: The Statistical Package Program for the Social Sciences (SPSS 15.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Groups were controlled in terms of conformity to normal distribution using graphical check and Shapiro Wilk test. Since the groups were distributed normally, the mean and SD parameters were used. The median (minimum-maximum) was used for groups that were not distributed normally. The independent samples t- test was used for comparison of groups that were distributed normally. The Mann-Whitney test was used to compare groups that were not distributed normally. The Spearman correlation test was used to find the correlation between demographic parameters and gustin levels. Logistic regression analysis was performed for the determination of parameters that can predict the development of HG. Receiver-operating characteristics (ROC) curve analysis was performed to determine the effect of gustin in the prediction of HG. A p-value of ≤ 0.05 was taken as significant.

Results

The study included 60 pregnant women in the first trimester of pregnancy. Thirty women with HG and 30 healthy pregnant women without any HG symptoms were included in the study. During the follow-up period, 6 women in the HG group and 14 women in the control group were lost. The remaining 24 women with HG and 16 healthy pregnant women were taken as a control group. The mean weight of the women was 66.9 ± 12.2 kg and 63.0 ± 11.9 kg at the beginning of the pregnancy and during the emesis period in the HG group, respectively. Groups were compared with each other in terms of age, gravida, parity, gestational age and BMI before and during pregnancy. There was no statistically significant difference between groups in terms of these demographic parameters except for pre-pregnancy BMI (Table 1). Prepregnancy BMI was significantly higher in the HG group than in the control group (p=0.031).

The groups were compared with each other for salivary CA6 levels. CA6 levels were lower in the HG group than in the control group. We did not find a significant statistical difference between groups (p= 0.149) (Table 1).

Correlation analysis between gustin levels and demographic parameters revealed a strong positive correlation between CA6 level and gestational week (Rho=0.640, p=0.003) (Table 2). Logistic regression analysis was done to find the most effective parameter in the prediction of HG. The analysis revealed that the CA6 level has a borderline significance in the prediction of HG (p=0.058). It was found that high CA6 levels decreased HG development risk by 35.6% (Odds ratio=0.644) ((Table 3).

The ROC analysis was performed to find the most effective parameter in the diagnosis of HG. The only use of CA6 level predicted 36.3% of HG cases. This was not statistically significant (AUC=0.363, p=0.148 (95%CI=0.182-0.543)) (Figure 1).

Table 1. Distribution of demographic data and gustin levels according to the groups

	Hyperemesis Group	Control Group	p
Age (years)	28.6 ± 4.5	29.7 ± 3.0	0.242
Gravida (n)	2 (1-6)	2 (1-4)	0.719
Parity (n)	0.5 (0-4)	1 (0-2)	0.910
Gestational Age (weeks)	9 (6-12)	11 (9-12)	0.032
Prepregnancy BMI (kg/m^2)	24.5 (18.7-39.9)	21.7 (18.2-30.5)	0.031
Pregnancy BMI (kg/m^2)	23.0 (15.5-38.1)	24.1 (18.7-32.6)	0.294
Salivary Gustin (ng/ml)	1.38 (0.0001-3.22)	2.14 (0.0001-6.38)	0.149

P<0.05 Statistically significant. BMI: Body mass index

Table 2. Results of correlation analysis between salivary CA6 levels and demographic parameters

		Age	Gravida	Parity	Prepregnancy BMI	Pregnancy BMI	Gestational Week
CA6 level	Rho	0.308	0.305	0.232	0.002	0.181	0.640
	p	0.060	0.063	0.161	0.988	0.331	0.003

P<0.05 Statistically significant. CA: Carbonic anhydrase

Table 3. Logistic regression analysis of gustin level for prediction of hyperemesis development

	B	S.E.	Sig.	Odds ratio
Gustin	-0.440	0.232	0.058	0.644
Constant	0.698	0.525	0.183	2.011

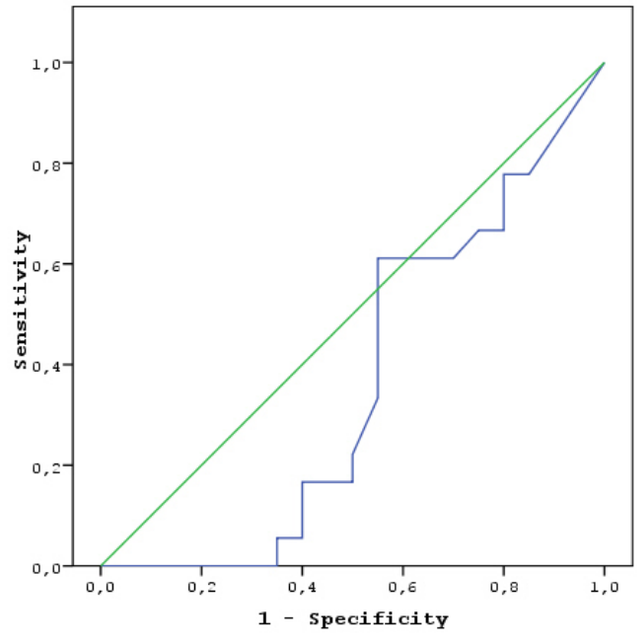

Figure 1. The ROC analysis was performed to find the most effective parameter in the diagnosis of HG

Discussion

Salivary fluid is an exocrine secretion consisting mostly of water and containing a variety of hormones, electrolytes, mucosal glycoproteins, enzymes and many other factors [8]. Nowadays, saliva is being used as an auxiliary diagnostic parameter. Sialometry and sialochemistry have been used to diagnose some diseases [9]. It is used for the determination of hormonal status in obstetrics and gynecology [10].

In this study, we want to use saliva for a different diagnostic purpose; in the prediction of HG. The test would be very simple, easy, non-invasive. It could also be used for the monitorization of HG and response to treatment. We found that salivary gustin has a limited value in the prediction of HG. Gustin levels predicted only 36.3% of HG patients.

Taste sensitivity is different among populations and may be one of the most important determinants influencing the food choice of the individual [11]. Gustin is a polypeptide hormone present in the saliva and is important in taste sensation. Abnormality in this salivary growth factor is considered responsible for the maintenance of taste bud function and the function of the olfactory epithelium. During pregnancy senses of smell and taste change, which may alter the eating habits of pregnancy. A change in sense of smell and taste may be caused by a change in gustin levels. In previous studies, it has been shown that decreased gustin in the nasal mucous is associated with abnormal olfactory function [5,6]. Inhibition of salivary gustin release is associated with gustatory buds abnormalities and changes in taste sensation, which is already present in pregnant women. In this study, although gustin levels were lower in HG cases, there was no significant difference between HG and control cases. Since the number of cases in this study is limited, larger series might give different results on the role of gustin in HG.

In normal physiology, the secretion of gut hormones, including salivary hormones, may change according to metabolic need and nutrition [12,13]. The pregnancy itself might alter salivary

hormone secretion and composition. In the 1st trimester, there is a negative energy balance, which could be induced by pregnancy-associated hormonal changes in GIS secretion. It might be a protective mechanism for the fetus. This is the time, in which mother and fetus need no additional energy, but they must be protected from any harmful substance or deleterious agent to keep developing fetus safe. Thus, most prominent symptoms occur during the 1st trimester of pregnancy. After the 1st-trimester, organogenesis is completed and physiological mechanisms are activated to increase maternal appetite to ensure adequate energy consumption for the mother and for the fetus. In this study, we found that gustin levels showed a small decrease in HG patients, which was not statistically significant. Since gustin is necessary for the normal function of the taste buds in the tongue, women will not be able to comprehend the tastes, as it happens in its absence or insufficient secretion. This might change the appetite and interest of women in different foods. After 1st trimester, adaptation and/or desensitization might develop, after which the symptoms disappear.

HG is seen more frequently in women with undesired pregnancy [14,15]. Some gut hormones act as neurotransmitters within the central nervous system to control food intake [16,17]. Their levels might be dysregulated due to stress. The hypothalamus is a crucial region for integrating signals from central and peripheral pathways and plays a major role in appetite regulation [18,19]. Psychological stress (an unplanned pregnancy, marital problems or pregnancy itself) can affect hormone levels in CNS, starting an activation from the cortex to hypothalamus, to the brain stem ending in the cranial nerves innervating salivary glands, changing the composition of saliva.

There are some limitations of our study. The first is the number of cases. Although we planned 30 cases for each group, due to the high dropout rates, case and control groups were formed from 24 and 16 patients, respectively. The small sample size decreased the power of the study.

The other important limitation is that we did not measure gustin levels at different gestational ages. They could be measured and compared with each other at the 1st, 2nd and 3rd trimester to see their relationship with appetite changes as pregnancy progress.

Another deficiency of the study is that other salivary factors which may affect appetite were not measured. The most important of them is Zinc. Zinc is an essential trace element that contributes to the active center of approximately 300 enzymes [20]. It is necessary for the function of gustin [21]. Deficiency of zinc also produces symptoms similar to deficiency of gustin. Measurement of zinc and other cofactors might be useful in the enlightening saliva-HG relation.

Conclusion

Due to the excessive number of variables, including genetic and environmental factors affecting appetite, the role of salivary gustin in the etiopathogenesis of HG and weight changes during pregnancy must be studied in the prospective studies, which will include broader groups of patients and a greater number of variables in this matter. Further study with larger series is required to fully understand the multiple signals regulating appetite in pregnancy and to contribute more to clinical practice.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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