RESEARCH ARTICLE

Maternal type 1 collagen N-terminal telopeptide levels in severe hyperemesis gravidarum

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Abstract

Background: Nausea and vomiting occur 50–90% during the first trimester of pregnancy. However, patients with hyperemesis gravidarum (HG) may be hospitalized at an incidence rate of 0.8–2% before the 20th week of gestational age. The symptoms generally start during the 5–6th gestational weeks, reaching the highest degree during the 9th week, and decline after the 16–20th weeks of gestation. Clinical findings are proportional to the severity of the disease and severe HG is characterized with dehydration, electrolyte imbalance, and nutritional deficiency as a result of vomiting.

Methods: The study population consisted of two groups of pregnant volunteers at 5–12 weeks of gestation: a severe HG group and a control group. The HG severity was scored using the Pregnancy-Unique Quantification of Emesis (and nausea) (PUQE). The serum levels of the maternal Ca, parathyroid hormone (PTH), Na, K, blood urea nitrogen (BUN), creatinine, vitamin D (25OHD3), and the maternal urine NTx levels were compared between the groups.

Results: In total, 40 volunteers were enrolled in this study: 20 healthy pregnant volunteers and 20 with severe HG. There were no statistically significant differences between the maternal characteristics. The first trimester weight loss of ≥5 kg was significantly higher in the severe HG group (p < 0.001), while the control group had a significantly higher sunlight exposure ratio than the severe HG group (p = 0.021). The urine NTx levels were significantly higher in the severe HG group (39.22 ± 11.68NTx/Cre) than in the control group (32.89 ± 8.33NTx/Cre) (p = 0.028). The serum Ca, PTH, Na, K, BUN, and creatinine levels were similar between the groups (p = 0.738, p = 0.886, p = 0.841, p = 0.957, p = 0.892, and p = 0.824, respectively). In the severe HG group, the serum 25OHD3 levels were significantly lower than in the control group (p < 0.001).

Conclusions: The data from this study indicated that severe HG is associated with increased urine NTx levels. However, large-scale studies are required to understand the clinical significance of this finding, as well as the long-term consequences of elevated urine NTx levels and the underlying mechanisms.

Trial registration: NCT02862496 Date of registration: 21/07/2016.

Keywords: Hyperemesis gravidarum, Vitamin D deficiency, Urine type 1 collagen N-terminal telopeptide, Urine Ntx
Background
Nausea and vomiting occur 50–90% during the first trimester of pregnancy. However, patients with hyperemesis gravidarum (HG) may be hospitalized at an incidence rate of 0.8–2% before the 20th week of gestational age [1, 2]. The symptoms generally start during the 5–6th gestational weeks, reaching the highest degree during the 9th week, and decline after the 16–20th weeks of gestation [3]. Clinical findings are proportional to the severity of the disease and severe HG is characterized with dehydration, electrolyte imbalance, and nutritional deficiency as a result of vomiting [4–6]. Deficiencies in certain vitamins that work as catalysts in the human body can lead to metabolic disorders.

Bone turnover is a dynamic and complex procedure, with bone resorption and bone formation being seen sequentially due to many different substances and factors. Biochemical markers, including protein and enzyme measurements, can be used to define bone resorption and formation [7]. Telopeptides are tiny protein particles found particularly in type I collagen that include 15–20 amino acids. Telopeptides bound to the ends of these amino acids are removed from the body through the urine as a result of collagen metabolism. During resorption, amino and carboxyl terminal fragments, called telopeptides, are bound to collagens with cross links and can be released into the circulation and excreted in the urine. Some recent studies have demonstrated that the type I collagen N-terminal telopeptide (NTx) level is more specific to bone tissue than any other resorption markers, showing more specificity if established earlier [8]. It can be used as a specific and stable indicator in the measurement of bone resorption.

It is well documented that sHG characterized by nutritional deficiency. We hypothesized that inadequate calcium and vitamin D intake may cause increased calcium mobilization from the maternal skeleton. Thus, in the present study, we aimed to determine the presence of maternal bone resorption in women with severe HG using the NTx levels in the maternal urine.

Methods
Study protocol
The present study was performed at Kayseri Education and Research Hospital in Kayseri, Turkey. The study was approved by the “Ethics Committee of Erciyes University” (decision number: 2016/345), which was carried out by the Declaration of Helsinki. All participants gave informed consent before the study.

The study population consisted of two groups of volunteers between 19 and 35 years of age who were between 5 and 12 weeks of gestation: an HG group and a healthy control group. HG was described as stated by the American College of Obstetricians and Gynecologists (ACOG) criteria: severe nausea and vomiting resulting in 5% weight loss (compared to pre-gestation) or vomiting more than three times per day and 3–5% weight loss with ketonuria. Those patients with the following other pathologies were excluded: gastroenteritis, pyelonephritis, bile duct diseases, hepatitis, urinary tract stones, hyperthyroidism, hyperparathyroidism, migraines and vestibular disease, and any other diseases causing NTx level changes (e.g., Paget’s disease and bone tumors) [3, 6]. In addition, those patients with any conditions causing osteoporosis or decreases in the bone mineral density were also excluded (smoking, celiac disease, inflammatory bowel disease, depression, pre-gestational diabetes, chronic kidney or liver disease, malignancy, and medication use, such as steroids, anticonvulsants, antiepileptics, heparin, or low molecular weight heparin) [9].

Pregnancy-Unique Quantification of Emesis (and nausea) (PUQE) questionnaire was used scoring the severity of each patient’s nausea and vomiting. The scores ≥13 points are classified as severe NVP/HG [10].

The blood samples were collected from the study population during the hospitalization of those patients diagnosed with severe HG. The blood samples from the volunteers in the control group were collected during their regular clinical visits. A5-mL blood sample was placed into a serum-separating tube to measure the serum Ca, PTH, Na, K, BUN, and creatinine levels. An additional 3-mL blood sample was placed into an EDTA tube to measure the serum 25OHD3 level. The blood samples were analyzed on the same day that they were obtained at the Kayseri Education and Research Hospital Biochemistry Laboratory. The 25OHD3 was measured using ultra high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). While no consensus exists, we used a common clinical cut-off based on the Endocrine Society recommendations to categorize the 25OHD3 levels: deficiency < 25 nmol/L, insufficiency = 25 to < 50 nmol/L, and normal = 50–75 nmol/L [11].

NTx levels can be measured in serum and urine. In the present study we analyzed in urine because we aimed to measure to be non-invasively. In order to measure the urinary NTx level and urine creatinin, 10 cc of morning urine were collected following 12 h of fasting overnight the day after hospitalization. In control group morning urine samples were obtained the day after blood samples collected following 12 h of fasting overnight. Urine NTx levels vary throughout the day therefore, urine samples were analyzed from morning urine that NTx excretion was the most constant. The urine samples were immediately stored at −80 °C, without blood contamination. The NTx levels were evaluated on the same day four months after the first urine sample was stored via an enzyme-linked immunosorbent assay.
by Cigdem Karakukcu. This was done to avoid any inconsistencies, and the samples were titrated with the urinary creatinine levels. All blood and urine samples were analyzed in Kayseri Education and Research Hospital Biochemistry clinic.

Statistics
The Shapiro-Wilk’s test was used to test the normality assumption of the data. Levene’s test was used for the variance in homogeneity assumption. The values were expressed as the mean ± standard deviation, median (25–75th percentile), or n (%). The parametric comparisons were made using a t-test or z-test, and the nonparametric comparisons were made using the Mann-Whitney U test. All of the comparisons were made with the PASW Statistics 18 program, and a p value of < 0.05 was considered to be statistically significant.

The sample size was determined using a two-way analysis, and the reference values (means, standard deviations, and reference sample sizes) were taken from a study conducted by Shieh et al. [12]. It was estimated that at least 20 participants were required in each group to detect a clinically significant difference between the two groups when assuming a power of 80% to assess the primary hypothesis and a type I error of 0.05. Assuming a 5% dropout rate, we recruited 40 women (20 participants per group).

Results
In total, 40 pregnant women were enrolled in this study: 20 healthy pregnant women and 20 with severe HG. The mean maternal age was 26.90 ± 2.36 years old in the control group and 26.95 ± 2.35 years old in the severe HG group (p = 0.947). The mean gestational age was 10.35 ± 1.23 weeks in the control group and 10.30 ± 1.22 weeks in the severe HG group (p = 0.898). The pre-pregnancy body mass index (BMI) was 24.67 ± 1.27 in the control group and 24.76 ± 1.35 in the severe HG group (p = 0.829). The first trimester weight loss ≥5 kg was significantly higher in the severe HG group (p < 0.001), and the control group had a significantly higher sunlight exposure ratio than the severe HG group (p = 0.021). Overall, there were no statistically significant differences between the nulliparity, caffeine intake, exercise regularly, and family history of osteoporosis. The comparisons of other maternal characteristics between the groups are shown in Table 1.

The biochemical parameters are compared in Table 2. The urine NTx levels were significantly higher in the HG group (39.22 ± 11.68 NTx/Creat nmolBiCE/nmol) when compared to the control group (32.89 ± 8.33NTx/Creat nmolBiCE/nmol)(p = 0.028). Serum Ca, PTH, Na, K, BUN, and creatinine levels were similar between the two groups (p = 0.738, p = 0.886, p = 0.841, p = 0.957, p = 0.892, and p = 0.824, respectively). In the severe HG group, the serum 25OHD3 levels were significantly lower than those in the control group (p < 0.001).

Discussion
During pregnancy, the physiological upregulation of calcium is of vital importance for the mineralization and development of the fetal skeleton, with approximately 20–30 g of calcium being transferred from the mother to the fetus [13]. This calcium demand is regulated by various compensatory mechanisms, including increased renal reabsorption, increased intestinal dietary calcium absorption, and calcium mobilization from the maternal skeleton [14]. The absorption of calcium in the maternal intestine doubles increasingly until the 12th week of gestation. The most important determinant of intestinal calcium absorption is vitamin D, which provides continuous maternal fetal calcium transfer [15, 16]. The healthy functioning of these compensatory mechanisms will ensure healthy maternal bone mineral density at the

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### Table 1: Comparison of maternal characteristics between the groups

|                        | Severe HG group (n = 20) | Control group (n = 20) | P value |
|------------------------|--------------------------|------------------------|---------|
| Maternal age (year)    | 26.95 ± 2.35             | 26.90 ± 2.36           | 0.947   |
| Gestational weeks      | 10.30 ± 1.22             | 10.35 ± 1.23           | 0.898   |
| Pre-pregnancy BMI (kg/m²) | 24.76 ± 1.34         | 24.67 ± 1.27           | 0.829   |
| First trimester weight loss ≥5 kg | 19(95%)                   | 0(0%)                  | < 0.001 |
| Nulliparity            | 13(65%)                  | 12(60%)                | 0.752   |
| Exercise regularly     | 0(0%)                    | 3(15%)                 | 0.083   |
| Family history of osteoporosis | 3(15%)                    | 3(15%)                 | 1       |
| Caffeine intake        | 0(0%)                    | 0(0%)                  | 1       |
| Sunlight exposure      | 0(0%)                    | 4(20%)                 | 0.021   |

HG: hyperemesis gravidarum, BMI: body mass index

The Shapiro-Wilk’s test was used to test the normality assumption of the data. Levene’s test was used for the variance in homogeneity assumption. The values were expressed as the mean ± standard deviation, median (25–75th percentile), or n (%). The parametric comparisons were made using a t-test or z-test, and the nonparametric comparisons were made using the Mann-Whitney U test. All of the comparisons were made with the PASW Statistics 18 program, and a p value of < 0.05 was considered to be statistically significant.
end of pregnancy and lactation. In presence of severe HG, due to inadequate intestinal compensatory mechanism, more calcium mobilized from the maternal skeleton. Thus, this clinical study aimed to investigate the maternal bone health in women with severe HG using the bone resorption marker NTx levels in the maternal urine.

Metabolic and nutritional disorders associated with the severity of the HG are likely to develop, and fetomaternal morbidity increases [17]. In the present study, we found that serum the 25OHD3 levels were significantly lower in the severe HG group when compared to the control group. These results could be explained by inadequate calcium and vitamin D intake due to nausea and vomiting. In the literature, more than 60% of these patients were determined to have thiamine, riboflavin, vitamin B6, vitamin A, retinal binding protein, and vitamin K deficiencies [18]. Chiossi et al. reported that HG patients developed Wernicke’s encephalopathy due to thiamine deficiencies [19]. Corona G et al. reported the development of osmotic demyelination syndrome in these patients due to their metabolic disorders and hyponatremia [20]. In another study, Jonakiraman et al. showed that dietary calcium supplementation during pregnancy reduced the NTx levels when compared to a placebo. In their study, the dietary calcium supplementation suppressed the maternal bone mobilization [28]. Similarly, Jonakiraman et al. showed that dietary calcium supplementation reduced the NTx levels in the third trimester [29]. In another study, Liu et al. reported that among 36 pregnant Chinese women with inadequate dietary calcium intakes, calcium supplementation significantly decreased the bone resorption markers [30].

Our findings can be interpreted as; Pregnancy may impact a woman’s bone mass, which is an important determinant of her subsequent risk of osteoporosis [31]. The possibility that the intrauterine programming of fetal bone growth may be an important determinant of

| Table 2 Comparison of biochemical parameters between the groups |
|---------------------------------------------------------------|
|                                                               |
| Serum BUN (5–15 mg/dL)                                         |
| Severe HG group (n = 20)                                       |
| Control group (n = 20)                                        |
| P value                                                       |
| 12.01 ± 1.09                                                  | 11.96 ± 1.21                                                  | 0.892 |
| 0.91 ± 0.09                                                  | 0.90 ± 0.08                                                  | 0.824 |
| 139(138–140)                                                  | 139(138–140)                                                  | 0.841 |
| 3.72 ± 0.13                                                  | 3.72 ± 0.14                                                  | 0.954 |
| 8.70(8.43–9.10)                                               | 8.75(8.40–9.05)                                              | 0.738 |
| 37.39 ± 12.93                                                 | 36.85 ± 10.96                                                | 0.886 |
| 18(90%)                                                     | 6(30%)                                                      | < 0.001 |
| 39.22 ± 11.68                                                 | 31.89 ± 8.33                                                 | 0.028 |

HG hyperemesis gravidarum, BUN blood urea nitrogen, PTH parathyroid hormone, NTx type 1 collagen N-terminal telopeptide
The Shapiro-Wilk’s test was used to test the normality assumption of the data. Levene’s test was used for the variance in homogeneity assumption. The values were expressed as the mean ± standard deviation, median (25-75th percentile), or n (%). The parametric comparisons were made using a t-test or z-test, and the nonparametric comparisons were made using the Mann-Whitney U test. All of the comparisons were made with the PASW Statistics 18 program, and a p value of < 0.05 was considered to be statistically significant.
osteoporosis is now being considered [32]. New evidence showed that maternal dietary deficiencies during pregnancy may be associated with a lower bone mass in her offspring later in life [33, 34]. Women’s with severe HG may have an increased risk for lower bone mass in offspring due to maternal dietary deficiency and increased maternal bone mobilization.

Conclusions

The data from the present study indicated that severe HG is associated with increased urine NTx levels. Large-scale studies are required to understand the clinical importance of this finding, in addition to the long-term consequences of elevated urine NTx levels and the underlying mechanisms.

Abbreviations

ACOG: American College of Obstetricians and Gynecologists; BUN: Blood urea nitrogen; HG: Hyperemesis gravidarum; NTx: type 1 collagen N-terminal telopeptide; PTH: Parathyroid hormone; PUQE: Pregnancy-Unique Quantification of Emesis; UHPLC-MS: Ultra high-performance liquid chromatography coupled with mass spectrometry

Acknowledgements

Not applicable.

Funding

This study was not funded by any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Availability of data and materials

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Authors’ contributions

ES, YM, MES conception and design of the study. ATT, ICM, MG, CK Data collection and analyzed the data. ES, GA, IIM drafted the first manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Erciyes University approved this research. Reference number: 2016/345. Written informed consent was obtained.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 10 November 2017 Accepted: 12 December 2018

Published online: 20 December 2018

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