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ORIGINAL ARTICLE

# Dynamic thiol-disulfide homeostasis in hyperemesis gravidarum

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**OBJECTIVE:** To determine serum thiol-disulfide homeostasis in hyperemesis gravidarum.
**STUDY DESIGN:** Twenty-six pregnant women with hyperemesis gravidarum and 37 healthy pregnant women were included in the study. Native thiol, disulfide and total thiol concentrations were measured with a novel automated method.
**RESULTS:** Serum disulfide levels were $15.68 \pm 4.41$ μmol $l^{-1}$ in the hyperemesis gravidarum group and $13.49 \pm 2.81$ μmol $l^{-1}$ in the healthy group ($P = 0.031$). Native thiol levels were $213.86 \pm 26.29$ μmol $l^{-1}$ in the hyperemesis gravidarum group and $232.18 \pm 19.21$ μmol $l^{-1}$ in healthy group ($P = 0.004$), and total thiol levels were $245.23 \pm 28.58$ μmol $l^{-1}$ in the hyperemesis gravidarum group and $259.17 \pm 19.94$ μmol $l^{-1}$ in the healthy group ($P = 0.038$).
**CONCLUSION:** Native and total thiol were deficient in the hyperemesis gravidarum group and this deficiency was correlated with the severity of the disease. The thiol-disulfide balance has shifted to the oxidative side. This metabolic disturbance may have a role in the pathogenesis of hyperemesis gravidarum.

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## INTRODUCTION

Nausea and vomiting in pregnancy are complaints seen in up to 80% of pregnant women in the first trimester of pregnancy.[1] Hyperemesis gravidarum (HG), a more severe condition, is seen in ~2% of pregnant women. Although severity varies, HG is characterized by dehydration, electrolyte disorders, acid-base imbalance, weight loss and ketonuria.[2] Nausea and vomiting during pregnancy and HG affect the quality of life of pregnant women and require in-patient treatment management.[3] Although nausea and vomiting are common complaints, the etiology is not clearly understood. It has been thought that multiple factors, such as hormonal variations (for example, β-hCG, estrogen and transient hyperthyroidism), genetic predisposition, immunological causes, gastrointestinal system dysmotility, *Helicobacter pylori* infection and psychological reasons, may lead to HG.[4–7] Although many factors have been described, the etiopathogenesis of HG has not been clearly explained so far.[8]

The imbalance between pro-oxidants and antioxidants results in oxidative stress. This ratio can be disrupted toward an increased levels of reactive oxygen species or a decrease in scavenging mechanisms. Oxidative stress has important roles in pregnancy complications.[9] Numerous mechanisms may lead to oxidative stress in pregnant women with HG. There is some evidence of the relationship between HG and oxidative stress.[10] First, the decreased nutritional intake is observed in patients with HG. As a result of insufficiency of dietary intake of vitamins, such as vitamin C and E and other energy sources and enhanced demands, imbalance occurs.[11,12] In addition, antioxidant enzyme activities, such as superoxide dismutase, glutathione (GSH) peroxidase and catalase, were found low in HG.[13] The other mechanism is the existence of *H. pylori*. In the presence of *H. pylori* the synthesis of reactive oxygen species has increased. The relationship between *H. pylori and HG* has been shown.[14] Last, the alteration in lipid profiles of patients with HG is related to the oxidative stress. Low levels of high-density lipoprotein (HDL) has been received in HG.[15,16] Thus, Paraoxonase-1 which is a HDL-associated antioxidant enzyme was found low in patients with HG.[15,16] Otherwise lipid peroxidation markers, such as malondialdehyde and lipid hydroperoxide levels, were obtained high in HG.[13,16]

Protein oxidation by the reactive derivatives leads to nitration of aromatic amino acids, oxidation of thiol groups and formation of advanced oxidation protein products and transformation of some amino-acid residues to the carbonyl derivatives. It is known that free radicals cause oxidation of –SH groups in sulfur-containing amino acids of proteins and this is the earliest observable signs of protein oxidation.[17] Thiols are in interaction with almost all physiological oxidants, and they are mentioned as essential antioxidant buffers. $H_2O_2$ has reactivity toward many amino acids in proteins, and cysteine residues are essential targets. Thus, the thiol group is susceptible to oxidation. GSH, accepted antioxidant and cytoprotectant, can scavenge hydrogen peroxide and hydroxyl anions.[18] Cysteine residues can catch radical oxygen species like the superoxide and hydroxyl radicals,[19] and also metallothioneins, the cysteine-rich class, are capable of binding heavy metals due to the presence of the sulfhydryl groups.

Thiols, also known as mercaptans, which consist of a sulfur atom and a hydrogen atom bound to a carbon atom, are functional sulfhydryl groups.[20] A very large part of the blood plasma thiol pools consist mainly of albumin and other proteins, such as GSH, thioredoxin, cysteine and homocysteine.[21] Thiol groups of proteins are oxidized by oxygen molecules present in the medium and are reversibly converted to disulfide bonds. Formed disulfide bonds can be reduced to thiol groups again. Thus, the thiol-disulfide balance is maintained.[22] Dynamic thiol-disulfide homeostasis has a critical role in antioxidant defense, detoxification,

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apoptosis, regulation of enzyme activity, transcription and cellular signal transduction mechanisms.[23,24]

Thiol-disulfide homeostasis is important. Only a single side of this double-sided balance has been measured since 1979.[25] Both variable levels are measured one by one and cumulatively with a novel and automated method.[26] Thus, the status can be evaluated completely.

We aimed to determine thiol-disulfide homeostasis, which has a vital role, in pregnant women with HG, and to investigate the relationship among homeostatic parameters and disease severity. To the best of our knowledge, this is the first study in this area.

## METHODS

### Study design

We enrolled 26 patients with HG admitted to the obstetrics and gynecology department. Inclusion criteria for the diagnosis of HG were severe and persistent nausea and vomiting that exist more than three times per day, at least one positive ketonuria on urinary dipstick and weight loss of more than 5% during the present pregnancy.[8] Disease severity was evaluated by amount of ketone bodies.[27,28] Pregnant women with multiple gestation, gestational trophoblastic disease, gestational diabetes mellitus, preeclampsia or other known probable vomiting causes, such as thyroid disorders or existing of any type of gastrointestinal, renal and hepatic diseases or with evidence of any systemic, infectious and inflammatory diseases (such as urinary infection, anemia, hypertension and so on), eating disorders, known psychiatric disease or taking medication or smoking habits and alcohol consumption, were excluded from the study. Gestational age was determined by last menstruation date and was confirmed with ultrasonography. Thirty-seven healthy pregnant women without nausea and vomiting constituted the control group. All subjects were informed. Written consents were obtained and the study was approved by the local ethics committee. Patient and healthy groups were matched in terms of maternal age and gestational age.

Venous blood samples were collected from the subjects and centrifuged at 2300× *g* for 10 min. Serum samples were separated and stored at − 80 °C until analysis. Serum thiol-disulfide homeostasis was determined with a recently developed novel and automatic measurement method[26] by using an automated clinical chemistry analyser (Roche, cobas 501, Mannheim, Germany). Native thiol (–SH) and total thiol (–SH+–S–S–) were measured directly, –S–S– and –S–S–/–SH, –S–S–/–SH+–S–S–, –SH/–SH+–S–S– results were obtained with calculation.

The levels of total protein and albumin were determined with commercially available assay kits (Roche, Mannheim, Germany) with an auto analyzer (cobas 501 Hitachi, Roche, Mannheim, Germany).

### Statistical analyses

The data were evaluated using visual (histograms, probability plots) and statistical methods (the Kolmogorov–Smirnov test and the Shapiro–Wilk test) to determine whether the data were normally distributed. Descriptive analyses were presented using mean and s.d. for the normally distributed variables. As the data were normally distributed, independent sample *t*-tests were conducted to compare the parameters among groups. Correlation analyses were managed using Pearson's correlation. An overall 5% type 1 error was used to infer statistical significance. Statistical analyses were performed using the SPSS software version 17 (SPSS Inc. Chicago, IL, USA).

## RESULTS

The clinical characteristics of the study group are shown in Table 1. Pregnant women with HG and healthy group were matched in terms of maternal age and gestational age. There were no statistically significant differences in age, gestational age in weeks and body mass index between patients and controls. Gravidity and parity were similar between the groups. Serum albumin and total protein levels of the HG group were lower than those of the control group ($P = 0.003$, 0.002, respectively).

The data including the thiol-disulfide profiles of patients with HG and healthy pregnants are given in Table 2 and Figures 1, 2, 3. Native thiol levels were significantly lower in the HG group when compared with the control group ($P = 0.004$). When two groups were compared based on the total thiol levels, there was a significant difference between the groups ($P = 0.038$) and total thiol levels were lower in patients than controls. In addition, mean disulfide values of the HG group were significantly higher than those of the control group ($P = 0.031$). In the HG study group disulfide/native thiol percent ratios and disulfide/total thiol percent ratios were found to be statistically higher and native/total thiol percent ratios were significantly lower than that of the pregnant control women ($P = 0.003$ in every three ratios).

Relationships between thiol-disulfide profiles and ketonuria were also assessed. As seen in Table 3, there were significant negative correlations between ketone levels and native and total thiol amounts ($r = -0.44$, $P = 0.001$; $r = -0.40$, $P = 0.002$, respectively). A significant positive correlation between ketone levels and disulfide amounts was found in HG group ($r = 0.29$, $P = 0.027$).

**Table 1.** Clinical characteristics of the study groups

| | *HG* (n = *26*) | *Control group* (n = *37*) | P-*value** |
|---|---|---|---|
| Age (years) | 27.38 ± 3.81 | 26.27 ± 5.11 | NS |
| Gestational age (week) | 9.46 ± 1.39 | 9.35 ± 1.29 | NS |
| BMI (kg m$^{-2}$) | 22.42 ± 2.9 | 23.51 ± 3.26 | NS |
| Gravidity | 2.04 ± 0.27 | 2.05 ± 0.21 | NS |
| Parity | 0.81 ± 0.22 | 0.68 ± 0.15 | NS |
| Albumin (g dl$^{-1}$) | 4.30 ± 0.42 | 4.61 ± 0.29 | 0.003 |
| Total protein (g dl$^{-1}$) | 7.21 ± 0.62 | 7.67 ± 0.35 | 0.002 |

Abbreviations: BMI, body mass index; HG, hyperemesis gravidarum; NS, not significant. Values are expressed as mean ± s.d. **P* value < 0.05 considered significant.

**Table 2.** Thiol-disulfide profiles of subjects

| *Parameters* | *Controls* (n = *37*) | *HG* (n = *26*) | P-*value** |
|---|---|---|---|
| –SH, μmol l$^{-1}$ | 232.18 ± 19.21 | 213.86 ± 26.29 | $P = 0.004$ |
| –SH+–S–S–, μmol l$^{-1}$ | 259.17 ± 19.94 | 245.23 ± 28.58 | $P = 0.038$ |
| –S–S–, μmol l$^{-1}$ | 13.49 ± 2.81 | 15.68 ± 4.41 | $P = 0.031$ |
| –S–S – / – SH, % | 5.8 ± 1.3 | 7.4 ± 2.2 | $P = 0.003$ |
| –S–S – / – SH+–S–S–, % | 5.2 ± 1.0 | 6.4 ± 1.6 | $P = 0.003$ |
| –SH/–SH+–S–S–, % | 89.1 ± 2.1 | 87.1 ± 3.3 | $P = 0.003$ |

Abbreviations: HG, hyperemesis gravidarum; –SH, native thiol; –SH+–S–S–, total thiol; –S–S–, disulfide Results were given as mean ± s.d. **P* value < 0.05 considered significant.

## DISCUSSION

Redox modulator system controls normal cellular functions. Exchanges in state of redox pairs influence the protein structure, function and interactions. Various sulfur forms with several functional groups are found in many oxidation states, such as thiols, disulfides, sulfinic, sulphenic and sulfonic acids and so on. These distinct forms participate in sulfur-based redox status.[29] Cysteine and cystine compose the major thiol-disulfide pair in human blood plasma. As a result cysteine has not only structural functions, but also can take roles in redox systems, such as thiol-disulfide exchange.[30] Like cysteine disulfides are members of the thiol-based regulatory redox system.[31]

Dynamic thiol-disulfide homeostasis has a critical role in the organism. Changes in the thiol-disulfide balance serve as components for antioxidant protection, detoxification, regulation of enzymatic activity and cellular signaling mechanisms.[23,24] Changes in thiol-disulfide homeostasis have been associated with various

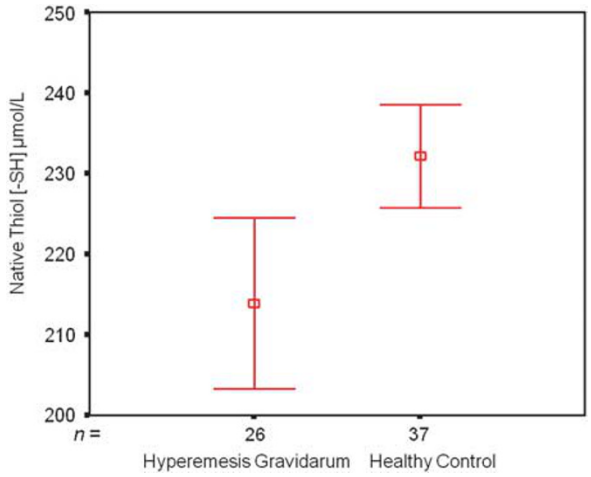


**Figure 1.** Serum native thiol levels ($X \pm \sigma_M$) in healthy controls and patients with HG. HG, hyperemesis gravidarum.

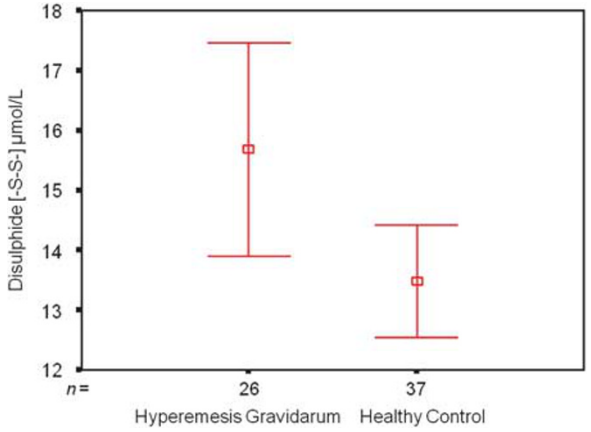


**Figure 2.** Serum disulfide levels ($X \pm \sigma_M$) of study groups.

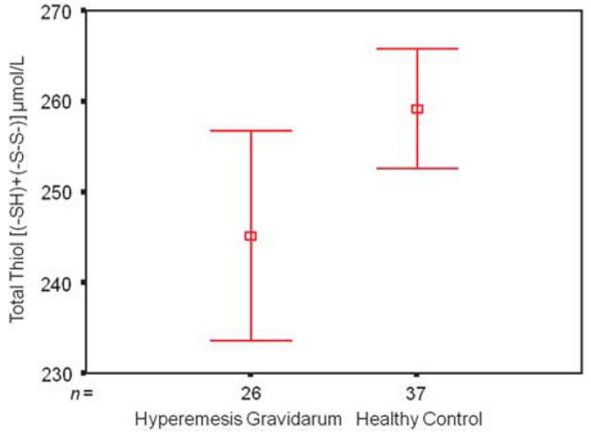


**Figure 3.** Serum total thiol levels ($X \pm \sigma_M$) in pregnant women with HG and healthy controls. HG, hyperemesis gravidarum.

diseases, such as diabetes mellitus, cancer, chronic kidney disease and liver disorders.[32–34]

Etiopathogenesis of HG remains unclear. Information on the physiopathological mechanisms is limited.[35] To the best of our knowledge, no report related to thiol-disulfide homeostasis in HG has been published. As seen in Table 2 and Figures 1, 2, 3 the serum native and total thiol levels were significantly lower and the disulfide levels were higher in pregnant women with HG than those of healthy pregnant women. This deficiency may originate from decreased inadequate nutritional intake despite increased demands during pregnancy. Despite the low-serum protein, albumin, native and total thiol levels, disulfide amounts were high in HG group when compared with the control group. This situation indicates that the balance has shifted to the oxidative side. The increase in the disulfide/total thiol and disulfide/native thiol ratios (–S–S–/ (–SH+–S–S–) and (–S–S–/–SH)) and the decrease in the native thiol/total thiol ratio (SH/(– SH+–S–S–)) show that the thiol-disulfide redox balance system shifted to the side of disulfide bond formation.

Fait *et al.*[10] found plasma GSH levels were significantly lower in pregnant women with HG compared with healthy pregnant women. GSH is the most abundant functional thiol compound presenting in the intracellular milieu. GSH is dominantly present in the reduced form. However, the measured plasma GSH concentration, a minor component of the plasma thiol pool, was a few micromoles. A more recently developed method shows hundred times more than that of GSH. Thus, the homeostatic status can be completely evaluated.

Onaran *et al.*[36] Yılmaz *et al.*[37] and Güney *et al.*[13] found a deficiency in the antioxidant status in HG. They speculated that HG may be associated with a lack of function in the antioxidant system. Erel showed that thiols are the largest antioxidant components of serum. [38] The diminished antioxidant status is parallel to our decreased thiol results.

Many mechanisms can lead to oxidative stress in HG such as decreased inadequate nutritional intake and increased needs during pregnancy. Celik *et al.*[11] compared a HG study group with healthy pregnant women to assess their dietary antioxidant levels. The researchers found significantly lower levels of vitamin A, E and C in women with HG.

Cysteine, which has a functional thiol group, is mostly found in high-protein food including animal and plant sources. Although classified as a non-essential amino acid, cysteine may be essential for infants, older adults and individuals with deficient nutritional intake or metabolic disease. Supplementation with SH-containing compounds may provide an adequate source to meet the metabolic needs.[39] We conclude that the substitution of thiol components should be discussed in HG.

Ketonuria indicates the catabolism of adipose storage, which can signal HG.[27,28] Ketonuria is commonly used in diagnosing HG. The relationship between severity of HG and the grade of ketonuria has been described.[27,28] There were inverse relationships between disease severity and native and total thiol amounts. There was also a positive correlation between disease severity and disulfide amounts (Table 3). On the basis of the results, supplementation of –SH-containing compounds can be discussed.

Besides the inadequacy of antioxidant system, Güney *et al.*[13] and Aksoy *et al.*[15] have found enhanced lipid peroxidation markers, which are closely related with oxidative status, in pregnant women with HG than those of the control group. These findings are compatible with our increased disulfide levels and disulfide/native thiol percent ratios and disulfide/total thiol percent ratios.

Thiol groups have a significant role in the cell by minimizing the toxic effects of oxygen-activation processes. Fundamentally sulfhydryl groups are associated with proteins. So, when thiol levels decreases in serum its antioxidant power will decrease too. In addition, protein levels of thiol-disulfide are related with other diseases processes in pregnancy. Shibata *et al.*[40] found the levels of the protein thiol-disulfide oxidoreductases were increased in pre-eclamptic placentae compared with normal placentae. Contrary to this, Llurba *et al.*[41] found serum thiol levels

**Table 3.** The relationship between the disease severity and homeostatic parameters

| | *–SH* (n = *26*) | *–SH+–S–S–*, (n = *26*) | *–S–S–*, (n = *26*) | *–S–S–/vSH* (n = *26*) | *–S–S–/–SH+–S–S–*, (n = *26*) | *–SH/–SH+–S–S–*, (n = *26*) |
|---|---|---|---|---|---|---|
| Ketone levels (1–3+) | *r* = *– 0.44*<br>*P* = *0.001* | *r* = *– 0.40*<br>*P* = *0.002* | *r* = 0.29<br>*P* = 0.027 | *r* = 0.30<br>*P* = 0.022 | *r* = 0.29<br>*P* = 0.028 | *r* = – 0.29<br>*P* = 0.028 |

Abbreviations: –SH, native thiol; –SH+–S–S–, total thiol; –S–S–, disulfide. The *r* value is the Pearson correlation coefficient. The *P* value is significant.

were significantly lower in preeclamptic patients compared with controls. Because reactive species organized near the sides of their formation, increases in the expression of protein levels of thiol-disulfide will protect the tissular oxidative damage and cannot prevent the oxidation of thiol groups in serum. Also Sahlin *et al.*[42] have reported that the mRNA levels of thioredoxin and glutaredoxin are decreased in pre-eclamptic placentae, and all Sawicki *et al.*[43] have performed proteomics on cytotrophoblast cells have found decreased thioredoxin. Likewise pre-eclampsia, Kharb[44] and Vural *et al.*[45] have found significantly lower-serum thiol levels in pregnant women with gestational diabetes mellitus compared with healthy pregnant women.

In conclusion, thiol-disulfide homeostasis is weakened in HG, and the balance shifts to the disulfide bond formation side. Substitution of thiol deficiency and correction of thiol-disulfide imbalance may be beneficial in managing treatment of the disease.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1 Niebyl JR. Clinical practice. Nausea and vomiting in pregnancy. *N Engl J Med* 2010; **363**: 1544–1550.
2 Miller F. Nause and vomiting in pregnancy: the problem of perception —is it really a disease? *Am J Obstet Gynecol* 2002; **186**(5 Suppl): 182–183.
3 Bailit JL. Hyperemesis gravidarum: epidemiologic findings from large cohort. *Am J Obstet Gynecol* 2005; **193**(3 Pt 1): 811–814.
4 Tan PC, Tan NC, Omar SZ. Effect of high levels of human chorionic gonadotropin and estradiol on the severity of hyperemesis gravidarum. *Clin Chem Lab Med* 2009; **47**(2): 165–171.
5 Tan JYL, Loh KC, Yeo GSH, Chee YC. Transient hyperthyroidism of hyperemesis gravidarum. *BJOG* 2002; **109**: 683–688.
6 Sandven I, Abdelnoor M, Nesheim BI, Melby KK. *Helicobacter pylori* infection and hyperemesis gravidarum: a systematic review and meta-analysis of case-control studies. *Acta Obstet Gynecol Scand* 2009; **88**(11): 1190–1200.
7 Tan PC, Vani S, Lim BK, Omar SZ. Anxiety and depression in hyperemesis gravidarum: prevalence, risk factors and correlation with clinical severity. *Eur J Obstet Gynecol Reprod Biol* 2010; **149**(2): 153–158.
8 Verberg MFG, Gillot DJ, Al-Fardan N, Grudzinskas JG. Hyperemesis gravidarum, a literature review. *Hum Reprod Update* 2005; **11**: 527–539.
9 Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. *Curr Opin Obstet Gynecol* 2006; **18**: 325–332.
10 Fait V, Sela S, Ophir E, Khoury S, Nissimov J, Tkach M *et al.* Hyperemesis gravidarum is associated with oxidative stress. *Am J Perinatol* 2002; **19**: 93–98.
11 Celik F, Guzel AI, Kuyumcuoglu U, Celik Y. Dietary antioxidant levels in hyperemesis gravidarum: a case control study. *Ginekol Pol* 2011; **82**(11): 840–844.
12 Van Stuijvenberg ME, Schabort I, Labadarios D, Nel JT. The nutritional status and treatment of patients with hyperemesis gravidarum. *Am J Obstet Gynecol* 1995; **172**: 1585–1591.
13 Guney M, Oral B, Mungan T. Serum lipid peroxidation and antioxidant potential levels in hyperemesis gravidarum. *Am J Perinatol* 2007; **24**: 283–289.
14 Cevrioglu AS, Altindis M, Yilmazer M, Fenkci IV, Ellidokuz E, Kose S. Efficient and non-invasive method for investigating *Helicobacter pylori* in gravida with hyperemesis gravidarum: *Helicobacter pylori* stool antigen test. *J Obstet Gynaecol Res* 2004; **30**: 136–141.
15 Aksoy H, Aksoy AN, Ozkan A, Polat H. Serum lipid profile, oxidative status, and paraoxonase 1 activity in hyperemesis gravidarum. *J Clin Lab Anal* 2009; **23**: 105–109.
16 Verit FF, Erel O, Celik H. Paraoxonase-1 activity in patients with hyperemesis gravidarum. *Redox Rep* 2008; **13**(3): 134–138.
17 Kayalı R, Çakatay U. Protein oksidasyonunun ana mekanizmaları. *Cerrahpaşa J Med* 2004; **35**(2): 83–89.
18 Sung CC, Hsu YC, Chen CC, Lin YF, Wu CC. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. *Oxid Med Cell Longev* 2013; **2013**: 301982.
19 Kumari M.V., Hiramatsu M., Ebadi M. Free radical scavenging actions of metallothionein isoforms I and II. *Free Radic Res* 1998; **29**: 93–101.
20 Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr* 2000; **72**(2 Suppl.): 653S–669SS.
21 Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med* 2013; **65**: 244–253.
22 Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med*. **15**; 2009; **47**(10): 1329–1338.
23 Circu ML. AwTY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* 2010; **48**(6): 749–762.
24 Biswas S, Chida AS, Rahman I. Redox modifications of protein–thiols: emerging roles in cell signaling. *Biochem Pharmacol* 2006; **71**(5): 551–564.
25 Ellman G, Lysko H. A precise method for the determination of plasma sulfhydryl groups. *Anal Biochem* 1979; **93**(1): 98–102.
26 Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014; **47**(18): 326–332.
27 Bashiri A, Neumann L, Maymon E, Katz M. Hyperemesis gravidarum: epidemiologic features, complications and outcome. *Eur J Obstet Gynecol Reprod Biol* 1995; **63**: 135–138.
28 Tan PC, Jacob R, Quek KF, Omar SZ. Pregnancy outcome in hyperemesis gravidarum and the effect of laboratory clinical indicators of hyperemesis severity. *J Obstet Gynaecol Res* 2007; **33**(4): 457–464.
29 Jacob C., Anwar A. The chemistry behind redox regulation with a focus on sulphur redox systems. *Physiol Plant* 2008; **133**: 469–480.
30 Hansen RE, Østergaard H, Winther JR. Increasing the reactivity of an artificial dithioldisulfide pair through modification of the electrostatic milieu. *Biochemistry* 2005; **44**: 5899–5906.
31 Wouters MA, George RA, Haworth NL. 'Forbidden' disulfides: their role as redox switches. *Curr Protein Pept Sci* 2007; **8**: 484–495.
32 Matteucci E, Giampietro O. Thiol signalling network with an eye to diabetes. *Molecules* 2010; **15**(12): 8890–8903.
33 Prabhu A, Sarcar B, Kahali S, Yuan Z, Johnson JJ, Adam KP *et al.* Cysteine catabolism:a novel metabolic pathway contributing to glioblastoma growth. *Cancer Res* 2014; **4**(3): 787–796.
34 Rodrigues SD, Batista GB, Ingberman M, Pecoits-Filho R, Nakao LS. Plasma cysteine/cystine reduction potential correlates with plasma creatinine levels in chronic kidney disease. *Blood Purif* 2012; **34**(3–4): 231–237.
35 Quinlan JD, Hill DA. Nausea and vomiting of pregnancy. *Am Fam Physician* 2003; **68**: 121–128.
36 Onaran Y, Kafali H, Duvan Cİ, Keskin E, Celik H, Erel O. Relationship between oxidant and antioxidant activity in hyperemesis gravidarum. *J Matern Fetal Neonatal Med* 2014; **27**(8): 825–828.
37 Yilmaz S, Ozgu-Erdinc AS, Demirtas C, Ozturk G, Erkaya S, Uygur D. The oxidative stress index increases among patients with hyperemesis gravidarum but not in normal pregnancies. *Redox Rep* 2014; **20**: 97–102.
38 Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004; **37**: 277–285.
39 Ingenbleek Y. The nutritional relationship linking sulfur to nitrogen in living organisms. *J Nutr* 2006; **136**: 1641S–1651S.
40 Shibata E, Ejima K, Nanri H, Toki N, Koyama C, Ikeda M. *et al.* Enhanced protein levels of protein thiol/disulphide oxidoreductases in placentae from preeclamptic subjects. *Placenta* 2001; **22**: 566–572.
41 Llurba E, Grataco E, Martın-Gallan P, Cabero L, Domınguez C. A comprehensıve study of oxıdatıve stress and antıoxıdant status ın preeclampsıa and normal pregnancy. *Free Radıcal Bıo Med* 2004; **37**: 557–570.

42 Sahlin L, Ostlund E, Wang H, Holmgren A, Fried G. Decreased expression of thioredoxin and glutaredoxin in placentas from pregnancies with pre-eclampsia and ıntrauterine growth restriction. *Placenta* 2000; **21**: 603–609.
43 Sawicki G, Dakour J, Morrish DW. Functional proteomics of neurokinin B in the placenta indicates a novel role in regulating cytotrophoblast antioxidant defences. *Proteomics* 2003; **3**: 2044–2051.
44 Kharb S. Total radical-trapping antioxidant potential in gestational diabetes. *Int J Gynaecol Obstet* 2008; **103**: 257–258.
45 Vural M, Camuzcuoglu H, Toy H, Cece H, Aydin H, Eren MA *et al*. Evaluation of the future atherosclerotic heart disease with oxidative stress and carotid artery intima media thickness in gestational diabetes mellitus. *Endocr Res* 2012; **37**(3): 145–153.