Relation of inflammatory markers with both presence and severity of hyperemesis gravidarum

Związek pomiędzy markerami stanu zapalnego a obecnością i nasileniem wymiotów ciężarnych

Raziye Keskin Kurt, Ayşe Güler, Dilek Benk Silfeler, Mustafa Doğan Özçil, Atilla Karateke, Ali Ulvi Hakverdi,

Mustafa Kemal University Medical School, Department of Obstetrics and Gynecology, Hatay, Turkey

Abstract

Objectives: *The aim of our study is to determine the newly introduced systemic inflammation marker, neutrophil lymphocyte ratio (NLR) in hyperemesis gravidarum (HG) patients and to investigate the association between severity of the disease and NLR.*

Method: *The study population consisted of 55 pregnant patients with HG and 50 pregnant women without complaints matched for gestational age as a control group. The HG patients were grouped as mild (n=16), moderate (n=19) and severe (n=20) according to Modified Pregnancy- Unique Quantification of Emesis and Nausea Scoring Index Questionnaire. Furthermore, hsCRP, neutrophils, lymphocytes, and NLR were evaluated with complete blood count.*

Results: *The HG group had significantly higher NLR values compared to the control group (2.69±1.81 vs 1.97±1.34, p=0.004). HsCRP levels were significantly higher among HG patients compared to the control group (1.95±2.2 vs 0.56±0.30, p<0.001). The subgroup analysis revealed statistically significant increases in NLR and hsCRP values with increased HG severity (p<0.001, p=0.002). The correlation analysis demonstrated a strong correlation between NLR and hsCRP levels (r: 0.703, p<0.001).*

Conclusion: *Our study results showed that NLR and hsCRP levels are increased in HG disease compared to gestational age matched control group subjects. Furthermore, NLR and hsCRP values are correlated with severity of disease. NLR could be used as a marker for both presence and severity of hyperemesis gravidarum.*

Key words: **hyperemesis gravidarum** / **inflammation** / **neutrophil to lymphocyte ratio** / / **hsCRPl** /

Streszczenie

Cel pracy: *Celem pracy była ocena nowo wprowadzonych markerów stanu zapalnego, wskaźnika neutrofili do limfocytów (NLR) u ciężarnych z wymiotami (HG) oraz zbadanie powiązań między ciężkością choroby a NLR.*

Corresponding author:
Raziye Keskin Kurt
Mustafa Kemal Üniversitesi Tıp Fakültesi,
Kadın Hastalıkları ve Doğum Anabilim Dalı, 31100, Hatay, TURKEY
ph./fax: +90 326 245 51 14, +90 326 245 53 57
e-mail: drraziyekeskinkurt@yahoo.com

Otrzymano: **12.12.2013**
Zaakceptowano do druku: **02.03.2014**

Metoda: *Grupę badaną stanowiło 55 ciężarnych z HG i 50 ciężarnych bez dolegliwości, jako grupa kontrolna dopasowana do grupy badanej pod względem wieku ciążowego. Pacjentki z wymiotami zostały podzielone na podgrupy w zależności od nasilenia objawów, tj. łagodne (n=16), średnie (n=19) i ciężkie (n=20), na podstawie zmodyfikowanego kwestionariusza oceny nudności i wymiotów w ciąży. Następnie oznaczono w krwi pełnej hsCRP, neutrofile, limfocyty i NLR.*

Wyniki: *Pacjentki z wymiotami miały znacząco wyższe wartości NLR w porównaniu do grupy kontrolnej (2,69±1,81 vs 1,97±1,34, p=0,004). Poziom hsCRP był istotnie wyższy w grupie HG w porównaniu do grupy kontrolnej (1,95±2,2 vs 0,56±0,30, p<0,001). Analiza w obrębie podgrup HG wykazała istotny wzrost wartości NRL i hsCRP wraz ze wzrostem nasilenia wymiotów (p<0,001, p=0,002). Dalsza analiza wykazała silną korelację pomiędzy poziomem NLR a hsCRP (r:0,703, p<0,001).*

Wnioski: *nasze badanie pokazuje, że poziom NLR i hsCRP jest zwiększony u pacjentek z wymiotami ciążowymi w porównaniu do zdrowych ciężarnych w tych samych tygodniach ciąży. Co więcej, NLR i hsCRP korelują z nasileniem choroby. NLR może być markerem obecności i ciężkości wymiotów ciężarnych.*

Słowa kluczowe: **wymioty ciężarnych** / **zapalenie** / **wskaźnik neutrofile** / / **limfocyty** / **hsCRP** /

Introduction

Most pregnant women experience nausea and vomiting during their pregnancy by a rate of 80% [1]. A more severe form of nausea and vomiting, called hyperemesis gravidarum (HG) is a state characterized by intractable vomiting during pregnancy, leading to dehydration, ketonemia, electrolyte imbalance and weight loss [2, 3]. HG incidence is reported as 0.5-2% in many researches but it varies with ethnicity [4]. In generally, HG is seen in between 8. and 12. weeks of pregnancy while 5% of cases persists throughout pregnancy [5].

Although the exact etiopathogenesis of HG is not known, there are many proposed mechanisms such as metabolic and hormonal changes, gastrointestinal dysmotility, psychological and immunological factors [6-9]. Throughout pregnancy some immunological changes occur to protect fetus and decidua from maternal immune system. If this physiological immune response changes, diseases related with pregnancy arise. It is suggested that HG originate from overactive immune response [10]. Inflammation is believed to play a considerable role in pathophysiologic mechanism of HG [11, 12]. Cytokines which are mediators of inflammation such as IL-6, TNF-α as well as CRP have been found to be increased in HG patients [9, 12, 13].

Neutrophil to lymphocyte ratio (NLR), easily available marker, is a newly introduced systemic inflammation marker that has been a strong prognostic factor in many diseases and most of cancers such as breast, kidney, colorectal and lung cancers [14-17]. It has been shown that NLR is superior to white blood cells in the prediction of adverse outcomes in patients with cardiovascular diseases and malignancies. NLR was found to positively correlated with inflammatory markers including tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 and CRP in many diseases [18].

The aim

The aim of our study is to determine the newly introduced systemic inflammation marker, NLR in HG patients and to investigate the association between severity of the disease and NLR.

Methods

The study population consisted of 55 pregnant patients with HG and 50 pregnant women without complaints matched for gestational age as a control group. The criteria for HG were severe vomiting (more than 2 times per day), weight loss more than 5% of body weight and the presence of at least one positive ketonuria. Patients with multiple gestation, urinary tract infection, gastrointestinal and thyroid disease, psychological disorders, BMI>35kg/m², diabetes mellitus were excluded from study. The study was approved by the ethics committee and informed consent was obtained from all participants.

All the patients filled out the 3-question Modified Pregnancy-Unique Quantification of Emesis and Nausea(PUQE) Scoring Index Questionnaire which quantifies nausea, vomiting, retching for the first trimester [19].

Total scores were calculated in between 4-15 points for HG patients. The HG patients were grouped as mild (≤6 points) (n=16), moderate (7-12) points) (n=19) and severe (≥ 13 points) (n=20) (Table I).

Gravity, parity of each participant was recorded. BMI was calculated as weight in kilograms divided by the square of the height in meters. Gestational age was determined using the first date of last menstrual period and confirmed by ultrasonography. Urine analysis for ketones was done for detection of starvation ketosis.

All blood samples were taken in the morning between 08:00 and 09:00 after overnight fasting. Blood samples were taken from antecubital vein with a 20 gauge needle. Complete blood counts with automated differential counts, which included total WBCs, neutrophils lymphocytes were measured using a Coulter LH 780 Hematology Analyzer (BeckmanCoulterIrelandInc, Mervue, Galway, Ireland). NLR was calculated as the ratio of the neutrophil to lymphocyte count.

High-sensitivity C-reactive protein (hs-CRP) was measured by using a BN2 model nephlometer (Dade-Behring, Marburg, Germany).

Table I. Modified Pregnancy- Unique Quantification of Emesis and Nausea Scoring Index Questionnaire.
Circle the answer that best suits your situation from the beginning of your pregnancy.

1. On average in a day, for how long do you feel nauseated or sick to your stomach?				
Not at all	1 hr	2-3 hr	4-6 hr	6 hr
(1)	(2)	(3)	(4)	(5)
2. On average in a day, how many times do you vomit or thrown up?				
7 times	5-6 times	3-4 times	1-2 times	I did not throw up
(5)	(4)	(3)	(2)	(1)
3. On average in a day, how many times do you have retching or dry heaves without bringing anything up?				
None	1-2 times	3-4 times	5-6 times	7 times
(1)	(2)	(3)	(4)	(5)

Total score (sum of replies to 1, 2, and 3): mild NVP, _6; moderate NVP, 7-12; severe NVP, _13.
NVP: nausea and vomiting of pregnancy

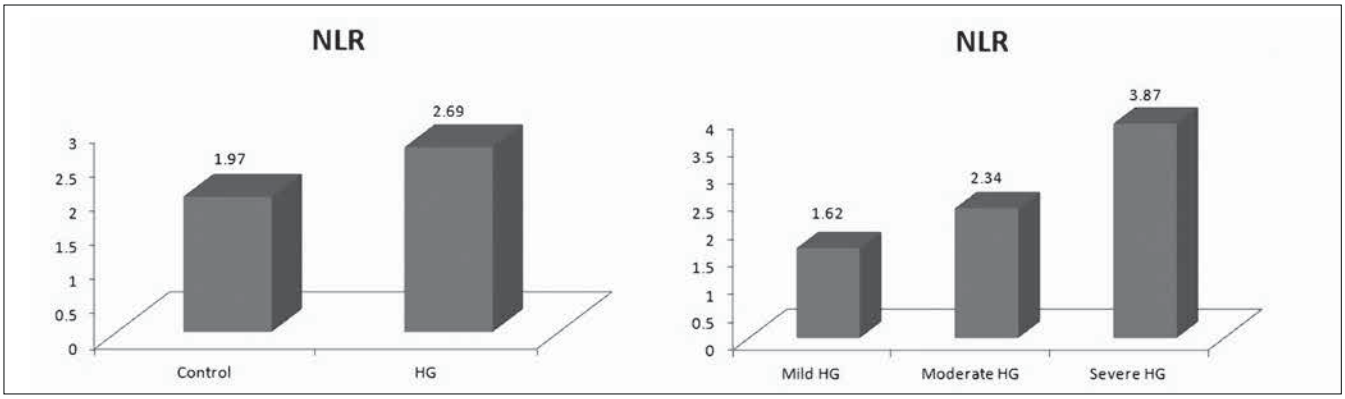

Figure 1. Comparison of NLR levels in the control and HG subgroups.

Statistics

Numerical variables were expressed as mean ± standard deviation, and categorical variables as percentage. Numerical variables with normal distribution were compared using student's t-test for the paired groups and one-way ANOVA (analysis of variance) for triplet groups while numerical variables with abnormal distribution were compared using Mann Whitney-U test for the paired groups and Kruskal-Wallis test for triplet groups. Tukey's test was chosen as the post-hoc test for ANOVA. Categorical variables were evaluated using chi-square test. Normality of distribution was assessed with Kolmogorov Smirnov test for numerical variables. The correlation between variables was evaluated using Pearson's correlation test. SPSS 20.0 package software (SPSS inch, Chicago, Illinois, USA) was used for the statistical analysis.

Results

Mean age was 29±2.8 years among the patients with HG included in the study whereas it was 30±3.1 years for the control group. BMI was 23.5±3.1 in the HG group and 24.3±3.3 in the control group. No statistically significant difference was found between the patients and the control group regarding age, parity, gestational age and BMI. Baseline characteristics and biochemical values are summarized in Table II.

HsCRP levels were significantly higher among HG patients compared to the control group (1.95±2.2 vs 0.56±0.30, p<0.001).

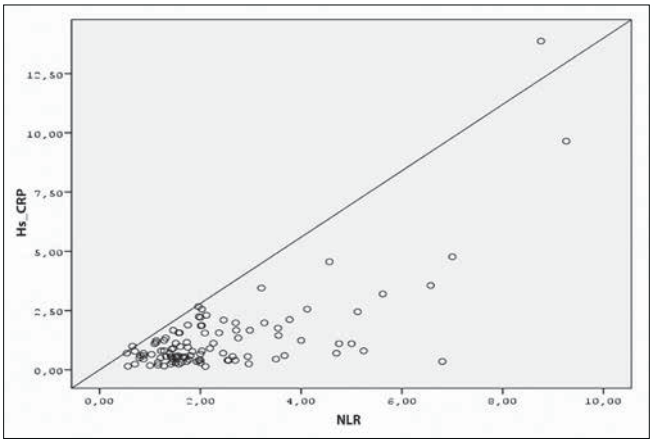

Figure 2. The correlation of NLR and hs-CRP in the population.

The HG group had significantly higher NLR values compared to the control group (2.69±1.81 vs 1.97±1.34, p=0.004) (Figure 1). The subgroup analysis revealed statistically significant increases in NLR and hsCRP values with increased HG severity (p<0.001, p=0.002, respectively) (Table III). The correlation analysis demonstrated a strong correlation between NLR and hsCRP levels (r: 0.703, p<0.001) (Figure 2).

Table II. Baseline clinical and laboratory characteristics of patient and control groups.

	HG patients (n:55)	Control (n:50)	P value
Age (years)	29+2.8	30±3.1	0,092
BMI (kg/m²)	23.5+3.1	24.3±3.3	0.194
Gestational Age (weeks)	9.4+3	8.8+2.5	0.413
Parity	2.1±0.5	2.4±0.6	0.441
Hscrp (mg/l)	1.95±2.2	0.56±0.30	**<0.001**
Leukocyte (x1000/ mm³)	7.58±1.95	6.32±1.33	**0.001**
Neutrophil (x1000/ mm³)	4.78±1.45	3.68±1.30	**<0.001**
Lymphocyte (x1000/ mm³)	2.12±0.66	2.3±0.81	0.213
NLR	2.69±1.81	1.97±1.34	**0.004**

BMI: body mass index, NLR: neutrophil to lymphocyte ratio, HG: hyperemesis gravidarum

Table III. Clinic and laboratory findings according to the of patient subgroups.

	Mild (n:16)	Moderate (n:19)	Severe (n:20)	P value
Age (years)	29.8±3.4	28.7±2.9	28.8±2.2	0.472
BMI (kg/m²)	22.4±2.4	23.8±3.2	24.1±3.1	0.254
Gestational Age (weeks)	9.0±0.5	9.8±3.3	9.3±2.2	0.710
Hscrp (mg/l)	0.96±0.44	1.52±0.82	3.15±3.24	**0.005**
Leukocyte (x1000/ mm³)	7.0±2.1	7.8±1.76	7.9±1.9	0.355
Neutrophil (x1000/ mm³)	3.45±0.63	4.72±0.99	5.89±1.4	**<0.001**
Lymphocyte(x1000/ mm³)	2.26±0.52	2.29±0.66	1.85±0.71	0.076
NLR	1.62±0.58	2.34±1.23	3.87±2.26	**<0.001**

BMI: body mass index, NLR: neutrophil to lymphocyte ratio

Discussion

Our study results showed for the first time in the literature that NLR and hsCRP levels are increased in patients with HG compared to control subjects matched for gestational age. Furthermore, NLR and hsCRP values are correlated with severity of the disease.

HG is a condition that adversely affects quality of life in pregnant women [20]. Severe nausea and vomiting is the 3rd most common cause of hospitalization during pregnancy [21]. Nausea and vomiting seen during the first trimester causes serious workforce loss among women [22]. In addition to the morbidity, HG may rarely lead to conditions with high mortality risk such as Wernicke's encephalopathy and central pontine myelinolysis [23]. It is therefore important to provide appropriate treatment; however, the current treatment of HG is empirical and suboptimal as the exact etiology remains unknown. Several mechanisms have been suggested to be involved in the etiopathogenesis of HG; however, none of them alone has provided a full explanation and the etiology therefore appears to be multifactorial [24].

Inflammation is thought to be playing an important role in the pathophysiological mechanism of HG [11, 12]. Yoneyama et al. demonstrated increased TNF-α levels in HG [25]. Besides Kaplan et al. revealed that TNF-α, a biologically active cytokine involved in immune disorders, may be associated with the pathogenesis and progression of hyperemesis gravidarum [13]. In addition, Kuscu et al. have shown increased levels of the inflammation marker, IL-6 in hyperemesis. They proposed that immunological activity seen in patients with HG and the dramatic response to short-term treatment with anti-inflammatory drugs (steroid therapy) may be the clues to consider HG as an inflammatory response during pregnancy [9]. Verit et al. found decreased levels of paraoxonase-1 in patients with HG and correlated this finding to increased oxidative stress and inflammation in HG, and they also demonstrated increased levels of the inflammatory marker, hsCRP in patients with HG [12]. Engin-ustun et al. showed increased CRP and vaspin levels in patients with HG and associated this finding with inflammation [11]. In the present study, the inflammation markers, hsCRP and NLR were found to be increased, supporting the role of inflammation in HG etiology. Furthermore, we for the first time in literature showed that hsCRP and NLR levels increase proportionally with the severity of HG.

NLR is a marker that has been recently shown to be associated with inflammation in a number of diseases [26]. Neutrophilia and lymphocytopenia are physiological responses of the immune system to various conditions including systemic inflammation and malignancy. Cho S et al. found increased NLR levels in endometriosis which is a chronic condition associated with inflammation, and highlighted that NLR may be a simple and readily available marker for the diagnosis of endometriosis [27]. Cho et al. showed increased NLR levels in epithelial ovarian cancer as a measure of the systemic inflammatory response, and demonstrated further increases in NLR with increasing stages of the disease [28]. Kim et al. showed superiority of NLR plus cervix length combination to cervix length alone in

predicting pre-term pregnancy, which is an obstetric condition associated with inflammation [29]. Granulocyte levels increase while lymphocyte levels decrease in later stages of pregnancy. Minagawa et al. found significantly increased granulocyte levels in HG, and showed that lymphocyte levels were decreased compared to normal pregnancy although the difference was not statistically significant [10]. Similar to the findings of Minagawa et al., we found increased neutrophil levels in the present study, and we for the first time showed that the inflammation marker, NLR also increases in HG.

Conclusion

In conclusion, NLR could be used as a marker for both presence and severity of hyperemesis gravidarum. HG patients have increased levels of hsCRP and NLR, indicating that inflammation may play an important role in the pathogenesis of the disease. Furthermore, NLR and CRP levels increase even further with the increasing severity of the disease. Although the association between HG and NLR remains unclear, we believe that the activation of inflammation associated with HG lead to increased NLR values. Nevertheless, further studies are required in order to fully understand the exact pathogenesis of HG.

NLR could be a marker in hyperemesis gravidarum.

Limitations

One of the major limitations of the present study is the relatively low number of patients. Additionally, hsCRP and NLR have been assessed as inflammatory markers whereas TNF-alpha and interleukin levels were not determined in the present study. Furthermore, it remains unclear whether the increase in inflammatory marker levels observed in HG is the underlying cause of the condition, or a result of the compensatory mechanism in response to HG [13]. The possibility that it may be a mere effect of disturbances connected with emesis and not its cause, needs to be taken into consideration as well.

Authors' Contribution

1. Raziye Keskin Kurt – concept, analysis and interpretation of data, article draft, corresponding author.
2. Ayşe Güler – concept, analysis and interpretation of data, acquisition of data, assumptions.
3. Dilek Benk Silfeler – study design, acquisition of data, article draft, analysis and interpretation of data.
4. Mustafa Doğan Özçil – concept, analysis and interpretation of data, acquisition of data.
5. Atilla Karateke – revised article critically, acquisition of data, study design, article draft.
6. Ali Ulvi Hakverdi – revised article critically, analysis and interpretation of data, article draft.

Authors' statement

This is to certify, that the publication will not violate the copyrights of a third party, as understood according to the Act in the matter of copyright and related rights of 14 February 1994, Official Journal 2006, No. 90, Clause 63, with respect to the text, data, tables and illustrations (graphs, figures, photographs);

there is no 'conflict of interests' which occurs when the author remains in a financial or personal relationship which unjustly affects his/her actions associated with the publication of the manuscript;

any possible relationship(s) of the author(s) with the party/parties interested in the publication of the manuscript are revealed in the text of the article;

the manuscript has not been published in or submitted to any other journal.

Source of financing:

The present study has no funding.

References

1. Gadsby R, Barnie-Adshead AM, Jagger C. A prospective study of nausea and vomiting during pregnancy. Br J Gen Pract. 1993;43, 245-248.
2. Fairweather DV. Nausea and vomiting in pregnancy. Am J Obstet Gynecol. 1968, 102, 135-175.
3. Celik F, Guzel AI, Kuyumcuoglu U, Celik Y. Dietary antioxidant levels in hyperemesis gravidarum: a case control study. Ginekol Pol. 2011, 82, 840-844.
4. Kallen B. Hyperemesis during pregnancy and delivery outcome: a registry study. Eur J Obstet Gynecol Reprod Biol. 1987, 26, 291-302.
5. Sherman PW, Flaxman SM. Nausea and vomiting of pregnancy in an evolutionary perspective. Am J Obstet Gynecol. 2002, 186, 190-197.
6. Goodwin TM, Hershman JM, Cole L. Increased concentration of the free beta-subunit of human chorionic gonadotropin in hyperemesis gravidarum. Acta obstetriObstet Gynecol Scand. 1994, 73, 770-772.
7. Baron TH, Ramirez B, Richter JE. Gastrointestinal motility disorders during pregnancy. Ann Intern Med. 1993, 118, 366-375.
8. Katon WJ, Ries RK, Bokan JA, Kleinman A. Hyperemesis gravidarum: a biopsychosocial perspective. Int J Psychiatry Med. 1980, 10, 151-162.
9. Kuscu NK, Yildirim Y, Koyuncu F, [et al.]. Interleukin-6 levels in hyperemesis gravidarum. Arch Gynecol Obstet. 2003, 269,13-15.
10. Minagawa M, Narita J, Tada T, [et al.]. Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. Cell Immunol. 1999, 196, 1-13.
11. Engin-Ustun Y, Tonguc E, Var T, [et al.]. Vaspin and C-reactive protein levels in hyperemesis gravidarum. Eur Rev Med Pharmacol Sci. 2013, 17, 138-140.
12. Verit FF, Erel O, Celik H: Paraoxonase-1 activity in patients with hyperemesis gravidarum (communications in free radical research). Redox Rep. 2008, 13, 134-138.
13. Kaplan PB, Gucer F, Sayin NC, [et al.]. Maternal serum cytokine levels in women with hyperemesis gravidarum in the first trimester of pregnancy. Fertil Steril. 2003, 79, 498-502.
14. Kaya A, Kaya Y, Topcu S, [et al.]. Neutrophil-to-Lymphocyte Ratio Predicts Contrast-Induced Nephropathy in Patients Undergoing Primary Percutaneous Coronary Intervention. Angiology. 2014, 65 (1), 51-56.
15. Walsh SR, Cook EJ, Goulder F, [et al.]. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol. 2005, 91, 181-184.
16. Azab B, Bhatt VR, Phookan J, [et al.]. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol. 2012, 19, 217-224.
17. Forget P, Machiels JP, Coulie PG, [et al.]. Neutrophil:Lymphocyte Ratio and Intraoperative Use of Ketorolac or Diclofenac are Prognostic Factors in Different Cohorts of Patients Undergoing Breast, Lung, and Kidney Cancer Surgery. Ann Surg Oncol. 2013, 20 (3), 650-660.
18. Turkmen K, Guney I, Yerlikaya FH, Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail. 2012, 34, 155-159.
19. Lacasse A, Rey E, Ferreira E, [et al.]. Validity of a modified Pregnancy-Unique Quantification of Emesis and Nausea (PUQE) scoring index to assess severity of nausea and vomiting of pregnancy. Am J Obstet Gynecol. 2008, 198, 71-77.
20. Lacasse A, Rey E, Ferreira E, [et al.]. Nausea and vomiting of pregnancy: what about quality of life? BJOG. Inter J Obstet Gynaecol. 2008, 115, 1484-1493.
21. Bennett TA, Kotelchuck M, Cox CE, [et al.]. Pregnancy-associated hospitalizations in the United States in 1991 and 1992: a comprehensive view of maternal morbidity. Am J Obstet Gynecol. 1998, 178, 346-354.
22. Mazzotta P, Maltepe C, Navioz Y, [et al.]. Attitudes, management and consequences of nausea and vomiting of pregnancy in the United States and Canada. Int J Gynaecol Obstet. 2000, 70, 359-365.
23. Netravathi M, Sinha S, Taly AB, [et al.]. Hyperemesis-gravidarum-induced Wernicke's encephalopathy: serial clinical, electrophysiological and MR imaging observations. JouJ Neurol Sci. 2009, 284, 214-216.
24. Sanu O, Lamont RF. Hyperemesis gravidarum: pathogenesis and the use of antiemetic agents. Exp Opin Pharmacother. 2011, 12, 737-748.
25. Yoneyama Y, Suzuki S, Sawa R, Araki T. Plasma adenosine concentrations increase in women with hyperemesis gravidarum. Clin Chem Acta. 2004, 342, 99-103.
26. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. Bratis Lek Listy. 2001, 102 (1), 5-14.
27. Cho S, Cho H, Nam A, [et al.]. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil Steril. 2008, 90, 2073-2079.
28. Cho H, Hur HW, Kim SW, [et al.]. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. Cancer Immunol Immunother. 2009, 58, 15-23.
29. Kim MA, Lee BS, Park YW, Seo K. Serum markers for prediction of spontaneous preterm delivery in preterm labour. Eur J Clin Invest. 2011, 4, 773-780.