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Role of mean platelet volume and neutrophil/lymphocyte ratio to predict single-dose methotrexate treatment success in tubal ectopic pregnancy

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Summary

Objective: The aim of the study was to evaluate the value of mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) to predict single-dose methotrexate (MTX) treatment success in ectopic pregnancy (EP). *Materials and Methods:* A total of 115 EP diagnosed and hemodynamically stable women were enrolled in the study and divided into two groups as group 1, the treatment success group (n = 78) and group 2, the treatment failure group (n = 37). The authors compared the groups in terms of MPV and NLR. *Results:* MPV and NLR levels were higher in MTX treatment successful group than in failure group.The cut-off values of MPV and NLR were determined as 10.1 fL and 1.82, respectively. These cut off values showed similar sensitivity and specificity in prediction of MTX treatment success. *Conclusion:* MPV and NLR can be used as reliable markers to predict single-dose MTX treatment success however further studies are needed.

Key words: Mean platelet volume; Neutrophil/lymphocyte ratio; Ectopic pregnancy.

Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and is responsible for pregnancy-related deaths in the first trimester [1]. Methotrexate (MTX) is the most popular agent for the treatment of EP worldwide. There are a greater percentage of women treated medically with single dose (50 mg/m^2) MTX which is a folate antagonist and acts on rapidly dividing cells at the implantation site, most notably trophoblast cells [2].

Over the years, many putative markers to predict MTX treatment success have been described in the literature but none of them could reach sufficient achievement, therefore researchers have been attempting to find more effective and sensitive markers to predict MTX treatment success.[3] Recent studies have highlighted that some inflammatory cytokines are increased both at the implantation site and systemic circulation in tubal ectopic pregnancies which play role in angiogenesis, inflammation, and immunity [4].

Neutrophil/lymphocyte ratio (NLR) is a inflammatory response marker in peripheral blood. NLR possesses diagnostic value in certain pathologies characterised by systemic or local inflammatory response, such as diabetes mellitus, coronary artery disease, ulcerative colitis, and inflammatory arthritis [5-7].

Mean platelet volume (MPV) is a marker of platelet count and platelet activity. Platelets have also been suggested to play important roles in immune and/or inflammatory processes [8]. MPV levels are higher in inflammatory conditions such as active rheumatoid arthritis, acute attack of familial Mediterranean fever, and active chronic obstructive pulmonary disease [9].

In tubal EP, either ruptured or not, inflammation occurs in the microenvironment of the fallopian tube which may effect inflammation markers [10]. In this trial the authors aimed to evaluate the value MPV and NLR to predict single-dose MTX treatment success in EP.

Materials and Methods

A total of 115 EPs diagnosed and hemodynamically stable women in Umraniye Medical and Research Hospital between 2011-2014 were enrolled in this retrospective study. The ethical approval was taken from Institutional Ethics Committee.

The diagnosis of EP was conducted in two ways, namely, by direct visualization of extra uterine gestational sac or by the occurrence of persistent/rising b-hCG values without intrauterine evidence of pregnancy by sonography or histology.

Patients with chronic liver disease, pre-existing blood dyscrasias, pulmonary disease, peptic ulcer disease and immunodeficiency, who had sensitivity to MTX, had an intrauterine pregnancy, an unruptured mass of > 3.5 cm, fetal cardiac activity detected, and a quantitative hCG level of > 15,000 mIU/ml were excluded from the study.Moreover the authors also excluded patients with non-adnexal EP, incomplete records, patients who were lost to follow-up evaluation and who had other infections, inflammatory disease, type 2 diabetes mellitus, prediabetes, smoking, hypertension, hypercholesterolemia, obesity, coronary heart

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disease, metabolic syndrome, stating and some antihypertensive use, and atrial fibrillation.

In this paper, the authors included the patients who received single-dose MTX treatment, had complete medical records that fulfilled the aforementioned criteria, and had days 0, 4, and 7 b-hCG values appropriately recorded after MTX administration.

Before any medication, five to seven cc of peripheral venous blood was collected into sterile ethylenediaminetetraacetic acid (EDTA) tubes from all patients. Hematological parameters were analysed within 30 minutes after collection for minimizing the effect of EDTA on platelets by using a haematology analyser. Leucocyte (/mm^3), neutrophil (/mm^3), lymphocyte (/mm^3), and platelet (/mm^3) counts were recorded. NLR was calculated using the results of these parameters. Haemoglobin levels (g/dL) and MPV(fL) were determined. MTX treatment was decided as successful in patients who had a 15% decline of b-hCG levels between the fourth and seventh days of treatment.

To evaluate the efficacy of MTX treatment, the study population was divided into two groups; namely group 1, the treatment success group (n =78) and group 2, the treatment failure group (n =37) and were compared in terms of MPV and NLR.

Statistical analyses

Statistical analysis was performed with SPSS 12.0 software. Data are expressed as mean ± standard deviation. Chi-square or Fisher's Exact test was used to compare categorical variables, and the Wilcoxon Rank Sum test was used to compare continuous variables. A p value < 0.05 was considered statistically significant. Pearson correlation test was also used.

Results

The mean age of the group 1 was 30 ± 4.4 years and the mean age of the group 2 was 28 ± 5.9 years. There was no significant difference among groups regarding the mean age ($p > 0.05$) The mean hemoglobin levels were 11± 1.2 g/ dl and 10 ± 2.6 g/dl in groups 1 and 2, respectively ($p > 0.05$).

Prediction of MTX treatment success with b-hCG

The mean day 1 b-hCG levels of group 1 was 1,348 mIU/ml and the mean day 1 b-hCG levels of group 2 was 3,960 mIU/ml. The mean initial b-hCG level was significantly lower in the treatment success group than in the treatment failure group ($p < 0.05$). The b-hCG levels increased between days 0 and 4 in 53.9 % of cases (62/115); b-hCG levels decreased between days 0 and 4 in 46 % of cases (53/115). The number of cases with decreasing b-hCG level on day 4 was significantly more in the treatment success group than the failure group (36 % *vs.* 14.9 %, respectively, $p < 0.05$) (Table 1).

Prediction of MTX treatment success with MPV

Platelet values and MPV of the groups are shown in Table 2. There were no statistically difference detected between groups in terms of platelet counts (264 ± 75.2 /mm^3 *vs.* 273 ± 65 /mm^3, respectively). In group 1 the MPV levels were statistically higher than in group 2 (9.1 ± 0.4 fL *vs.*11.5 ± 0.7 fL, $p < 0.05$). Optimal cut off value was obtained at a level of 10.1 fL with 62% sensitivity and 66% specificity, respectively.

Table 1. — *MTX treatment success prediction with b-hCG levels.*

	Group 1 (MTX treatment successful) (n=78)	Group 2 (MTX treatment failure) (n= 37)
Hemoglobin (mg/dL)	11 ± 1.2	10 ± 2.6
Day 1 b-hCG (mIU/ml)	1348	3960
Declining of b-hCG values between days 1 and 4 (%)	36	14.9

Table 2. — *Comparison of MPV, NRL, and platelet counts between groups.*

	Group 1 (MTX treatment successful) (n=78)	Group 2 (MTX treatment failure) (n= 37)
Platelet count /mm^3	264 ± 75.2	273 ± 65
MPV (fL)	11.5 ± 0.7	9.1 ± 0.4
NRL	2.14 ± 0.04	1.76 ± 0.1

Prediction of MTX treatment success with NLR

NLR was calculated and assessed in both the groups, and it was found that NLR was higher in MTX treatment successful group than in failure group (2.14 ± 0.04 *vs.* 1.76 ± 0.1, $p < 0.05$) (Table 2). Optimal cut off value was obtained at a level of 1.82 with 59% sensitivity and 64% specificity, respectively.

Discussion

Single-dose MTX regimen constitutes a safe and effective treatment modality for tubal EP in selected patients.In the literature there are many studies reporting success rates of single-dose MTX administration varying from 52% to 94% among patients with asymptomatic EP [11]; however which patients will benefit from single-dose MTX treatment is still controversial.This study investigated the value of MPV and NLR to predict single-dose MTX treatment success in EP. The main finding of this study was higher MPV and NLR levels in tubal EPs may be used as predictors of MTX treatment success.

There are many suggested predictors for MTX treatment failure which include high b-hCG concentration (5,000 IU/ml), fetal cardiac activity, larger ectopic size (four cm), and sonographic evidence of free peritoneal fluid [3]. In another study, it is suggested that b-hCG levels, alone or combined with serum progesterone levels, have an important predictive value [12]. In the present study the authors excluded the patients with fetal cardiac activity, larger ectopic size (four cm), and sonographic evidence of free peritoneal fluid, but also found that the treatment failure rate was increasing significantly when the initial serum b-hCG level was higher than 3,000 mIU ($p < 0.05$).

Previous studies have demonstrated that inflammation and microenviromental changes occur in fallopian tube in

tubal EPs [10]. This inflammation is suggested to activate platelets which are well known sources of growth factors. Some of these factors are vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), transforming growth factor-beta (TGF-β), and they have important roles in causing alterations in MPV [13]. In an activated state, some morphological changes occur and platelets form pseudopodias and become spherical in shape. Turgut *et al.* also showed that patients with ectopic pregnancy had significantly higher MPV levels [14]. In the present study, the authors found that MPV was higher in MTX treatment successful group when compared to treatment failure group. According to their hypothesis, due to inadequate inflammation and lower MPV levels, angiogenic cytokine levels and blood flow were lower in MTX failure group. For this reason they suggest that MTX may not have reached a sufficient concentration in EP tissue in this group. As far as they know, the present study is the first to evaluate the role of MPV in prediction of MTX treatment success.

NLR obtained by dividing neutrophil count to lymphocyte count, is considered as an other inflammatory marker [5]. In the present study, NLR was calculated and assessed in both groups, and it was found that NLR was lower in MTX failure group.This result proves the inadequate inflammatory state in MTX failure group when compared with MTX successful group. In concordance with the present study, Kim *et al.* reported NLR as a reliable marker for placental inflammation prediction [15]. The study of Sönmez *et al.* also emphasized that NLR is a simple and inexpensive method in order to evaluate inflammatory status in patients with acute coronary syndrome [6]. There are several studies assessing NLR in various diseases including ulcerative colitis and hepatic cirrhosis, familial Mediterranean fever, cardiovascular diseases, and malignancies [7].

Early b-hCG changes after MTX therapy were also accepted as a predictor of treatment outcome after MTX. In a trial, it was reported that declining b-hCG values of at least 20 % between days 1 and 4 during MTX treatment have been associated with a positive predictive value of 97% for treatment success [16]. In this present study the authors also found a correlation between early b-hCG levels after MTX therapy and treatment success. Adjustment of MPV and NLR may increase predictive value of early b-hCG changes after MTX therapy. This may be a new aim for a new study.

In conclusion, this was a preliminary study to evaluate the predictive value of MPV and NLR in single-dose MTX treatment success of tubal EP and the authors concluded that MPV and NLR can be used as reliable markers to predict single-dose MTX treatment success without any clinical signs, but it is necessary to be studied in different cohort groups.

References

[1] Shaw J.L.V., Dey S.K., Critchley H.O.D., Horn A.W.: "Current knowledge of the aetiology of human tubal ectopic pregnancy". *Hum. Reprod. Update,* 2010, *16*, 432.

[2] Guvendag Guven E.S., Dilbaz S., Dilbaz B., Aykan Yildirim B., Akdag D., Haberal A.: "Comparison of single and multiple dose methotrexate therapy for unruptured tubal ectopic pregnancy: a prospective randomized study". *Acta. Obstet. Gynecol. Scand*., 2010, *89*, 889.

[3] Practice Committee of American society for reproductive medicine.: "Medical treatment of ectopic pregnancy". *Fertil. Steril., 2008, 90*, S206.

[4] Varma R., Gupta J.: "Tubal ectopic pregnancy"'. *BMJ Clin. Evid.*, 2009, *2009*, pii: 1406.

[5] Imtiaz F., Shafique K., Mirza S.S., Ayoob Z., Vart P., Rao S.: "Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population". *Int. Arch. Med.,* 2012, *5*, 2.

[6] Sönmez O., Ertaş G., Bacaksız A., Tasal A., Erdoğan E., Asoğlu E., *et al.*: "Relation neutrophil-to-lymphocyte ratio with the presence and complexity of coronary artery disease: an observational study". *Anadolu Kardiyol Derg.,* 2013, *13*, 662. doi: 10.5152/akd.2013.188. Epub 2013 Jul 31.

[7] Torun S., Tunc B.D., Suvak B., Yildiz H., Tas A. Sayilir A., *et al.*: "Assessment of neutrophillymphocyteratio in ulcerative colitis: a promising marker in predicting disease severity". *Clin. Res. Hepatol. Gastroenterol.*. 2012, *36*, 491.

[8] Wagner D.D., Burger P.C.: "Platelets in inflammation and thrombosis". *Arterioscler. Thromb. Vasc. Biol.,* 2003, *23*, 2131.

[9] Gasparyen A.Y., Ayvazyen L., Mikhalidis D.: "MPV: a link between thrombosis and inflammation?" *Curr. Pharm. Des*., 2011, *17*, 47.

[10] Tay J.I., Moore J., Walker J.J.: "Ectopic pregnancy". *BMJ,* 2000, *320*, 916.

[11] Sagiv R., Debby A., Feit H., Cohen-Sacher B., Keidar R., Golan A.: "The optimal cutoff serum level of human chorionic gonadotropin for efficacy of methotrexate treatment in women with extrauterine pregnancy". *Int. J. Gynaecol. Obstet.,* 2012, *116*, 101.

[12] Corsan G.H., Karacan M., Qasim S., Bohrer M.K., Ransom M.X., Kemmann E.: "Identification of hormonal parameters for successful systemic single-dose methotrexate therapy in ectopic pregnancy". *Hum. Reprod*., 1995, *10*, 2719.

[13] Cabar F.R., Fettback P.B., Pereira P., Zugaib M.: "Serum markers in the diagnosis of tubal pregnancy". *Clinics*, 2008, *63*, 701.

[14] Turgut A., Sak M.E., Ozler A.: "Alterations of peripheral blood cells in tubal ectopic pregnancy". *Ginekol. Pol.,* 2013, *84*, 193.

[15] Kim M.A., Lee Y.S., Seo K.: "Assessment of predictive markers for placental inflammatory response in preterm births". *PLos One*, 2014, *9*, e107880.

[16] Agostini A., Blanc K., Ronda I., Romain F., Capelle M., Blanc B.: "Prognostic value of human chorionic gonadotropin changes after methotrexate injection for ectopic pregnancy". *Fertil. Steril.,* 2007, *88*, 504.

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