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Authors: Busra Korpe, Caner Kose, Gokce Naz Kucukbas, Sevki Celen, Yaprak Engin Ustun

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Determining treatment success in tubal ectopic pregnancies – the predictive value of the delta neutrophil index in single-dose methotrexate therapy

Busra Korpe [1], Caner Kose [1], Gokce Naz Kucukbas [2], Sevki Celen [3], Yaprak Engin Ustun [4]

[1] Department of Gynecology and Obstetrics, Ankara Etlik City Hospital, Ankara, Turkey

[2] Department of Perinatology, Kocaeli City Hospital, Kocaeli, Turkey

[3] Department of Perinatology, Ankara Etlik City Hospital, Ankara, Turkey

[4] Women's Health Training and Research Hospital, University of Health Sciences Etlik Zubeyde Hanım, Ankara, Turkey

Corresponding author: Caner Kose, e-mail: dr.canerkose@gmail.com

ORCID

BK: https://orcid.org/0000-0002-4315-5518

CK: https://orcid.org/0000-0002-3044-4804

GNK: https://orcid.org/0000-0002-2755-3700

SC: https://orcid.org/0000-0001-7033-3474

YEU: https://orcid.org/0000-0002-1011-3848

ABSTRACT

Introduction and aim. This study assessed the significance of the Delta neutrophil index (DNI) in predicting the effectiveness of single-dose methotrexate (MTX) treatment for tubal ectopic pregnancy.

Material and methods. In this retrospective study, 214 women diagnosed with tubal ectopic pregnancies and treated with MTX between 2019 and 2022 were included. Group 1 comprised 88 MTX responders, while group 2 consisted of 86 non-responders necessitating surgery. HCG and DNI levels were monitored on days 1, 4, and 7 of MTX treatment and compared between the groups.

Results. Group 1 exhibited significantly lower HCG and DNI levels compared to group 2 on days 1, 4, and 7 ($p<0.001$). Both HCG and DNI levels on days 1 and 4 of MTX treatment were significant predictors of treatment failure, with the highest AUC observed for DNI on day 4. In multivariate logistic regression analysis, elevated DNI levels on day 1 of MTX treatment were associated with a 5.8-fold increased risk, and DNI levels on day 4 were associated with a 4.44-fold increased risk of MTX treatment failure.

Conclusion. DNI emerges as a valuable marker for predicting the success of single-dose methotrexate treatment in tubal ectopic pregnancies, demonstrating superior predictive power compared to HCG.

Keywords. clinical prediction, ectopic pregnancy outcomes, methotrexate efficacy, neutrophil response, prognostic indication

Introduction

An ectopic pregnancy is defined as a pregnancy implanted outside the uterine cavity, with the majority of such cases occurring in the fallopian tube.[1] Ectopic pregnancy poses a significant threat to the life of the mother and is a leading cause of maternal morbidity among women of reproductive age.[2] Confirming the presence of an ectopic pregnancy typically involves periodic measurements of human chorionic gonadotropin (HCG) levels and transvaginal ultrasound examinations.[3-4] The routine use of ultrasonography has greatly improved the diagnosis of ectopic pregnancies, allowing for the selection of medical treatment in a majority of cases. The liberal use of methotrexate (MTX) in medical management has significantly reduced the need for surgical intervention and associated complications in the treatment of ectopic pregnancies.[5-6] In this context, single-dose MTX has emerged as a safe and effective method, with a crucial early indicator of treatment success being a substantial reduction in HCG levels between days 4 and 7 of MTX therapy.[6]

Numerous studies in the literature have sought to identify markers that predict the success of MTX treatment, but none have achieved a satisfactory level of accuracy. Therefore, the quest for more effective and sensitive markers of medical treatment success remains an ongoing challenge. Some inflammatory cytokines known to play roles in angiogenesis, inflammation, and immunity have been detected at both the implantation site and in the systemic circulation of tubal ectopic pregnancies.[7-11]

The delta neutrophil index (DNI) is a novel marker of inflammation that can be readily assessed through routine complete blood count (CBC) testing.[10-11] DNI measures the percentage of immature granulocytes in the bloodstream. In cases of infection or inflammation, increased production of myeloid cell lines leads to an elevated presence of immature granulocytes.[12-15] Recent research has explored the potential of DNI to predict various inflammatory conditions.[12-18]

The detection of certain inflammatory cytokines at the implantation site and in systemic circulation in ectopic pregnancy has prompted our interest in exploring the association between DNI and this condition.[7-11] Investigating DNI as a marker of inflammatory processes in ectopic pregnancies may provide valuable insights for determining treatment success and refining management strategies. Therefore, this study aimed to examine DNI in ectopic pregnancies, marking the first instance in the literature. This represents a crucial step in enhancing our understanding of treatment success and improving management strategies for this condition.

Aim

The primary objective of this study is to investigate the role of DNI in determining the success of single-dose MTX treatment in cases of tubal ectopic pregnancy.

Material and methods

This retrospective cohort study analyzed the medical records of pregnant women diagnosed with tubal ectopic pregnancy at a tertiary care center between 2019 and 2022. The study protocol received approval from the Institutional Review Board (IRB) on June 23, 2022 (IRB approval number: 11-23.06/09/2022). All participating women provided informed consent upon hospital admission, granting permission for their medical records to be used in future studies. The study adhered to the principles outlined in the Declaration of Helsinki.

Ectopic pregnancy was diagnosed when ultrasound imaging revealed a gestational sac located in the fallopian tubes, in conjunction with the absence of a gestational sac in the uterus and HCG levels exceeding 1500 mIU/mL, as per diagnostic criteria.

Patients admitted to the hospital with a diagnosis of tubal ectopic pregnancy undergo routine physical examinations, pelvic and transvaginal ultrasonographies, complete blood counts, and liver and kidney function tests. Patients' medical history and detailed medical background are questioned. Vital signs are closely monitored during hospitalization. Following comprehensive evaluations, decisions regarding follow-up, medical treatment, or surgical options are made in consultation with the patient based on their suitability. Women with tubal ectopic pregnancy who were clinically stable and exhibited no signs of tubal rupture or bleeding were selected for MTX treatment. However, MTX treatment was contraindicated for women with hypersensitivity reactions to this medication, pregnant or breastfeeding women, individuals with pre-existing blood disorders such as bone marrow hypoplasia, leukopenia, thrombocytopenia, or significant anemia, as well as those with rheumatoid arthritis or psoriasis, chronic liver disease, liver cirrhosis, alcoholic hepatitis, or chronic alcoholism.

On the day of admission, women who were not clinically stable, exhibited signs of tubal rupture or bleeding, and did not meet the criteria for MTX treatment were selected for surgical intervention.

All women in the study cohort received a single intramuscular dose of MTX at a dosage of 50 mg/m^2, following established guidelines.[19] No other medical treatment interventions were applied beyond MTX. Patients were monitored until the ectopic pregnancy was resolved.

As the 2018 ACOG (American College of Obstetricians and Gynecologists) guideline, successful MTX treatment was defined as a decrease in HCG levels of 15% or more when comparing days 4 and 7, with HCG levels dropping to <0.5 mIU/mL during follow-up, and no need for additional MTX or surgery.[19] Exclusion criteria encompassed women with non-tubal ectopic pregnancies, those exhibiting ectopic fetal heart activity, individuals with HCG levels exceeding 10,000 mIU/mL, and those with a tubal mass greater than 4 cm. Additionally, individuals requiring a repeat dose of MTX, surgery for pain or hemodynamic instability before day 4 of MTX treatment, those with heterotopic pregnancies, or individuals with chronic inflammatory or autoimmune diseases were also excluded from the study.

Figure 1 illustrates the study's flowchart. The study comprised a total of 174 women, selected from a pool of 331 women diagnosed with tubal ectopic pregnancies. These women were divided into two groups based on their response to MTX treatment. Group 1 included 88 women who responded to MTX treatment, while group 2 comprised 86 women who necessitated surgical intervention after day 4 of MTX treatment. HCG levels, CBC, renal function tests, and liver function tests, were conducted on days 1, 4, 7, 14, and 21 of MTX treatment. Day 1 was considered the initial day of MTX administration. All blood tests were processed at the same laboratory. DNI was determined using the ADVIA 2120 automated hematology analyzer. Data regarding demographic characteristics, obstetric history, physical examination findings, and laboratory results were extracted from patient records and the hospital's database. The choice of surgical procedure involved either laparoscopy or laparotomy.

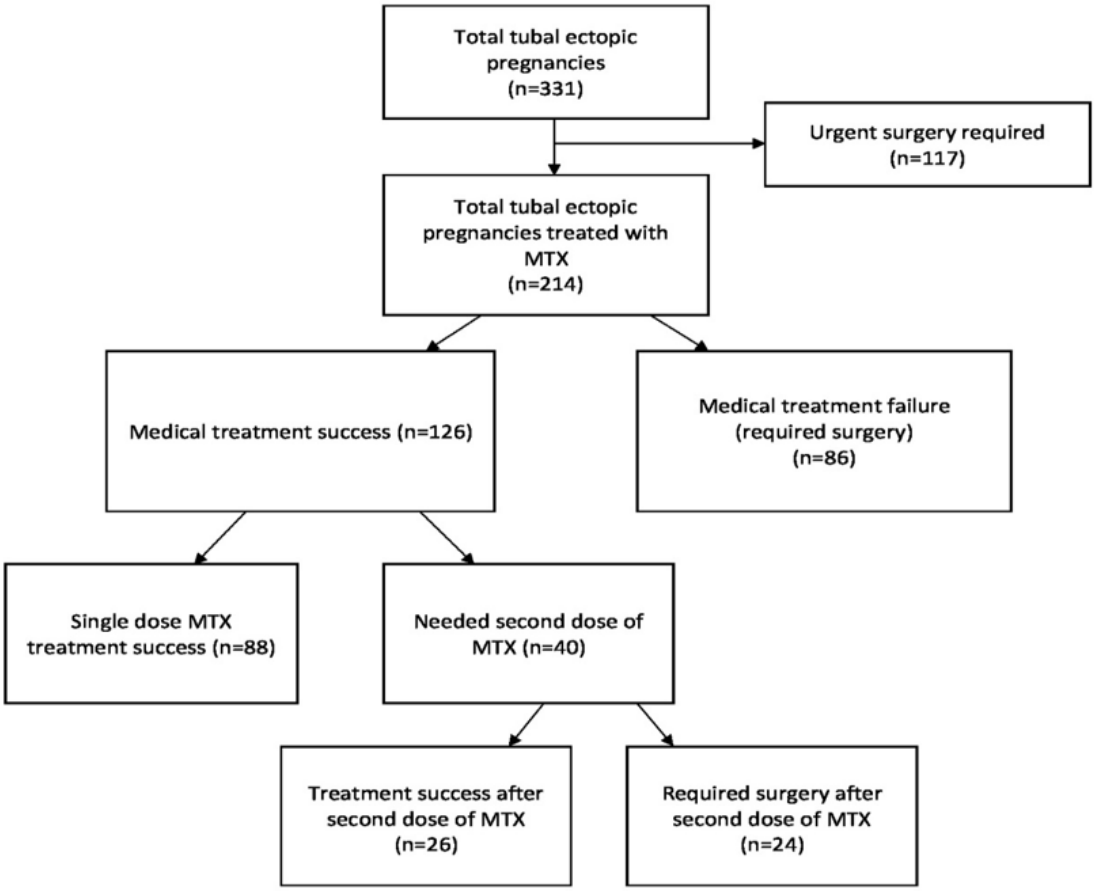

Fig. 1. Flow chart of the study

Statistical analysis

The IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Descriptive statistics were used to summarize the data. The comparison of nominal data was conducted using the independent samples t-test and presented as mean ± standard deviation. For ordinal variables, we employed the $\chi 2$ test, and Fisher's exact test was utilized for variables with limited sample sizes. The

receiver operating characteristic (ROC) curve was used to determine cut-off values, sensitivity, specificity, and the area under the curve (AUC). Significance was considered at a 95% confidence interval (CI) with a p-value of <0.05. To identify independent risk factors for medical treatment failure, both univariate and multivariate logistic regression analyses were performed.

Results

A total of 174 women were diagnosed with tubal ectopic pregnancies, with 88 women categorized in Group 1, indicating a response to MTX treatment, and 86 women assigned to Group 2, who did not respond to MTX treatment and required surgical intervention. The success rate of single-dose MTX treatment was 50.5%.

Table 1 presents a comparison of age, BMI, laboratory parameters, and sonographic measurements between the two groups. No statistically significant differences were observed in terms of age, body mass index (BMI), CBC results, renal and liver function tests. There was a significant difference in HCG levels (1219.22±1118.82 vs. 3493.66±4488.96 IU/L, 1541.7±1728.29 vs. 4709.79±6504.37 IU/L, 1363.58±1363.51 vs. 2945.75±2241 IU/L, $p < 0.001$) and DNI levels (0.99±0.61% vs. 2.4±1.51%, 0.90±0.57% vs. 4.92±2.98%, 1.07±0.58% vs. 2.98±1.89%, $p<0.001$) (Table 1). When comparing the differences in HCG and DNI levels between the 1st and 4th days of MTX treatment within the groups, a significant difference was observed in both HCG and DNI levels between these time points ($p<0.001$).

Table 1. Comparison of demographic, laboratory and ultrasonographic findings of groups*

	MTX success group (n=88)	**MTX failure group (n=86)**	**p**
Age (y)	30.12±4.23	30.51±5.1	0.587
BMI (kg/m^2)	25.75±4.44	25.46±4.22	0.656
Endometrial thickness (mm)	9.38±4.35	10.7±4.71	0.056
Adnexal mass size (cm)	1.55±0.95	1.5±1.13	0.721
Hb (mg/dL)	12.37±1.22	12.4±1.18	0.882
WBC	7901±2275	8024±2091	0.711
PLT	263±58	262±56	0.920
AST	16.2±6.29	16.26±6.11	0.947
ALT	16.98±8.63	16.75±8.54	0.858
LDH	224.94±101.26	201.3±78.22	0.087
BUN (mg/dL)	11.75±5.97	11.45±4.65	0.716
Creatinine (mg/dL)	0.6±0.08	0.61±0.09	0.255

1st day HCG (mIU/mL)	1219.22±1118.82	3493.66±4488.96	<0.001
4th day HCG (mIU/mL)	1541.7±1728.29	4709.79±6504.37	<0.001
7th day HCG (mIU/mL)	1363.58±1363.51	2945.75±2241	<0.001
14th day HCG (mIU/mL)	710.67±606.32	780.87±899.51	0.654
1st day DNI (%)	0.99±0.61	2.4±1.51	<0.001
4th day DNI (%)	0.90±0.57	4.92±2.98	<0.001
7th day DNI (%)	1.07±0.58	2.98±1.89	<0.001
14th day DNI (%)	1±0.96	1.17±0.95	0.232

* BUN – blood urea nitrogen, DNI – delta neutrophil index, HCG – human chorionic gonadotropin

Table 2 presents a comparison of the medical, obstetric, and gynecologic histories of the two groups, revealing no significant differences between them.

Table 2. Comparison of medical and obstetric history of groups*

	MTX success group (n=88)	**MTX failure group (n=86)**	
	n (%)	**n (%)**	**p**
History of ectopic pregnancy	15 (17)	10 (11.6)	0.308
Obstetric history			
Gravidity	3 (1–6)	3 (1–6)	0.287
Parity	1 (0–3)	1 (0–4)	0.144
Abortion	0 (0–3)	0 (0–3)	0.346
Smoking	24 (27.3)	34 (39.5)	0.086
History of PID	1 (1.1)	5 (5.8)	0.092
Contraceptive method	6 (6.8)	6 (7)	0.967
Current IUD user	4 (4.5)	6 (7)	0.491
Additional disease	7 (8)	6 (7)	0.806
Operation history	29 (33)	38 (44.2)	0.128
C- section	17	22	0.798
Salpingectomy	10	11	0.685
Appendectomy	2	1	0.545
Cholecystectomy	–	3	0.912
Inguinal hernia operation	–	1	0.934
Adnexal mass	83 (94.3)	83 (96.5)	0.490

Free intraabdominal fluid	18 (20.5)	18 (20.9)	0.938

* PID – pelvic inflammatory disease, IUD – intrauterine device

ROC analyses of HCG and DNI levels on the 1st and 4th days of single-dose MTX treatment are presented in Figure 2 and Figure 3. Notably, both HCG and DNI levels on the 1st and 4th days of MTX treatment were found to be significant in predicting MTX treatment failure. For detailed values, Table 3 provides the optimal cut-off points, AUC, sensitivity, and specificity values for HCG and DNI levels on the 1st and 4th day of MTX treatment.

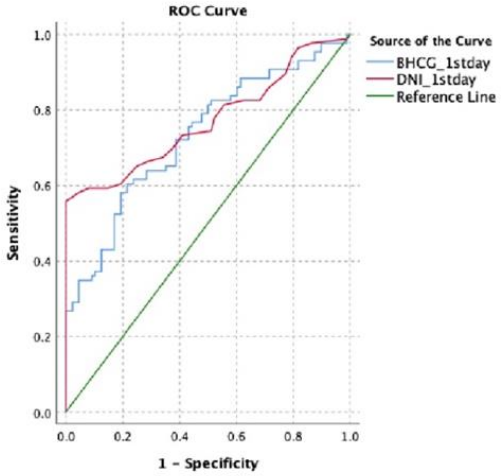

Fig. 2. ROC analysis of HCG and DNI levels on 1st day of treatment

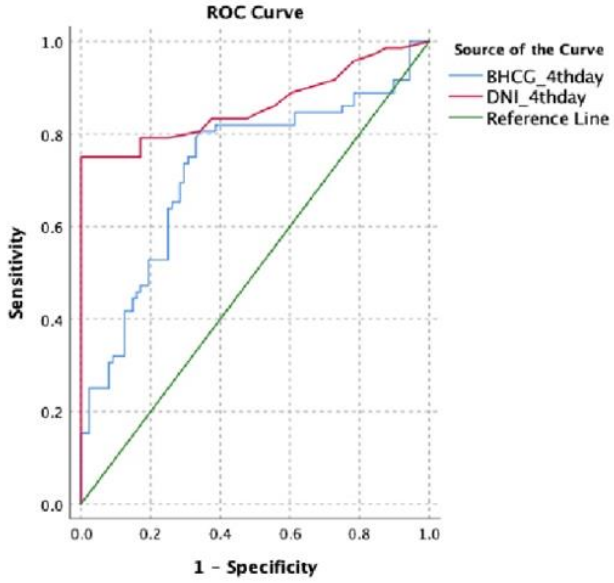

Fig. 3. ROC analysis of HCG and DNI levels on 4th day of treatment

Table 3. AUC values and cuff off points of DNI and HCG for MTX failure*

						95% confidence interval	
	Cut value	**AUC**	**p**	**Sensitivity**	**Specificity**	**Lower bound**	**Upper bound**
1st day DNI (%)	1.35%	0.771	<0.001	67.4%	65.9%	0.686	0.844
4th day DNI (%)	1.35%	0.862	<0.001	79.2%	75%	0.797	0.926
1st day HCG (mIU/mL)	1687	0.731	<0.001	65.1%	64.8%	0.654	0.812
4th day HCG (mIU/mL)	1838	0.724	<0.001	72.2%	70.5%	0.642	0.807

* DNI – delta neutrophil index

The correlation analysis revealed a weak, positive correlation between HCG and DNI levels. In univariate analysis, elevated HCG and DNI levels on the 1st and 4th day of MTX treatment emerged as independent risk factors for MTX treatment failure. Moving forward, multivariate logistic regression analysis demonstrated that elevated DNI levels on the 1st day of MTX treatment carried a 5.8-fold increased risk, while DNI levels on the 4th day of MTX treatment were associated with a 4.447-fold higher risk of MTX treatment failure (Table 4).

Table 4. Univariate and multivariate analysis and DNI and HCG*

	Univariate		**Multivariate**	
	Odds ratio (95% CI)	**p**	**Odds ratio (95% CI)**	**p**
1st day DNI (%)	3.120 (2.114–4.602)	<0.001	5.8 (2.325–14.468)	<0.001
4th day DNI (%)	2.927 (1.990–4.304)	<0.001	4.447 (2.307 –8.575)	<0.001
1st day HCG (mIU/mL)	1.001 (1–1.001)	<0.001	1.001 (1–1.001)	0.294
4th day HCG (mIU/mL)	1 (1–1.001)	<0.001	1.001 (1–1.001)	0.440
Initial HCG (mIU/mL)	1.634 (0.945–2.893)	0.456		
Smoking status	1.744 (0.921–3.300)	0.088	–	
History of ectopic pregnancy	0.640 (0.270–1.517)	0.311	–	
History of PID	5.37 (0.614–46.953)	0.129	–	
History of IUD	1.575 (0.429–5.789)	0.494	–	
Contraception usage	1.025 (0.317–3.312)	0.967	–	
Adnexal mass	1.667 (0.386–7.201)	0.494	–	

* DNI – delta neutrophil index, IUD – intrauterine device, PID – pelvic inflammatory disease

Discussion

Maternal morbidity and mortality remain pressing healthcare concerns, particularly in many underdeveloped and some developing countries. Notable progress has been made through early diagnosis and medical intervention using methotrexate in cases of ectopic pregnancy. Predicting the success of MTX treatment not only helps prevent complications like tubal rupture, optimizes patient follow-up, but also alleviates the anxiety experienced by affected women.

Our study delved into the association between DNI and tubal ectopic pregnancy, prompted by the detection of certain inflammatory cytokines at the implantation site and in systemic circulation.[7-11] Investigating DNI as a marker of inflammatory processes in ectopic pregnancies may provide valuable insights for determining treatment success and refining management strategies. This represents a significant advancement in the literature, as it marks the first instance of examining DNI in this context. By identifying high DNI levels on both day 1 and day 4 as potential indicators of MTX treatment success, our findings offer a promising avenue for improving patient outcomes.

Prior research has suggested that higher pretreatment HCG levels are associated with increased rates of treatment failure.[7,22-23] However, in our study, no significant correlation was observed between pretreatment HCG levels and treatment outcomes. This discrepancy could be attributed to our selection criteria, which included only women eligible for MTX treatment with HCG concentrations less than 10,000 mIU/ml. However, our study contributes to the ongoing discussion on optimal tubal treatment criteria, highlighting the need for further research to establish universally applicable guidelines.

Helmy et al. noted that there is no clear-cut endpoint for the initial serum HCG level that can be universally employed as a cutoff value for determining optimal tubal treatment. Nevertheless, they found that women with an initial HCG level exceeding 2,121 mIU/ml were more likely to experience MTX treatment failure than their counterparts.[11] In a study conducted by Levin et al., a decrease in HCG concentration from day 1 to day 4, coupled with a slight increase in HCG concentration one day before MTX treatment, was identified as a predictor of medical treatment success for ectopic pregnancy.[7] It was also demonstrated that an elevation in HCG levels before MTX administration is associated with treatment failure.[20-22] Levin et al. reported low success rates for treatment with a single dose of MTX in two separate studies.[7,22] Our results similarly reflect a low success rate of MTX treatment (50.5%), likely influenced by our stringent inclusion and exclusion criteria. Predictors of single-dose MTX treatment were evaluated, with the requirement for repeated MTX dosing considered indicative of treatment failure.

Previous studies have consistently highlighted the presence of inflammation and alterations in the tubal microenvironment in cases of tubal ectopic pregnancies. This inflammatory response triggers the activation of numerous growth factors.[23] Cekmez et al. conducted research investigating the role of systemic inflammatory markers in predicting the success of single-dose MTX treatment. They observed that mean

platelet volume (MPV) and neutrophile-lymphocyte ratio (NLR) levels were lower in the treatment failure group.[10] Another studies found that HCG, NLR, and platelet- lymphocyte ratio (PLR) levels were found to be associated with tubal rupture.[24,25] Our study adds to this body of evidence by identifying DNI as a potential predictor of MTX treatment failure. However, it's important to acknowledge the limitations of our retrospective design and reliance on medical records data.

DNI's potential to distinguish between complicated and noncomplicated appendicitis has also been explored in previous studies. Shin et al. discovered that DNI stands out as the sole marker significantly predicting perforation, and its predictive capability for perforation tends to increase with age.[26] Meanwhile, Kim et al. examined the predictive value of DNI for acute appendicitis in children. They noted that the median levels of DNI and CRP were higher in the complicated group, although initial CRP values emerged as the sole predictor of complications.[27] The CRP value has not been assessed in our study as it is not a routinely examined parameter in cases of ectopic pregnancy.

In our study, we identified that DNI levels exceeding 1.35% serve as a reliable predictor of MTX treatment failure. Notably, DNI levels were found to be higher in the MTX treatment failure group. This could potentially be attributed to the increased presence of immature granulocytes in circulation, resulting from inflammation in the fallopian tube. In our multivariate regression analyses, both DNI levels on day 1 and day 4 independently emerged as risk factors for predicting MTX treatment failure. The observed increase in DNI between days 1 and 4 may signify a heightened biological response in cases of ectopic pregnancy, possibly associated with the lower success rates of MTX treatment.

The main limitations of our study are attributed to its retrospective design and the reliance on data extracted from medical records. To provide a more robust understanding of the predictive role of DNI in women with ectopic pregnancy, future studies with a prospective nature and a larger sample size are warranted.

Conclusion

Nonetheless, our findings suggest that DNI emerges as a valuable marker for predicting the success of single-dose methotrexate treatment in tubal ectopic pregnancies, demonstrating superior predictive power compared to HCG. It could prove invaluable in determining which women are likely to benefit from MTX treatment. Early prediction has the potential to optimize treatment timing, reduce patient burden, and enhance overall patient care.

Declarations

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Author contributions

Conceptualization, B.K. and C.K.; Methodology, B.K. and G.N.K; Software, B.K and C.K..; Validation, B.K., C.K. and G.N.K.; Formal Analysis, B.K.; Investigation, C.K.; Resources, S.C.; Data Curation, B.K. and C.K.; Writing – Original Draft Preparation, B.K.; Writing – Review & Editing, Y.E.U and S.C.; Visualization, S.C.; Supervision, Y.E.U.; Project Administration, B.K.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The study protocol received approval from the Institutional Review Board (IRB) on June 23, 2022 (IRB approval number: 11-23.06/09/2022).

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