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Original Research	250 words	5,500 words (~22 pages) [‡]	NA	30
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Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

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Original researches should have the following sections;

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

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Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report



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outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNT_h) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Evaluation of sexual dysfunction and its associated risk factors in the male partners of the infertile couples using International Index of Erectile Function

İnfertil çiftlerin erkek partnerlerinde cinsel işlev bozukluğunun ve bununla ilişkili risk faktörlerinin Uluslararası Erektile Fonksiyon İndeksi kullanılarak değerlendirilmesi

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Abstract

Objective: Sexual dysfunction is a major health concern in infertile men. This research aims to evaluate the sexual dysfunction and its associated risk factors in the male partners of infertile couples.

Materials and Methods: The cross-sectional study was performed on 204 male partners of infertile couples that were referred to Fatemeh Zahra Infertility & Reproductive Center, Babol, Iran, in 2015. Sexual dysfunction was evaluated using The International Index of Erectile Function (IIEF). Logistic and linear regression tests were used for statistical analyses. Statistical significance was considered with a p value less than 0.05.

Results: The mean total IIEF score was 58.30±8.52. The lowest mean of IIEF domains was related to sexual desire and then orgasmic function in the male partners of the infertile couples. Erectile function contributed to the greatest amount of unique variance in the model for sexual function ($p<0.001$, $R^2=69.8\%$). The strongest correlation value was between the domains of overall satisfaction and intercourse satisfaction. There was a positive statistically significant association between sexual function with wife marital intimacy ($p<0.002$) and wife sexual function ($p<0.001$). There was a significant association between sexual dysfunction with job conditions ($p<0.037$, $OR=0.094$), and coitus count ($p<0.009$, $OR=6.146$). After adjusting for other variables, there was a significant association between sexual function and wife sexual function ($p<0.005$). Also, after adjusting for other variables, there was a significant association between sexual dysfunction and coitus count ($p<0.004$, $OR=2.496$), and job condition ($p<0.046$, $OR=0.081$).

Conclusion: By considering sexual dysfunction and some related factors, early screening is required for distinguishing predictor factors of sexual dysfunction.

Keywords: Infertility, male sexual dysfunction, risk factors for sexual dysfunction

Öz

Amaç: Cinsel işlev bozukluğu, infertil erkeklerde önemli bir sağlık sorunudur. Bu araştırma, infertil çiftlerin erkek partnerlerindeki cinsel işlev bozukluğunu ve bununla ilişkili risk faktörlerini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Kesitsel çalışma, 2015 yılında Fatemeh Zahra İnfertilite ve Üreme Merkezi, Babol, İran'a sevk edilen infertil çiftlerin 204 erkek partnerleri üzerinde gerçekleştirildi. Cinsel işlev bozukluğu Uluslararası Erektile Fonksiyon (IIEF) İndeksi kullanılarak değerlendirildi. İstatistiksel analizlerde lojistik ve lineer regresyon testleri kullanıldı. İstatistiksel anlamlılık 0,05'ten düşük p değeri olarak kabul edildi.

Bulgular: Ortalama toplam IIEF skoru 58,30±8,52 idi. IIEF alanlarının en düşük ortalaması, infertil çiftlerin erkek partnerlerindeki cinsel istek ve daha sonra orgazmik fonksiyonla ilişkiliydi. Erektile fonksiyon, cinsel fonksiyon modelinde en büyük benzersiz varyansa katkıda bulunmuştur ($p<0,001$, R^2 : %69,8). En güçlü korelasyon değeri, genel memnuniyet ve cinsel ilişki memnuniyeti alanları arasındaydı. Cinsel işlev ile kadının yakınlığı ($p<0,002$) ve

PRECIS: There were risk factors for sexual dysfunction in the male partners of the infertile couples and these able to affect sexual health.

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Öz

kadının cinsel işlevi ($p<0,001$) arasında istatistiksel olarak anlamlı bir ilişki vardı. Cinsel işlev bozukluğu ile iş koşulları [$p<0,037$, odds ratio (OR): 0,094] ve koitus sayısı ($p<0,009$, OR: 6,146) arasında anlamlı bir ilişki vardı. Diğer değişkenler için düzeltmeler yapıldıktan sonra, cinsel işlev ile kadın cinsel işlevi arasında anlamlı bir ilişki vardı ($p<0,005$). Ayrıca, diğer değişkenler için düzeltmeler yapıldıktan sonra, cinsel işlev bozukluğu ile koitus sayısı ($p<0,004$, OR: 2,496) ve iş durumu ($p<0,046$, OR: 0,081) arasında anlamlı bir ilişki vardı.

Sonuç: Cinsel işlev bozukluğu ve bazı ilgili faktörler göz önünde bulundurularak, cinsel işlev bozukluğunun yordayıcı faktörlerini ayırt etmek için erken tarama gereklidir.

Anahtar Kelimeler: Kısırlık, erkek cinsel işlev bozukluğu, cinsel işlev bozukluğu için risk faktörleri

Introduction

Infertility is clinically described as the inability to conceive after 12 months of intercourse without using birth control⁽¹⁾. Fertility problems can be observed in men and women in which the impaired fertility is experienced by 7-17% of couples; however, in over one-third of cases, the cause of infertility has been attributed to the men^(2,3). Infertility is a major crisis in the lives of infertile couples and associated with a heavy psychological burden, underlying the male sexual function⁽⁴⁻⁶⁾. Male sexual activity is an essential factor for the fertility⁽⁷⁾. There exist a reasonable connection between infertility and sexual disorder⁽⁶⁾. The sexual dysfunction can affect fertility and vice versa⁽⁸⁾. Several researches have examined the effect of infertility on the sexual experience^(9,10). It was found that the infertility might threaten the sexuality, competence, and male identity in infertile men⁽¹⁰⁻¹²⁾. In the infertile men, sexual dysfunction is an intricate issue⁽¹³⁾ that may have a deep impact on the quality of their sexual life⁽¹⁴⁾. Men with infertility experience a crisis that may have an injurious outcome on their sexual function with the perception of sexual inefficacy⁽¹⁵⁾. Many infertile men believe that infertility is associated with the loss of virility and masculinity, resulting in the sexual problems⁽¹³⁾. Since the sexual function is necessary for the reproduction⁽⁷⁾, identifying the sexual dysfunction and their risk factors are crucial in the infertile men before treatment of infertility⁽¹⁶⁾. Diagnosis of sexual disturbances in the male partners of the infertile couples can not only increase sexual function but also enhances natural pregnancy⁽⁷⁾. Many factors such as demographic conditions, poor relationships, biological and physical or emotional causes are known to be associated with the sexual dysfunction⁽¹⁷⁻¹⁹⁾. Moreover, the effect of cultural and social factors as well as the relationship between such aspects have not been well evaluated in the infertile men⁽¹⁷⁾, thus, more study will be required. Basically, careful assessments of male sexual dysfunction in understanding its detrimental consequences and in distinguishing its risk factors are important for prophylaxis efforts, which can be useful for counseling of the infertile partners.

To the best of our knowledge, there are a few studies in the literature on the infertile men to clarify the risk factors that shape the development of sexual dysfunction. However, more studies have been applied to establish the incidence rate of this topic in a healthy population. As sexual dysfunction is likely common in the infertile men, the evaluation of this matter is of the utmost importance. Therefore, this study aims to evaluate

the sexual dysfunction along with the potential risk factor in Iranian infertile men.

Materials and Methods

This cross-sectional study was done at Fatemeh Zahra Infertility and Reproductive Health Center of Babol Medical Sciences University, Iran in 2015. The duration of the present study was five months. Of the 220 eligible infertile men, 204 accepted to enroll in the project. This present study was conducted for the determination of sexual dysfunction and associated risk factors in the male partners of the infertile couples. All subjects were informed about the aims and details of the research and the secrecy of the data. Inclusion criteria were the ability of reading and writing, living with wife, history of >1 year of infertility, not having remarriage in couple, without any previous sterility, and not having a foster child. Exclusion criteria were major life events in the past months (death or difficult sickness in the family), currently using antidepressant and psychotropic drugs, physical and psychiatric problems, not having a stable sexual life for four previous weeks. The male partners of the infertile couples completed a demographic characteristics form, The International Index of Erectile Function (IIEF) questionnaire. The IIEF, which is an international index of erection function, was translated and validated into the Persian language. The IIEF Iranian version is valid and reliable for the Iranian population. The Cronbach's alpha was from 0.73 to 0.99⁽²⁰⁻²¹⁾. Its five domains includes sexual desire (2 items), erectile function (6 items), orgasmic function (2 items), intercourse satisfaction (3 items), and overall satisfaction (2 items). The score of each item ranges from 0/1 (no sexual dysfunction) to 5 (normal range). The range of subscale score including 2-10 for sexual desire; 1-30 for erection function, 0-10 for orgasmic function, 0-15 for intercourse satisfaction, and 2-10 for overall satisfaction. The total IIEF score is obtained from the sum of 15 items in the five domains that evaluate the sexual function. The range of the total score was 5-75. Lower values of this questionnaire represent worse sexual dysfunction. More studies have indicated its suitability to evaluate the sexual function in men. Also, in present study was used the instruments of Marital Intimacy Need Questionnaire and Female Sexual Function index for detecting of association between the male sexual function with the female marital intimacy and the female sexual function. These questionnaires were given to infertile women to be completed by their husbands.

The project with code number 1828 was approved by the Ethics Committee of the Babol University of Medical Sciences, Babol, Iran (approval number: 3326, date: 9/11/2013). Consent form was completed by all subjects.

Statistical Analysis

Simple and multiple linear and logistic regression analysis models were estimated to calculate predictor factors for the sexual dysfunction among infertile men. Pearson's correlation coefficient was used to determine the correlation between quantity data. All data analysis was conducted using SPSS, version 21 with $p < 0.05$ indicating significance.

Results

The mean age of male partners of the infertile couples and their wives was 31.77 ± 5.47 (range from 20 to 50) and 27.82 ± 5.70 (range from 17 to 43) years. The duration of marriage was 6.21 ± 4.04 (range from 20 to 50) years. The residency in the majority of subjects was the private house (70.8%). The economic status of most the male partners of the infertile couples was moderate level (66%). The highest educational level in most of the subjects and their wives was high school diploma (33.8%, 40.1%). The mean and standard deviation of the duration of marriage was 6.21 ± 4.04 (range from 2 to 20). The etiology of infertility in the majority of subjects was associated with male factors (37.1%). 92.1% of the infertile men had no child.

The mean and standard deviation of the IIEF was 58.30 ± 8.52 (range from 27 to 75). The highest mean in IIEF domains is related to erectile function (23.27 ± 4.26) (range from 9 to 30) and then intercourse satisfaction (10.70 ± 2.42) (range from 0 to 15). The lowest mean in IIEF domains is related to sexual desire (7.75 ± 1.52) (range from 4 to 14) and then orgasmic function (7.89 ± 1.97) (range from 2 to 10). The mean of Overall satisfaction domain was (8.88 ± 1.56) (range from 2 to 13). Standardized beta values showed that erectile function contributed to the greatest amount of unique variance to the model for infertile men sexual function ($R^2 = 69.8\%$), and followed by intercourse satisfaction ($R^2 = 54\%$), overall satisfaction ($R^2 = 41.3\%$), sexual desire ($R^2 = 39.6\%$), and orgasmic function ($R^2 = 31.8\%$) ($p < 0.001$). The strongest correlation value was determined between overall satisfaction and intercourse satisfaction. There was a high correlation value between the domains of erectile function with intercourse satisfaction (Table 1).

A simple linear regression was done to assess the predictive nature of demographic characteristics on male sexual function. The results of the regression analysis are summarized in Table 2. There was not a significant association between the sexual function and age, wife's age, the age difference of spouses, duration of the marriage, duration of infertility, and Body Mass index (BMI). There was a positive statistically significant association between the sexual function and wife's marital intimacy ($p < 0.002$) and wife's sexual function ($p < 0.001$).

Furthermore, after adjusting other variables, there was a positive significant association between a sexual function with wife sexual function ($p < 0.05$), and a trend towards a positive significant association between a sexual function with wife marital intimacy ($p < 0.57$).

There was not a significant association between the sexual dysfunction with housing, economic status, wife's educational level, current settlement type, educational level, infertility causes, treatment effort, infertility type, and previous using assisted reproductive technology (ART). There was a significant association between the sexual dysfunction with job conditions. The risk of sexual dysfunction was 0.094-fold less in employee than unemployed infertile men ($p < 0.037$, $OR = 0.094$). Also, there was a trend towards a significant association between the sexual dysfunction with the wife's job. The risk of sexual dysfunction was 0.5-fold lower in infertile men when the job of their wives was employed than housewives ($p < 0.083$, $OR = 0.500$). There was a significant association between sexual dysfunction with Coitus Count when the frequency of coitus was monthly than > 3 times/week, the risk of sexual dysfunction was 2-fold higher ($p < 0.009$, $OR = 2.172$). Also, when the frequency of coitus was 1-2 times/week than > 3 times/week, the risk of sexual dysfunction was 6 -fold higher ($p < 0.009$, $OR = 6.146$). After adjusting other variables, there was a significant association between sexual dysfunction with job condition ($p < 0.046$, $OR = 0.081$), and coitus count ($p < 0.004$, $OR = 2.496$) (Table 3).

Discussion

This study displayed the low average amount of IIEF among the male partners of the infertile couples. The low IIEF scores indicated more sexual dysfunction in subjects. A similar study showed that many male partners of the infertile couples reported decreased sexual function⁽⁷⁾. The average IIEF in the study of Moazeni-Bistgani and Mohammad-Alibeigi⁽²²⁾ was similar with the our study, while the infertile men's score was observed to be higher than other similar studies in total

Table 1. The International Index of Erectile Function domains intercorrelations

IIEF	Sexual desire	Erectile function	Orgasmic function	Intercourse satisfaction
Sexual desire	1	-	-	-
Erectile function	0.440	1	-	-
Orgasmic function	0.277	0.324	1	-
Intercourse satisfaction	0.368	0.471	0.279	1
Overall satisfaction	0.321	0.378	0.255	0.526

(Pearson r; Range = -1.00 – +1.00). Correlation is significant at the 0.001 level (2-tailed)
IIEF: The International Index of Erectile Function

Table 2. Simple and multiple linear regression analysis of the International Index of Erectile Function with the other variables in the male partners of the infertile couples

Factors	Simple linear regression					Multiple linear regression			
	B standardized	R ²	p	95%CI		B standardized	p	95% CI	
				Low	Up			Low	Up
Wife age	-0.010	0.000	0.889	-0.223	0.194	Constant	0.001	22.102	50.980
Age	-0.61	0.004	0.390	-0.311	0.122	0.042	0.672	-0.241	0.373
Age difference of spouses	-0.062	0.004	0.381	-0.383	0.147	-0.065	0.395	-0.414	0.164
Duration of marriage	-0.093	0.009	0.188	-0.489	0.096	-0.070	0.600	-0.706	0.409
Duration of infertility	-0.093	0.009	0.188	-0.553	0.110	-0.072	0.538	-0.717	0.374
BMI	0.032	0.001	0.658	-0.204	0.322	0.146	0.683	-0.201	0.306
Wife marital intimacy	0.222	0.049	0.002	0.015	0.062	0.212	0.057	-0.001	0.051
Female sexual function	0.254	0.064	0.001	0.244	0.802	0.212	0.005	0.133	0.762

IIEF: The International Index of Erectile Function, CI: Confidence interval, BMI: Body mass index

IIEF and also subdomains of sexual function such as sexual desire, orgasmic function, intercourse satisfaction, and overall satisfaction. According to a study (2015), the mean of total IIEF score was 45.7 ± 7.5 . Our study revealed a mean just higher than Turkish men diagnosed with infertility⁽²³⁾. We believe that the difference in the average amount of IIEF and its subdomains of the infertile men in our protocol, compared with other studies, one reason is problems with the methodological issues and the other one cultural context.

Our findings suggest that the lowest average score in IIEF domains is related to sexual desire and then orgasmic function, which was nearly in line with the result of the study that it had done in Turkey⁽²³⁾. A review of literature presented that in infertile men, hypoactive sexual desire and lack of sexual satisfaction were the most prevalent types of sexual dysfunction⁽⁵⁾. In another study, the average sexual desire and then orgasmic function scores were lower compared to other subscales of IIEF, which was similar to our study⁽²²⁾. Also, Lotti and Maggi⁽⁵⁾ (2018) had reported that hypoactive sexual desire and sexual satisfaction were the most prevalent types of sexual dysfunctions in infertile men, while McCabe et al.⁽²⁴⁾ (2016) showed that erectile disorder and premature ejaculation (Orgasmic disorders) were the most frequent sexual disorders in men.

The gathered data showed that in more than half of the cases, erectile function and then intercourse satisfaction contributed to the greatest amount of unique variance to the model for sexual function. A review of the literature indicated that the domains of sexual function such as arousal, and orgasm strongly were related to sexual satisfaction⁽²⁵⁾. Another study showed that the men who experienced erection dysfunction had more negative expectations related to sexual function, and consider themselves as incompetent and weakly⁽²⁶⁾. As erectile dysfunction is the most common sexual dysfunction in the male partners of the

infertile couples, therefore; it can be considered as predicted sexual function to a considerable degree. It suggests that the various domains of sexual function are considered in sexual satisfaction and sexual function.

The results indicated that one of the most surprising results in our study was the strength of the correlation between overall satisfaction and intercourse satisfaction. Furthermore, there was a high correlation value between the domains of erectile function with intercourse satisfaction. The studies reported that more sexual satisfaction is related to the high frequency of sexual activity⁽²⁷⁾. Several similar studies presented that more frequency of sexual function was associated with sexual satisfaction in men^(28,29).

In this study, there were some interesting results by analyzing the factors impressing sexual dysfunction. This allowed us to identify the risk factors of sexual dysfunction in the male partners of the infertile couples.

Demographic Characteristics

Neither the age of men and women nor the age difference of spouses, duration of the marriage, duration of infertility, and BMI significantly contributed to the model for sexual function in infertile men. Also, there was not a significant association between the sexual dysfunction with housing, economic status, wife's educational level, current settlement type, educational level, infertility causes, treatment effort, infertility type, and previous using ART. The study by Muller et al.⁽³⁰⁾ demonstrated that sexual satisfaction was not associated with age, duration of the relationship, duration of treatment, and having a child in infertile men. We believe that the difference in significant association between a sexual function with some of the demographic characteristics in the infertile men in our protocol, compared with other studies, is caused by the variety of customs and cultures in our population. We think that the

Table 3. Binary and multiple logistic regression analysis of IIEF with the other variables in the male partners of the infertile couples

Factor		Binary logistic regression				Multiple logistic regression			
		OR	p	95%CI		OR	p	95%CI	
				Low	Up			Low	Up
Housing	Owner	-	-	-	-	-	-	-	-
	Tenant	1.367	0.314	0.744	2.510	1.290	0.484	0.632	2.630
Job condition	Unemployed (R*)	-	0.082	-	-	-	0.175	-	-
	Worker	0.205	0.154	0.023	1.813	0.206	0.174	0.021	2.009
	Employee	0.094	0.037	0.010	0.870	0.081	0.046	0.007	0.959
	Self-employed and other	0.132	0.065	0.015	1.133	0.152	0.105	0.016	1.489
Wife job	Housekeeper	-	-	-	-	-	-	-	-
	Employee	0.500	0.083	0.228	1.095	0.610	0.277	0.250	1.488
Economic status	Low (R*)	-	0.284	-	-	-	0.351	-	-
	Moderate	0.583	0.115	0.298	1.140	0.783	0.532	0.363	1.688
	High	0.617	0.354	0.223	1.710	1.641	0.450	0.454	5.929
Wife Educational level	University (R*)	-	0.558	-	-	-	0.858	-	-
	Literacy	1.983	0.157	0.768	5.122	1.587	0.412	0.526	4.783
	High school	1.256	0.585	0.554	2.851	1.085	0.870	0.411	2.863
	diploma	1.351	0.397	0.673	2.709	1.215	0.641	0.535	2.760
Coitus Count	>3 time/week	-	0.004	-	-	-	0.002	-	-
	1-2 times/week	6.146	0.009	1.577	23.947	7.445	0.007	1.733	31.983
	Monthly	2.172	0.009	1.209	3.900	2.496	0.004	1.332	4.676
Current settlement type	Urban	-	-	-	-	-	-	-	-
	Rural	0.829	0.509	0.475	1.447	0.718	0.343	0.362	1.424
Constant						3.833	0.379		
Educational level	University (R*)	-	0.818	-	-	-	-	-	-
	High school	1.285	0.522	0.596	2.772	-	-	-	-
	diploma	1.409	0.375	0.661	3.002	-	-	-	-
Infertility cause	Female factors (R*)	-	0.612	-	-	-	-	-	-
	Male factors	0.992	0.985	0.441	2.232	-	-	-	-
	Female/Male factors	1.524	0.400	0.571	4.065	-	-	-	-
	Unexplained factors	1.422	0.408	0.617	3.276	-	-	-	-
Treatment effort	First time	-	-	-	-	-	-	-	-
	Several times	1.274	0.408	0.718	2.261	-	-	-	-
Infertility type	Primary	-	-	-	-	-	-	-	-
	Secondary	1.420	0.272	0.760	2.652	-	-	-	-
ART	Yes	-	-	-	-	-	-	-	-
	No	1.053	0.857	0.599	1.853	-	-	-	-

OR: Odds ratio, CI: Confidence interval, R: Reference, IIEF: The International Index of Erectile Function

characteristics of the Iranian society may have an important role in the average amount of sexual dysfunction.

Wife Sexual Function

The results of our study represented a positive association between a sexual function with wife sexual function. On the other hand, one of the key results in this study was that wife sexual function that was significantly related to sexual function for the male partners of the infertile couples. Favorable sexual function in the wife can be a sign of high sexual function in the husband. Alan et al.⁽³¹⁾ reported that female sexual function was a significant predictor of male partner sexual function. Female sexual performance can have positive effects on male sexual function. A similar study represented the interdependence of sexual satisfaction between partners, so that sexual complaints in husband often contributing to problems in sexual satisfaction or/and sexual function for wife and vice versa⁽³²⁾. These results highlight the interaction between a female sexual function with male sexual function. The concept that the promotion of sexual function in the infertile men might be an important marker of female sexual health is emerging.

Wife Marital Intimacy

The current study also showed that sexual function significantly was associated with wife marital intimacy. More wife marital intimacy was associated with improved sexual function in the infertile men. It is considered that sexual function is influenced by the marital intimacy of one's partner. On the other hand; couple intimacy may alter sexual behavior. Studies presented that infertility is associated with the effects in couples and one partner's response influences her or his partner's response⁽³³⁾. Theiss (2011) indicated that a lack of sexual intimacy was correlated with lower sexual satisfaction in married couples⁽³⁴⁾. Findings from the same study showed that one of the psychological causes of sexual dysfunction is relationship or marital problems⁽³⁵⁾. Emmanuel et al.⁽³⁶⁾ reported that male sexual performance ability is vital in the marital relationship so that the lack of it can lead to a failure in the relationships. Basically, socio-cultural and interpersonal factors play an important role in developing sexual concern, which can lead to sexual difficulty and sexual dysfunction⁽³²⁾. In our opinion, wife marital intimacy can consider proxies for sexual function in the male partners of the infertile couples.

Job Condition/Wife Job

This present study shows that occupation is related to sexual dysfunction, as unemployed infertile men and women face complaints in sexual function more than employed individuals. The risk of sexual dysfunction in employed men was 0.094-fold less compared to unemployed men. Also, there was a trend towards a significant association between the sexual dysfunction with the wife's job. The risk of sexual dysfunction was 0.5-fold less when the occupation of the

infertile men's wives was an employee than a housekeeper. Sexual performance in men and women undergoing infertility is positively affected by job conditions. Principally, one of the barriers to sexual satisfaction can be a lack of occupational support. The review of the literature showed that social factors such as occupation can influence the sexual dysfunction⁽²⁹⁾. Pasha et al.⁽³⁷⁾ showed that there was poor marital intimacy in husbands who were unemployed than those with job. Basically, favorable job, appropriated social status, and good financial situation can lead to improved sexual and marital satisfaction. Unemployment and not having occupation may have an important effect on marital satisfaction^(38,39). Also, for both infertile men and women, the job can consider the most common source of financial support, which is needed for paying heavy costs of infertility treatment. High cost of infertility treatment can lead to persistence concerns. In fact, having a job increases the chance that individuals can pay the cost of infertility treatment. On the other hand, financial support and occupation are essential for having desirable sexual performance. Alirezaie et al.⁽⁴⁰⁾ indicated that income and high costs of infertility treatment have an influence on sexual function. Low-income individuals have a sexual complaint 4 times more compared with high-income individuals. To confirm this statement, Audu⁽⁴¹⁾ found the income effect on sexual function. Low income is a risk factor for sexual disturbance. Difficulty in paying costs of infertility treatment raised the chance of sexual problem up to nine times⁽⁴²⁾. The authors offer two possible explanations for this result: At first, it seems that job is one of the important factors that a person is able to cover the costs of infertility treatment. Second, the lower socioeconomic situation is also associated with less physical and mental health, which can be linked with the sexual dysfunction.

Coitus Count

Gathered data from the present study found that the sexual dysfunction was higher among infertile men who had a low frequency of intercourse. The rate of sexual dysfunction was 2-fold higher when the frequency of coitus was monthly than >3 times/week. The results of a study on the subject showed that the quality of couples' relationships may influence the sexual dysfunction⁽²⁹⁾. Data from a similar study revealed that more frequency of sexual activity can lead to high sexual satisfaction⁽²⁷⁾. These data are important because they will determine whether appropriate intercourse frequency can be suggested to these infertile couples to improve sexual function and fertility. Perlis N et al.⁽⁴³⁾ in the study as "Coital frequency and infertility: which male factors predict less frequent coitus among the infertile couple?" reported that the infertile men with better erectile function had 1-12 times more frequent coitus. Erectile dysfunction can be considered as a risk factor for less frequent intercourse. Therefore, coital frequency should be assessed in infertility protocol.

Strengths and Weaknesses

The present study has several strengths and weaknesses. We used a validated, internationally established questionnaire IIEF to assess the sexual dysfunction. Providing the correct answer to question about sexual issues was a limitation of the research, which was somewhat reduced by giving confidence to patients about the confidentiality of information. It is not possible to consider whether the study subjects representatives of the male partners of the infertile couples in general.

Conclusion

From the data obtained in the male partners of the infertile couples, we can observe that sexual dysfunction is common in unemployed men and their housewives, wife's sexual dysfunction, poor wife's marital intimacy, and monthly intercourse. Therefore, our findings strongly suggest the routine clinical investigation of risk factors for sexual function in the male partners of the infertile couples. Good understanding of the risk factors of sexual dysfunction is essential to assay male infertility and sexual complaints.

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Ethics

Ethics Committee Approval: The project with code number 1828 was approved by the Ethics Committee of the Babol University of Medical Sciences, Babol, Iran (approval number: 3326, date: 9/11/2013).

Informed Consent: Consent form was completed by all subjects.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Z.B., H.P., M.F., F.K.H., H.S.H., Design: Z.B., H.P., M.F., F.K.H., H.S.H., Data Collection or Processing: H.P., M.F., Analysis or Interpretation: H.P., M.F., Z.B., Literature Search: H.P., F.K.H., H.S.H., Writing: H.P., M.F.

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References

- Brugh VM 3rd and Lipshultz LI. Male factor infertility: evaluation and management. *Med Clin North Am* 2004;88:367-85.
- Mosher WD. Infertility trends among U.S. couples:1965-1976. *Fam Plann Perspect* 1982;14:22-7.
- Pasha H, Basirat Z, Faramarzi M, Kheirkhah F. Comparative Effectiveness of Antidepressant Medication versus Psychological Intervention on Depression Symptoms in Women with Infertility and Sexual Dysfunction. *Int J Fertil Steril* 2018;12:6-12.
- Pasha H, Basirat Z, Faramarzi M, Kheirkhah F. Pharmacological and Non-pharmacological Therapeutic Strategies for Improvement of State-Trait Anxiety: A Randomized Controlled Trial Among Iranian Infertile Women with Sexual Dysfunctions. *Crescent Journal of Medical and Biological Sciences* 2017;4:47-53.
- Lotti F, Maggi M. Sexual dysfunction and male infertility. *Nat Rev Urol* 2018;15:287-307.
- Lenzi A, Lombardo F, Salacone P, Gandini L, Jannini EA. Stress, sexual dysfunctions, and male infertility. *J Endocrinol Invest* 2003;26:72-6.
- Song SH, Kim DS, Yoon TK, Hong JY, Shim SH. Sexual function and stress level of male partners of infertile couples during the fertile period. *BJU Int* 2016;117:173-6.
- Berger MH, Messori M, Pastuszak AW, Ramasamy R. Association Between Infertility and Sexual Dysfunction in Men and Women. *Sex Med Rev* 2016;4:353-65.
- Peterson BD, Newton CR, Tal Feingold MA. Anxiety and sexual stress in men and women undergoing infertility treatment. *Fertil Steril* 2007;88:911-4.
- Elliott S. The relationship between fertility issues and sexual problems in men. *Can J Hum Sex* 1998;7:295-303.
- Sezgin H, Hocaoglu C. Psychiatric aspects of infertility. *Curr Approaches Psychiatry* 2014;6:165-84.
- Kilic M, Apay SE, Beji NK. Infertility and culture. *I.U.F.N.Hem Derg.* 2011;19:109-15.
- Reder F, Fernandez A, Ohl J. Does sexuality still have a place for couples treated with assisted reproductive techniques? *J Gynecol Obstet Biol Reprod* 2009;38:377-88.
- Eden KJ, Wylie KR. "Quality of sexual life and menopause". *Women's Health* 2009;5: 385-96.
- Bechoua S, Hamamah E, Scalici. Male infertility: an obstacle to sexuality? *Andrology* 2016;4:395-403.
- Dooley M, Dineen T, Sarma K, Nolan A. The psychological impact of infertility and fertility treatment on the male partner. *Hum Fertil (Camb)* 2014;17:203-9.
- McCabe MP, Sharlip ID, Lewis R, Atalla E, Balon R, Fisher AD. Risk Factors for Sexual Dysfunction among Women and Men: A Consensus Statement from the Fourth International Consultation on Sexual Medicine 2015. *J Sex Med* 2016;13:153-67.
- McCabe MP, Cobain MJ. The impact of individual and relationship factors on sexual dysfunction among males and females. *J Sex Marital Ther* 1998;13:131-43.
- Oksuz E, Malhan S. The prevalence of male sexual dysfunction and potential risk factors in Turkish men: a Web-based survey. *Int J Impot Res* 2005;17:539-45.
- Rosen RC, Cappelleri JC, Gendrano N. The International Index of Erectile Function (IIEF): a state-of-the-science review. *Int J Impot Res* 2002;14:226-44.
- Pakpour AH, Zeidi IM, Yekaninejad MS, Burri A. Validation of a translated and culturally adapted Iranian version of the International Index of Erectile Function. *Journal of Sex & Marital Therapy* 2014;40:541-51.
- Moazeni-Bistghani M, Mohammad-Alibayghi F. Effect of vasectomy on sexual function in men. *J Shahrekord Univ Med Sci* 2011;13:42-47.
- Ozkan B, Orhan E, Aktas N, Coskun ER. Depression and Sexual Dysfunction in Turkish Men Diagnosed with Infertility. *Urology* 2015;85:1389-93.
- McCabe MP, Sharlip ID, Lewis R, Atalla E, Balon R, Fisher AD, et al. Incidence and Prevalence of Sexual Dysfunction in Women and Men: A Consensus Statement from the Fourth International Consultation on Sexual Medicine 2015. *J Sex Med* 2016;13:144-52.
- Hurlbert DF. A comparative study using orgasm consistency training in the treatment of women reporting hypoactive sexual desire. *J Sex Marital Ther* 1993;19:41-55.

26. Nobre PJ, Pinto-Gouveia J. Dysfunctional sexual beliefs as vulnerability factors for sexual dysfunction. *J Sex Res* 2006;43:68-75.
27. Schoenfeld EA, Loving TJ, Pope MT, Huston TL, Stulhofer A. Does sex really matter? Examining the connections between spouses' nonsexual behaviors, sexual frequency, sexual satisfaction, and marital satisfaction. *Arch Sex Behav* 2017;46:489-501.
28. Smith AMA, Patrick K, Heywood W, Pitts MK, Richters J, Shelley JM, et al. Body mass index, sexual difficulties and sexual satisfaction among people in regular heterosexual relationships: A populationbased study. *Intern Med J* 2012;42:641-51.
29. Dundon CM, Rellini AH. More than sexual function: Predictors of sexual satisfaction in a sample of women age 40-70. *J Sex Med* 2010;7:896-904.
30. M.J. Muller, G Schiling, G. Haidl. Sexual satisfaction in male infertility, *Archives of Andrology* 1999;42:3:137-43.
31. Alan W. Shindel, Christian J. Nelson, Cathy K. Naughton, Michael Ohebshalom and John P. Mulhall. Sexual Function and Quality of Life in the Male Partner of Infertile Couples: Prevalence and Correlates of Dysfunction. *The Journal of Urology* 2008;179:1056-9.
32. Brotto L, Atallah S, Johnson-Agbakwu C, Rosenbaum T, Abdo C, Byers ES, et al. Psychological and Interpersonal Dimensions of Sexual Function and Dysfunction. *Sex Med* 2016;13:538-71.
33. Chachamovich J, Chachamovich E, Fleck MP, Cordova FP, Knauth D, Passos E. Congruence of quality of life among infertile men and women: findings from a couple-based study. *Hum Reprod* 2009;24:2151-7.
34. Theiss J. Modeling dyadic effects in the associations between relational uncertainty, sexual communication, and sexual satisfaction for husbands and wives. *Communic Res* 2011;38:565-84.
35. Sexual Dysfunction in Males: <https://my.clevelandclinic.org/health/diseases/9122-sexual-dysfunction-in-males>.
36. Amoo EO, Omideyi AK, Fadayomi TO, Ajayi MP, Oni GA. Male reproductive health challenges: appraisal of wives coping strategies Idowu. *Reprod Health* 2017;14:90.
37. Pasha H, Basirat Z, Esmailzadeh S, Faramarzi M, Adibrad H. Marital Intimacy and Predictive Factors Among Infertile Women in Northern Iran. *J Clin Diagn Res* 2017;11:13-7.
38. Joja OD, Dinu D, Paun D. Psychological aspects of male infertility. An overview. *Procedia - Social and Behavioral Sciences* 2015;187:359-63.
39. Story LB, Repetti R. Daily occupational stressors and marital behavior. *J Fam Psychol* 2006;20:690-700.
40. Alirezai S, Ozgoli G, Alavi Majd H. Evaluation of Factors Associated with Sexual Function in Infertile Women. *Int J Fertil Steril* 2018;12:125-9.
41. Audu BM. Sexual dysfunction among infertile Nigerian women. *J Obstet Gynaecol* 2002;22:655-7.
42. Cayan S, Akbay E, Bozlu M, Canpolat B, Acar D, Ulusoy E. The prevalence of female sexual dysfunction and potential risk factors that may impair sexual function in Turkish women. *Urol Int* 2004;72:52-7.
43. Perlis N, Lo KC, Grober ED, Spencer L, Jarvi K. Coital frequency and infertility: which male factors predict less frequent coitus among infertile couples? *Fertil Steril* 2013;100:511-5.



Evaluation inflammatory markers of hemogram parameters in primary ovarian insufficiency

Primer ovaryen yetersizlik hastalarında hemogramdaki enflamatuvar parametrelerin değerlendirilmesi

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Abstract

Objective: In most of primary ovarian insufficiency (POI) cases, etiologic factors have not been fully elucidated. Recent studies have revealed that inflammatory agents play an important role in the etiopathogenesis of POI. Therefore, the aim of this study was to investigate the role of inflammatory markers of hemogram parameters in POI.

Materials and Methods: The study compared 47 healthy women and 47 women diagnosed as having POI retrospectively by scanning electronic and written recording systems. Complete blood counts, day-3 hormone profiles levels of all subjects were analyzed. The neutrophil-lymphocyte ratio (NLR), red cell distribution width (RDW), platelet ratio (RPR), platelet lymphocyte ratio (PLR), and mean platelet volume (MPV) mean platelet lymphocyte ratio (MPLR) were calculated from the complete blood count parameters.

Results: White blood cell and MPV values, platelet, and lymphocyte counts were significantly higher in the POI patients ($p<0.001$, $p=0.042$, $p=0.038$, $p=0.049$, respectively), RPR was significantly lower than the control group ($p=0.011$), but there were no significant differences in hemoglobin, RDW, NLR, PLR, and MPLR ($p=0.454$, $p=0.057$, $p=0.635$, $p=0.780$, $p=0.126$, respectively). The neutrophil count of the study group was higher than in the control group ($p=0.057$). Bivariate correlation analyses showed no correlations between blood parameters and hormone levels. The area under the receiver operating characteristic curve for RPR in POI was 0.652, with a threshold value 0.053, sensitivity=63% and specificity=63.

Conclusion: Inflammatory markers of hemogram detected higher in patients with POI then control subjects.

Keywords: Primary ovarian insufficiency, inflammation, hemogram

Öz

Amaç: Pek çok primer ovaryen yetersizlik (POI) olgusunda yeterli etiyolojik değerlendirme yapılmamaktadır. Mevcut çalışmalar POI etyopatogenezinde enflamatuvar belirteçlerin rolü olduğunu göstermiştir. Bu çalışmanın amacı POI hastalarında hemogram parametrelerindeki enflamatuvar belirteçlerinin rolünü araştırmaktır.

Gereç ve Yöntemler: Çalışmada 47 sağlıklı kadın ile 47 POI hastası kadının hastane kayıtları retrospektif olarak karşılaştırılmıştır. Hemogram, ve 3. gün bakılan hormon profilleri değerlendirilmiştir. Hemogram bulgularından elde edilen nötrofil lenfosit oranı (NLR), eritrosit dağılım genişliği (RDW) platelet oranı (RPR), platelet lenfosit oranı (PLR) ve ortalama platelet hacmi (MPV) ortalama platelet lenfosit oranı (MPLR) hesaplanmıştır.

Bulgular: Lökosit ve ortalama MPV değerleri, platelet ve lenfosit sayıları POI hastalarında daha yüksek saptanmıştır (sırası ile $p<0.001$, $p=0.042$, $p=0.038$, $p=0.049$), RPR ise kontrol grubuna göre daha düşük saptanmıştır ($p=0.011$). Hemoglobin, RDW, NLR, PLR ve MPLR ($p=0.454$, $p=0.057$, $p=0.635$, $p=0.780$, $p=0.126$) açısından ise fark saptanmamıştır. Hasta grubunda nötrofil sayısı daha yüksek bulunmuştur ($p=0.057$). Bivariate korelasyon analizlerinde hemogram parametreleri ile hormonal düzeyler arasında bir korelasyon saptanmamıştır. POI hastalarında RPR için eğri altında kalan alan 0.652, sensitivite: %63 and spesifite: 63 bulunmuştur.

Sonuç: POI olgularında hemogramda enflamasyonda artan belirteçler kontrol grubuna göre daha yüksek saptanmıştır.

Anahtar Kelimeler: Primer ovaryen yetersizlik, enflamasyon, hemogram

PRECIS: Inflammatory markers of hemogram detected higher in patients with POI then control subjects.

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Introduction

Primary ovarian insufficiency (POI), also known as premature ovarian failure prior to 2008, is defined as the presence of reduced ovarian functions in women younger than 40 years, characterized by oligo- or amenorrhea, sub- or infertility, loss of residual follicles in the gonads, low estradiol levels, and high (menopausal) follicle-stimulating hormone (FSH) levels^(1,2). The risk of POI in women before the age of 40 is 1%⁽³⁾. POI is seen in one ten thousandth in the 18-25 age group, one thousandth in the 25-30 age group, and one percent in the 35-40 age group. Fifteen percent of patients with POI are familial, suggesting an underlying genetic factor^(4,5).

In the case of oligo- or amenorrhea (primary or secondary) for 4-6 months, the presence of hypergonadotropic hypogonadism in any woman younger than 40 years is sufficient for the diagnosis of POI⁽⁶⁾. However, symptoms of hypoestrogenism, the decrease in ovarian functions, and pace of events may affect the clinical picture. Thus, clinical manifestations of POI are diverse, including underlying etiologic factors, and the clinical manifestations of hypoestrogenism, such as hot flashes, dyspareunia, sleep disorders, decreased libido or vaginal dryness. Therefore, clinical manifestations of POI are diverse, including clinical signs of underlying etiologic factors and symptoms of hypoestrogenism such as hot flashes, dyspareunia, sleep disorders, decreased libido or vaginal dryness^(7,8).

A number of reasons for the etiopathogenesis of POI have been elucidated. Some of them are genetic anomalies, metabolic diseases, autoimmunity, iatrogenic events such as chemotherapy, infections, and environmental factors. However, in most cases the etiologic factor cannot be known and therefore these cases are called spontaneous or idiopathic⁽¹⁾. Recent studies have revealed that inflammatory agents play an important role in the etiopathogenesis of POI. In ovarian biopsies of patients with POI, it was shown that there are inflammatory cells associated with lymphocytic infiltration and other immune responses⁽⁹⁻¹³⁾. Inflamm-aging, defined as inflammation caused by aging, and inflammatory cells reacting at ovaries as a result of autoimmunity play an important role in idiopathic and unknown cases^(14,15). It has also been demonstrated in studies that estrogen depletion leads to pro-inflammatory processes secondary to an increase in proinflammatory markers, and oral estrogen treatment leads to a reduction in inflammatory marker concentrations⁽¹⁶⁻²⁰⁾. Inflammation, which plays a role in the etiopathogenesis of POI, and increased inflammatory markers triggered by decreased estrogen after POI development enter a cycle that triggers each other.

The aim of this study was to reveal the pathophysiologic role of inflammation and inflammatory markers in POI by comparing inflammatory markers including neutrophils, lymphocytes, and the values of hemoglobin (HGB), white blood cell (WBC), mean platelet volume (MPV), red cell distribution width (RDW), neutrophil lymphocyte ratio (NLR), RDW platelet ratio (RPR), platelet lymphocyte ratio (PLR), mean platelet lymphocyte

ratio (MPLR) with a control group, to determine whether these markers could be used in the diagnosis and to investigate relationships between these markers and hormones such as FSH and luteinizing hormone (LH).

Materials and Methods

This study was conducted as a retrospective and comparative cross sectional study at Çanakkale Onsekiz Mart University Hospital between January 2016 and December 2018 and Çanakkale State Hospital between January 2016 and December 2017. The study group comprised 47 patients with POI who were admitted to these clinics, and the control group consisted of 47 healthy females. All procedures performed in human studies were conducted in accordance with the ethical standards of the institutional and/or national research committee and the ethical standards of the 1964 Helsinki Declaration. The study was approved by Çanakkale Onsekiz Mart University Local Ethics Committee (approval number: 02.05.2018/09).

Patients aged between 20 and 40 years who were diagnosed as having POI were included in the study group. Women aged <40 years with elevated FSH levels >25 mIU/mL and accompanying 4-6 months of amenorrhea or oligomenorrhea periods, as identified in European Society of Human Reproduction and Embryology guideline, were considered as having POI⁽⁶⁾. Exclusion criteria of the study group were polycystic ovary syndrome, pregnancy, chronic medical disease, hyperprolactinemia, presence of hypothalamic or pituitary disease, hyperthyroidism, chemotherapy, and pelvic surgery prior to study and abnormal karyotype analysis. The inclusion criteria for healthy individuals in the control group were in the age range of 20-40 years, regular menstrual cycles, no use of drugs including contraceptive hormone therapies, and no known chronic disease.

From all of the participants, blood samples taken after 8-10 hours of fasting were collected in a tube with ethylenediamine tetraacetic acid (EDTA) and in a gel tube without anticoagulant. Complete blood counts in the tubes with EDTA were analyzed using an auto hematology analyzer. In this study, we evaluated the analytical performance of automated cellular analyzers for BF analysis: the UniCel DxH 800 (Beckman Coulter, Brea, CA, USA). Plasma levels of FSH, LH, prolactin, and thyroid-stimulating hormone in the gel tubes were measured by electrochemiluminescence immunoassay on a Cobas 6000 analyzer (Roche Diagnostics Germany) after centrifuging and separating their serum. NLR, RPR, PLR, and MPLR were calculated from the complete blood count parameters.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) statistics 20 software. The data were reported as the mean $\pm$ SD and median (minimum-maximum). The Shapiro-Wilk tests, skewness and kurtosis values, histograms, and detrended normal q-q plots were used for normality analyses of parameters. Student's t-test

was used for comparisons between groups of parameters with normal distribution, and the Mann-Whitney U test was used for parameters with non-normal distribution. According to parameter suitability Pearson's (for normal distribution) or Spearman's correlation tests were used for correlation analyses. $P < 0.05$ was considered as statistically significant.

Results

The average and median age of patients with POI and the control group were 31.6 ± 7.05 and 35 (range, 20-40) years and 30.3 ± 5.38 and 31 (range, 20-39) years. The baseline characteristics and hormone levels of both groups are shown in Table 1.

The mean levels (with standard deviations) and median levels (with minimum and maximum) of blood parameters of 47 patients with POI and 47 healthy individuals are depicted in Table 2. When the parameters in the complete blood count were analyzed, it was found that values of WBC and MPV, platelet and lymphocyte counts were significantly higher in the POI patients

($p < 0.001$, $p = 0.042$, $p = 0.038$, $p = 0.049$, respectively), RPR was significantly lower than the control group ($p = 0.011$), but there were no significant differences in HGB, RDW, NLR, PLR, and MPLR ($p = 0.454$, $p = 0.057$, $p = 0.635$, $p = 0.780$, $p = 0.126$, respectively). The neutrophil count of the study group was higher than in the control group. However, even though there was not a significant relationship, the p value was very near to 0.05 ($p = 0.057$).

Bivariate correlation analyses were conducted to determine relationships between the hormone levels (FSH and LH) and parameters of complete blood count in patients with POI (Table 3). However, no correlations were found between blood parameters and hormone levels in patients with POI.

We also performed receiver operating characteristic (ROC) curve analysis for RPR in the POI group. The area under the ROC curve for RPR in POI was 0.652 (Figure 1), with a threshold value 0.053. It discriminated patients with POI lower than 0.053 with a sensitivity=63% and specificity=63% (95% CI: 0.540-0.764; $p = 0.011$).

Table 1. Baseline characteristics and hormone levels of the primary ovarian insufficiency patients and the control group

	Study group (n=47)		Control group (n=47)		p
	Mean $\pm$ SD	Median (min-max)	Mean $\pm$ SD	Median (min-max)	
FSH	63.4 ± 25.9	59.3 (29-125)	7.3 ± 2.7	6.8 (2.5-14.3)	$<0.001^1$
LH	30.6 ± 19.3	25.8 (3.3-89.6)	8.6 ± 4.2	7.3 (3-19.9)	$<0.001^1$
PRL	15.2 ± 8.5	13.1 (6.3-58.5)	16.9 ± 9.2	15.1 (3.3-57.5)	0.143 ²
TSH	2.5 ± 1.3	2.2 (0.1-6.6)	2.3 ± 1.2	2.1 (0.5-5.6)	0.533 ¹
Glucose	87.6 ± 16.4	89 (8.8-115)	89.7 ± 9.7	90.4 (70.7-110)	0.666 ²

SD: Standard deviation, Min: Minimum, Max: Maximum, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, PRL: Prolactin, TSH: Thyroid-stimulating hormone, ¹Student's t-test, ²Mann-Whitney U test

Table 2. Blood parameters of the primary ovarian insufficiency patients and the control group

	Study group (n=47)		Control group (n=47)		p
	Mean $\pm$ SD	Median (min-max)	Mean $\pm$ SD	Median (min-max)	
WBC	9.1 ± 2.3	9 (5.1-15.9)	7.4 ± 2.8	6.9 (4.2-22.4)	$<0.001^2$
HGB	13.1 ± 3.4	12.5 (9.2-33.4)	12.7 ± 1.5	13 (8.2-14.5)	0.454 ²
Platelet	294.7 ± 77.8	278 (156-456)	262.5 ± 72.4	250 (109-515)	0.042 ²
MPV	8.7 ± 0.9	8.7 (6.9-10.6)	8.3 ± 1.1	8.4 (5.4-11)	0.038 ¹
Neutrophil	6.1 ± 5.7	4.7 (1.3-28.7)	4.9 ± 5.2	3.7 (0.2-29.3)	0.057 ²
Lymphocyte	2.4 ± 0.7	2.5 (0.7-3.9)	2.1 ± 0.8	2.2 (1.1-4.6)	0.049 ²
RDW	14.2 ± 2.6	13.5 (11.8-28.7)	14.8 ± 3.5	13.8 (12-28.7)	0.233 ²
NLR	2.95 ± 3.42	1.74 (0.41-16.9)	2.63 ± 2.94	1.75 (0.04-17.06)	0.754 ²
PLR	133.4 ± 53.5	121.3 (58.4-320)	139.5 ± 65.4	130.5 (45.4-400.9)	0.780 ²
RPR	0.051 ± 0.017	0.048 (0.03-0.111)	0.06 ± 0.02	0.054 (0.34-0.126)	0.011 ²
MPLR	4.11 ± 2.1	3.61 (2.15-15.14)	4.43 ± 1.63	4.21 (1.76-7.54)	0.126 ²

SD: Standard deviation, Min: Minimum, Max: Maximum, WBC: White blood cell, HGB: Hemoglobin, MPV: Mean platelet volume, RDW: Red cell distribution width, NLR: Neutrophil lymphocyte ratio, RPR: Platelet ratio, RDW: red cell distribution width, PLR: Platelet lymphocyte ratio, MPLR: MPV lymphocyte ratio, ¹Student's t-test, ²Mann-Whitney U test

Discussion

The etiology is not known in 90% of POI cases and it is thought that autoimmunity and inflammation may play an important role in ovarian dysfunction in most of these unknown cases.

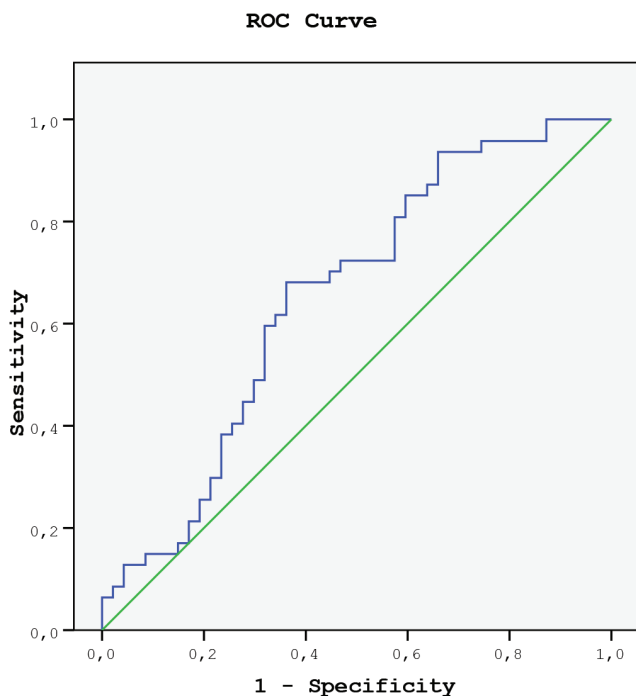


Figure 1. Receiver operating characteristic curve of platelet ratio to predict cases with primary ovarian insufficiency

ROC: Receiver operating characteristic

Table 3. Correlations between follicle-stimulating hormone, luteinizing hormone, and blood parameters

R	FSH		LH	
	R	p	R	p
WBC	0.126	0.400 ²	0.209	0.159 ²
HGB	0.101	0.498 ²	-0.049	0.741 ²
Platelet	0.097	0.516 ²	-0.072	0.632 ²
MPV	-0.179	0.230 ¹	-0.171	0.250 ¹
Neutrophil	-0.225	0.129 ²	-0.118	0.430 ²
Lymphocyte	0.225	0.128 ²	0.115	0.443 ²
RDW	-0.227	0.124 ²	-0.056	0.710 ²
NLR	-0.242	0.101 ²	-0.102	0.495 ²
PLR	-0.141	0.344 ²	-0.126	0.400 ²
RPR	-0.168	0.258 ²	0.026	0.864 ²
MPLR	-0.248	0.093 ²	-0.093	0.535 ²

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, WBC: White blood count, HGB: Hemoglobin, MPV: Mean platelet volume, RDW: Red cell distribution width NLR: Neutrophil lymphocyte ratio, PLR: Platelet lymphocyte ratio, RPR: RDW platelet ratio, MPLR: MPV lymphocyte ratio, ¹Pearson's correlation test, ²Spearman's correlation test

In recent years, some studies have been published on the increasing importance of inflammatory agents in ovarian diseases. Interests in this issue have been raised by the detection of xanthogranulomatous inflammation in a case with POI by Singh et al.⁽²¹⁾. In an experimental study on mice by Altuntas et al.⁽²²⁾, it was demonstrated that the targeting action of inhibin-alpha on autoimmunity was initiated by CD4 T helper cells, by stimulating B cells. Said et al.⁽²³⁾ also found that in primary ovarian failure induced by ionizing radiation and resveratrol, the expression of proinflammatory factors was increased and anti-inflammatory factors were inhibited. Tung et al.⁽²⁴⁾ showed that in loss of immune regulation in a mouse model, B lymphocytes stimulated by proinflammatory T lymphocytes and autoantibodies against oocyte antigens played important roles in the pathogenesis of POI. Huang et al.⁽¹⁴⁾ claimed in their review article that increased inflammatory cytokines and decreased anti-inflammatory cytokines played a crucial role in aging-related inflammation and POI. In addition, a decrease in the body's estrogen concentration during POI can lead to the activation of the pro-inflammatory pathway and B cells, tumor necrosis factor- α , interferon- γ , interleukin 1 and natural killer cells involved in this pathway⁽¹⁶⁾.

Randomized controlled studies investigating the role of inflammatory markers in etiopathogenesis of POI is not sufficiently available in the literature. The first study on this subject was conducted by Miyake et al.⁽²⁵⁾. In this study, subtypes of lymphocytes and autoantibodies of peripheral blood lymphocytes were investigated in 20 patients with POI. They found that the total lymphocyte count and CD4 T lymphocyte count were increased and CD8 T lymphocytes count was decreased in patients with POI compared with women in the same age group. However, these differences were not significant. Although lymphocyte subgroups were not investigated in our study, the lymphocyte count was significantly increased in the POI patients. In recent years, there have been studies showing that the lymphocyte count increased significantly in patients with POI; however, there are also studies in which no significant differences were found. In a study conducted by Yildirim et al.⁽²⁶⁾ on investigating inflammatory markers including 43 patients in the study group and 41 women in the control group, they showed that lymphocyte counts were significantly increased in the study group, but there were no significant differences in neutrophil counts and values of WBC and MPV. In a study conducted by Sanverdi et al.⁽²⁰⁾ on 96 patients POI and 110 healthy women, although the lymphocyte count was significantly increased and neutrophil count was significantly decreased, no significant changes were observed in parameters including MPV, RDW, and platelet count. However, İlhan et al.⁽³⁶⁾ and Akdemir et al.⁽²⁷⁾ found no significant differences between neutrophil and lymphocyte counts in their studies on complete blood counts in patients with POI⁽²⁰⁾. In our results, values of WBCs and MPV, platelets, and lymphocyte counts were significantly increased in patients with POI, but

there were no significant changes in values of HBG, RDW, and neutrophil count compared with the control group. While the above-mentioned studies showed no significant correlations between hormone levels and inflammatory parameters, there were also no significant correlations between them in our study. The NLR, RPR, PLR, and MPLR have been reported as laboratory markers for inflammation and diseases related to inflammatory processes⁽²⁸⁾. This NLR is a method that is frequently used in recent years to determine the degree of inflammation because it is easy to measure, frequent, and cheap⁽²⁹⁻³⁶⁾. In the literature, the predictive value of NLR in diabetes mellitus, thyroid dysfunction, essential hypertension, renal disease, heart valve disease, autoimmune diseases, and malignancies including ovarian, lung, renal, and colorectal cancers has been investigated⁽²⁹⁻⁴⁰⁾. The PLR has also been found to be an independent risk factor for survival in patients with malignancies such as pancreatic and colorectal cancer⁽³⁷⁻⁴⁰⁾. The RPR has resulted in a valuable new laboratory test for predicting mortality in acute pancreatitis, hepatic fibrosis, and cirrhosis^(41,42). The PLR was also found to be an independent risk factor for survival in patients with malignancies such as pancreatic and colorectal cancer. The predictive value of NLR in POI and whether it can be used in the diagnosis of POI has been a frequent research topic in recent studies. İlhan et al.⁽³⁶⁾ used NLR, PLR, and RPR as diagnostic markers in patients with POI and they found that only NLR showed a statistically significant difference between the groups. ROC analysis showed that NLR could be a marker for the diagnosis of POI. In this study, NLR was found to be significantly increased in patients with POI, whereas Sanverdi et al.⁽²⁰⁾ and Yildirim et al.⁽²⁶⁾ found that NLR was significantly decreased in women with POI. In the study conducted by Akdemir et al.⁽²⁷⁾, there was no significant difference in NLR rates between the groups, as we found. Sanverdi et al.⁽²⁰⁾ also found that MPLR was significantly higher in the POI group and MPLR might be a marker for the diagnosis of POI. When the results of this literature and our results are evaluated together, it can be concluded that the use of NLR in the early diagnosis of POI is not correct, but according to our ROC analysis, RPR can be used for the diagnosis of POI.

Conclusion

In conclusion, inflammatory markers of hemogram were detected higher in patients with POI than in control subjects. Even though the role of inflammatory cytokines in the etiopathogenesis of POI has been elucidated in experimental studies, it has not been adequately demonstrated in clinical studies. More studies with more patient groups are needed to reveal the role of inflammation and inflammatory markers of hemogram in the pathogenesis of POI.

Ethics

Ethics Committee Approval: The study was approved by Çanakkale Onsekiz Mart University Local Ethics Committee (approval number: 02.05.2018/09).

Informed Consent: This is a retrospective study and no need a informed consent.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Surgical and Medical Practices: B.D., S.S.D., Concept: B.D., Data Collection or Processing: Analysis or Interpretation: K.Ö.K., S.S.D., Literature Search: B.D., S.P., F.S., Writing: B.D., S.P.

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References

1. Rudnicka E, Kruszevska J, Klicka K, Kowalczyk J, Grymowicz M, Skórska J, et al. Premature ovarian insufficiency - aetiopathology, epidemiology, and diagnostic evaluation. *Prz Menopauzaln*. 2018;17:105-8.
2. Bukulmez O. Definitions and Relevance: Diminished Ovarian Reserve, Poor Ovarian Response, Advanced Reproductive Age, and Premature Ovarian Insufficiency. *Diminished Ovarian Reserve and Assisted Reproductive Technologies*: Springer; 2020. p. 55-61.
3. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;67:604-6.
4. Franic-Ivanisevic M, Franic D, Ivovic M, Tancic-Gajic M, Marina L, Barac M, et al. Genetic Etiology of Primary Premature Ovarian Insufficiency. *Acta Clin Croat* 2016;55:629-35.
5. Cramer DW, Xu H. Predicting age at menopause. *Maturitas*. 1996;23:319-26.
6. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. *Hum Reprod* 2016;31:926-37.
7. Torrealday S, Kodaman P, Pal L. Premature Ovarian Insufficiency - an update on recent advances in understanding and management. *F1000Res* 2017;6:2069.
8. Rebar RW. Premature ovarian failure. *Obstet Gynecol*. 2009;113:1355-63.
9. Sen A, Kushnir VA, Barad DH, Gleicher N. Endocrine Autoimmune Diseases and Female Infertility. *Nat Rev Endocrinol* 2014;10:37-50
10. Sammaritano LR. Menopause in Patients With Autoimmune Diseases. *Autoimmun Rev* 2012;11:A430-6.
11. Petriková J, Lazurova I. Ovarian failure and polycystic ovary syndrome. *Autoimmunity Reviews* 2012;11:A471-8.
12. Carp HJA, Selmi C, Shoenfeld Y. The Autoimmune Bases of Infertility and Pregnancy Loss. *J Autoimmun* 2012;38:J266-74.
13. Reato G, Morlin L, Chen S, Furmaniak J, Smith BR, Masiero S, et al. Premature Ovarian Failure in Patients With Autoimmune Addison's Disease: Clinical, Genetic, and Immunological Evaluation. *J Clin Endocrinol Metab*. 2011;96:E1255-61.
14. Huang Y, Hu C, Ye H, Luo R, Fu X, Li X, et al. Inflamm-Aging: A New Mechanism Affecting Premature Ovarian Insufficiency. *Journal of Immunology Research*. 2019;1-7
15. Sharif K, Watad A, Bridgewood C, Kanduc D, Amital H, Shoenfeld Y. Insights Into the Autoimmune Aspect of Premature Ovarian Insufficiency. *Best Pract Res Clin Endocrinol Metab* 2019;101323.
16. Straub RH. The Complex Role of Estrogens in Inflammation. *Endocr Rev* 2007;28:521-74.

17. Fröhlich M, Mühlberger N, Hanke H, Imhof A, Döring A, Pepys MB, et al. Markers of inflammation in women on different hormone replacement therapies. *Annals of Medicine* 2003;35:353-61.
18. Lacut K, Oger E, Le Gal G, Blouch M-T, Abgrall J-F, Kerlan V, et al. Differential Effects of Oral and Transdermal Postmenopausal Estrogen Replacement Therapies on C-reactive Protein. *Thrombosis and haemostasis* 2003;90:124-31.
19. Silvestri A, Gebara O, Vitale C, Wajngarten M, Leonardo F, Ramires JA, et al. Increased Levels of C-reactive Protein After Oral Hormone Replacement Therapy May Not Be Related to an Increased Inflammatory Response. *Circulation* 2003;107:3165-9.
20. Sanverdi I, Kilicci C, Cogendez E, Abide Yayla C, Ozkaya E. Utility of Complete Blood Count Parameters to Detect Premature Ovarian Insufficiency in Cases with Oligomenorrhea/Amenorrhea. *J Clin Lab Anal* 2018;32:e22372.
21. Singh N, Dadhwal V, Sharma KA, Mittal S. Xanthogranulomatous Inflammation: A Rare Cause of Premature Ovarian Failure. *Arch Gynecol Obstet* 2009;279:729-31.
22. Altuntas CZ, Johnson JM, Tuohy VK. Autoimmune Targeted Disruption Of The Pituitary-Ovarian Axis Causes Premature Ovarian Failure *J Immunol* 2006;177:1988-96.
23. Said RS, El-Demerdash E, Nada AS, Kamal MM. Resveratrol Inhibits Inflammatory Signaling Implicated in Ionizing Radiation-Induced Premature Ovarian Failure Through Antagonistic Crosstalk Between Silencing Information Regulator 1 (SIRT1) and poly (ADP-ribose) Polymerase 1 (PARP-1). *Biochem Pharmacol* 2016;103:140-50.
24. Tung KS, Garza KM, Lou Y, Bagavant H. Autoimmune Ovarian Disease: Mechanism of Induction and Prevention. *J Soc Gynecol Investig* 2001;8:S49-51.
25. Miyake T, Sato Y, Takeuchi S. Implications of Circulating Autoantibodies and Peripheral Blood Lymphocyte Subsets for the Genesis of Premature Ovarian Failure. *J Reprod Immunol* 1987;12:163-71.
26. Yıldırım G, Tokmak A, Kokanal MK, Sarkaya E, Züngün C, Inal HA, et al. Association Between Some Inflammatory Markers and Primary Ovarian Insufficiency. *Menopause* 2015;22:1000-5.
27. Akdemir N, Bostanci MS, Tozlu F, Özden S, Ünal O, Cevrioglu AS. Neutrophil/lymphocyte ratio as new-inflammatory biomarkers Primary ovarian insufficiency (POI) patients. *Journal of The Turkish German Gynecological Association* 2016;17:6.
28. Toktas O, Aslan M. Mean platelet volume, red cell distribution width, neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in the diagnosis acute appendicitis. *Eastern Journal Medicine* 2017;22:5-9.
29. Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The Baseline Ratio of Neutrophils to Lymphocytes is Associated With Patient Prognosis in Advanced Gastric Cancer. *Oncology* 2007;73:215-20.
30. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, 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.
31. Azab B, Jaglall N, Atallah JP, Lamet A, Raja-Surya V, Farah B, et al. Neutrophil-lymphocyte Ratio as a Predictor of Adverse Outcomes of Acute Pancreatitis. *Pancreatology* 2011;11:445-52.
32. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The Systemic Inflammation-Based Neutrophil-Lymphocyte Ratio: Experience in Patients With Cancer. *Crit Rev Oncol Hematol*. 2013;88:218-30.
33. Stotz M, Gerger A, Eisner F, Szkandera J, Loibner H, Ress A, et al. Increased Neutrophil-Lymphocyte Ratio Is a Poor Prognostic Factor in Patients With Primary Operable and Inoperable Pancreatic Cancer. *Br J Cancer* 2013;109:416-21.
34. Zheng Y-B, Zhao W, Liu B, Lu L-G, He X, Huang J-W, et al. The Blood Neutrophil-To-Lymphocyte Ratio Predicts Survival in Patients With Advanced Hepatocellular Carcinoma Receiving Sorafenib. *Asian Pac J Cancer Prev* 2013;14:5527-31.
35. Tanoglu A, Karagoz E. Predictive Role of the Neutrophil-To-Lymphocyte Ratio in Patients With Advanced Hepatocellular Carcinoma Receiving Sorafenib. *Asian Pac J Cancer Prev* 2014;15:1063.
36. İlhan M, İlhan G, Gök AFK, Bademler S, Verit Atmaca F, Ertekin C. Evaluation of Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio and Red Blood Cell Distribution Width-Platelet Ratio as Early Predictor of Acute Pancreatitis in Pregnancy. *J Matern Fetal Neonatal Med* 2016;29:1476-80.
37. Betterle C, Volpato M, Smith BR, Furmaniak J, Chen S, Greggio NA, et al. I. Adrenal Cortex and Steroid 21-Hydroxylase Autoantibodies in Adult Patients With Organ-Specific Autoimmune Diseases: Markers of Low Progression to Clinical Addison's Disease. *J Clin Endocrinol Metab* 1997;82:932-8.
38. van Der Voort D, van Der Weijer P, Barentsen R. Early Menopause: Increased Fracture Risk at Older Age. *Osteoporosis Int* 2003;14:525-30.
39. Schmidt PJ, Cardoso GM, Ross JL, Haq N, Rubinow DR, Bondy CA. Shyness, Social Anxiety, and Impaired Self-Esteem in Turner Syndrome and Premature Ovarian Failure. *Jama* 2006;295:1374-6.
40. Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature Menopause or Early Menopause: Long-Term Health Consequences. *Maturitas*. 2010;65:161-6.
41. Chen B, Ye B, Zhang J, Ying L, Chen Y. RDW to Platelet Ratio: A Novel Noninvasive Index for Predicting Hepatic Fibrosis and Cirrhosis in Chronic Hepatitis B. *PLoS one*. 2013;8:e68780.
42. Çetinkaya E, Şenol K, Saylam B, Tez M. Red cell distribution width to platelet ratio: New and promising prognostic marker in acute pancreatitis. *World J Gastroenterol* 2014;20:14450-4.



Effects of laparoscopic cystectomy on ovarian reserve in patients with endometrioma and dermoid cyst

Endometriomalı ve dermoid kistli hastalarda laparoskopik kistektominin ovaryan rezerv üstüne etkisi

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Abstract

Objective: To compare the effects of laparoscopic cystectomy on ovarian reserve between women with endometrioma and dermoid cyst.

Materials and Methods: Thirty-six patients were diagnosed as having endometrioma (group A) and 32 patients with dermoid cyst (group B) using ultrasonography. Preoperative anti-mullerian hormone (AMH) levels were measured and unilateral antral follicle counts (AFC) were calculated for the ovary side containing the cyst. Laparoscopic cystectomy was performed using the stripping technique for all participants. After 3 months, all participants were re-evaluated between the third and sixth day of their menstrual cycle to determine AFC and AMH levels.

Results: The mean serum preoperative AMH level and AFC level were significantly lower in group A than in group B ($p=0.001$, $p=0.002$), respectively. At 3 months after the surgery, serum AMH levels decreased significantly in group A from 2.04 ± 0.68 to 1.47 ± 0.55 ($p=0.001$), and from 2.60 ± 0.57 to 2.17 ± 0.56 in group B ($p=0.001$). In group A, unilateral (operated side) AFC levels decreased significantly from 4.05 ± 1.24 to 2.16 ± 0.94 ($p=0.001$), and in group B, it decreased significantly from 4.93 ± 0.94 to 3.40 ± 0.87 ($p=0.001$). The decrease in AMH levels was significantly higher in group A than in group B ($p=0.033$). The decrease in AFC levels was also significantly higher in group A than in group B ($p=0.044$).

Conclusion: Laparoscopic stripping has destructive effects on serum AMH levels and the operated side AFC levels after surgery for patients with endometrioma and dermoid cysts, and laparoscopic excision of endometrioma has more destructive effects on ovarian reserve than dermoid cysts.

Keywords: Antral follicle count, anti-mullerian hormone, ovarian reserve, laparoscopic cystectomy, endometrioma

Öz

Amaç: Laparoskopik kistektominin ovaryan rezerv üzerine etkisini endometriomalı ve dermoid kistli hastalarda kanıtlamaktır.

Gereç ve Yöntemler: Ultrasonografik olarak preoperatif tanı konulmuş 36 endometriomalı (grup A) ve 32 dermoid kistli (grup B) hasta çalışmaya alındı. Preoperatif anti-mullerian hormon (AMH) ve kistin olduğu overdeki antral folikül sayıları (AFC) ölçüldü. Tüm hastalara laparoskopik kistektomi stripping tekniği uygulanarak yapıldı. Ameliyattan 3 ay sonra tüm hastaların AMH ve AFC ölçümleri siklusun 3. ve 6. günleri arası tekrar yapıldı.

Bulgular: Preoperatif serum AMH seviyeleri ve opere olan taraftaki AFC değerleri grup A'da grup B'de göre anlamlı derecede düşük saptandı [$(p=0,001)$, $(p=0,002)$], sırasıyla. Ameliyattan 3 ay sonra serum AMH seviyesi grup A'da $2,04\pm0,68$ 'den $1,47\pm0,55$ 'e ($p=0,001$), grup B'de $2,60\pm0,57$ 'den $2,17\pm0,56$ 'ya ($p=0,001$) düştü. AFC seviyesi grup A'da ameliyattan 3 ay sonra $4,05\pm1,24$ 'ten $2,16\pm0,94$ 'e, grup B'de $4,93\pm0,94$ 'den $3,40\pm0,87$ 'ye ($p=0,001$) düşmüştür. AMH seviyesindeki düşüş grup A'da grup B'ye göre anlamlı derecede daha fazla olarak görüldü ($p=0,033$). AFC değerindeki düşüş grup A'da grup B'ye göre anlamlı oranda daha fazla idi ($p=0,044$).

Sonuç: Laparoskopik stripping tekniği hem endometriomalı hem de dermoid kistli hastalarda serum AMH ve opere olan taraftaki AFC seviyeleri üstünde yıkıcı etkilere sahiptir. Endometriomaların laparoskopik olarak çıkartılması ovaryan rezerv üzerine dermoid kistlere göre daha yıkıcı etkilere sahiptir.

Anahtar Kelimeler: Antral folikül sayısı, anti müllerien hormon, ovaryan rezerv, laparoskopik kistektomi, endometrioma

PRECIS: Laparoscopic excision of endometrioma has more destructive effects on ovarian reserve than dermoid cysts.

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Introduction

Endometriomas are found in 17-44% of women with endometriosis, causing dysmenorrhea, chronic pelvic pain, and increased infertility risk⁽¹⁾. Mature ovarian cystic teratomas, called dermoid cysts, are the most common benign ovarian cysts in reproductive age women and constitute up to 20% of all ovarian tumors⁽²⁾. Laparoscopic cystectomy is considered the first-line choice for endometrioma and dermoid cysts^(3,4) and associated with better pain control and shorter hospital stay. Laparoscopic stripping is the standard laparoscopic method for these ovarian tumors⁽⁵⁾.

Many tests and markers are used to determine the ovarian reserve⁽⁶⁾, such as serum levels of estradiol, follicle-stimulating hormone, anti-müllerian hormone (AMH), inhibin-B, and antral follicle count (AFC), of which AMH is the best marker⁽⁷⁾. Compared with AMH, AFC may be a more accurate marker of post-surgery ovarian reserve due to the laterality of diseases⁽⁸⁾, consequently, AMH and AFC are widely used.

Many studies reported increased risk of ovarian damage and decreased serum AMH levels after laparoscopic cystectomy in women with endometrioma⁽⁹⁻¹¹⁾. Although some studies reported that laparoscopic surgery did not damage the ovaries⁽⁸⁾, and laparoscopic cystectomy generally harmed the ovaries, resulting in decreased post-surgery ovarian reserve. Similarly, laparoscopic dermoid cyst excision may reduce the ovarian reserve⁽¹²⁾; however, it is unclear whether ovarian reserve reduction is dependent of the histological type of ovarian cyst after laparoscopic surgery, and results are conflicting⁽¹³⁻¹⁵⁾. Thus, this study aimed to compare the effects of laparoscopic cystectomy on ovarian reserve between women with endometrioma and dermoid cysts.

Materials and Methods

Design

This prospective observational study was conducted at Okan University Faculty of Medicine Hospital between January 2016 and August 2019. The study was reviewed by the Ethics Committee of İstanbul Medeniyet University Faculty of Medicine (approval number: 01435-2019) and was conducted in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. Written informed consent was obtained from all participants.

Participants

Female patients aged 18-35 years with endometrioma and/or dermoid cysts measuring ≥ 4 cm diagnosed using ultrasonography were selected for the study. Among the identified patients, 68 patients who underwent laparoscopic surgery due to unilateral endometrioma or unilateral dermoid cyst were analyzed. Of these patients, 36 were diagnosed as having endometrioma and 32 with dermoid cysts. Women who had a history of ovarian surgery, oligomenorrhea or amenorrhea, with more than one

unilateral cyst or bilateral cysts, those who were diagnosed as having polycystic ovary syndrome, endocrinologic diseases such as hyperprolactinemia, hypothyroidism or hyperthyroidism, and taking medications such as gonadotropin-releasing hormone analogs or oral contraceptives, which may affect ovarian reserve, in the previous six months were excluded from the study.

Procedures

Between the third and sixth day of the menstrual cycle, transvaginal or transrectal ultrasonography was performed to all patients to determine the type and size of the endometrioma and dermoid cyst. On the same day, blood samples of the participants were collected to determine preoperative AMH levels. Unilateral AFCs were calculated for the ovary side containing the cyst. The diagnosis criteria of endometrioma determined using ultrasonography were as follows: detection of the cystic structure with homogeneously decreased internal echogenicity without papillary structure and associated with weak vascularization, or the presence of a cystic structure with homogeneously decreased internal echogenicity with an echogenic portion without any flow⁽¹⁶⁻¹⁸⁾. Moreover, the diagnostic criteria for dermoid cysts based on ultrasonography were as follows: the presence of a shadowing echodensity, regionally diffuse bright echogenicity, hyperechoic lines and dots, and a fat-fluid level^(19,20). After measuring the cysts in three dimensions, the mean diameter of the endometrioma or dermoid cysts was also measured⁽²¹⁾. The AFC was defined as the number of follicles with a diameter of 2-9 mm in the ovary where the cyst was located. A 5-9-MHz endovaginal probe (Voluson E6 General Electric, Milwaukee, Wauwatosa, USA) was used for the ultrasonographic evaluation in all patients, which was performed by the same surgeon.

After determining the cysts' location, all participants underwent laparoscopic surgery. All laparoscopic operations were performed by the same surgery team.

Four ports were used in the operation. After a sub-umbilical vertical incision, an 11 mm trocar was inserted, and pneumoperitoneum was provided by insufflation of CO₂ (14 mmHg). Then, two left lateral 5 mm trocars and a right lateral 5 mm trocar were inserted. After exploration, the cyst was determined, and cyst wall stripping was used for all patients. To remove the cyst, the cyst wall was identified first and removed from the ovary by traction using graspers. For hemostasis, minimal bipolar electrocoagulation was carefully employed to minimize damage to the ovarian vascularity. For reconstruction, the ovary was sutured as necessary. Specimens were extracted from the abdomen using an endobag, and all samples were sent to the laboratory for pathologic examination. No major complications were reported during surgery. After pathologic evaluation, the preoperative diagnoses of the cysts were confirmed.

At three months after the operation, all participants were re-examined between the third and sixth day of their menstrual

cycle to determine AFC and AMH levels. The preoperative and postoperative AFC and AMH levels of group A and group B were compared. The two groups were matched by age and body mass index (BMI). Moreover, the differences in AFC and AMH levels were compared between patients who underwent laparoscopic surgery for endometrioma and dermoid cysts.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Student's t-test and the Mann-Whitney U test were used for comparisons between groups, where appropriate. To determine the correlation between AMH and endometrioma size, Pearson's correlation test was performed, and Spearman's correlation test was employed to determine the correlation between AFC and endometrioma size. P values <0.5 were considered statistically significant. Data are shown as mean $\pm$ standard error of the mean.

Results

The endometrioma group (group A) comprised 36 patients, and the dermoid cyst group (group B) was composed of 32 patients. Demographic parameters, AMH, and AFC levels among the groups are shown in Table 1. The two groups were matched for age and BMI. The mean cyst size of the two groups was also comparable. The mean serum preoperative AMH level was significantly lower in group A than in group B ($p=0.001$). Moreover, the mean preoperative AFC level was significantly lower in group A than in group B ($p=0.002$).

Postoperative serum AMH and AFC levels are shown in Table 2. At three months after surgery, serum AMH levels decreased significantly in group A from 2.04 ± 0.68 to 1.47 ± 0.55 ($p=0.001$). Further, in group B, serum AMH levels decreased significantly from 2.60 ± 0.57 to 2.17 ± 0.56 ($p=0.001$) at 3 months after the surgery. In group A, the unilateral (operated side) AFC level decreased significantly from 4.05 ± 1.24 to 2.16 ± 0.94 ($p=0.001$), and in group B, it decreased significantly from 4.93 ± 0.94 to 3.40 ± 0.87 ($p=0.001$).

The results of the comparison of the differences in AMH and AFC levels between the groups are shown in Table 3. The decrease in AMH levels was significantly higher in group A than in group B ($p=0.033$). The decrease in AFC levels was also

significantly higher in group A than in group B ($p=0.044$).

The correlation between AMH (before surgery) and endometrioma cyst size is shown in Figure 1. In addition, a significant negative correlation was found between the endometrioma size and serum AMH levels ($r=-0.413$, $p=0.012$). The correlation between AFC and endometrioma size is presented in Figure 2. Furthermore, we found a significant negative correlation between AFC levels and endometrioma size ($r=-0.448$, $p=0.006$); however, we found no significant correlation between dermoid cyst size and AMH levels ($r=-0.198$, $p=0.278$).

Discussion

In this study, we evaluated the ovarian reserve markers of patients who had undergone laparoscopic cystectomy for endometrioma or dermoid cysts at two time points (i.e., before and after surgery). We detected that the preoperative and postoperative AMH and AFC levels in patients with endometrioma were lower than those in patients with dermoid cysts. In addition, the decrease rate in AMH and AFC levels was higher in patients with endometrioma than in those with dermoid cysts. We also found a negative correlation between endometrioma size and ovarian reserve markers.

Although the mechanism is still not clearly known, endometriomas are confirmed to reduce fecundability. However, previous studies showed conflicting results on the relationship between endometrioma and ovarian reserve. Streuli et al.⁽²²⁾ evaluated the AMH levels of patients with endometrioma and reported similar AMH levels in patients with endometrioma and healthy controls. Uncu et al.⁽²³⁾ compared the AMH and AFC levels of 30 women with endometrioma and healthy controls and showed decreased AMH and AFC levels in women with endometrioma. Similarly, Pacchiarotti et al.⁽²⁴⁾ reported lower AMH levels in patients with endometrioma than in healthy controls. Kim et al.⁽²⁵⁾ evaluated the AMH and AFC levels of

Table 2. Anti-mullerian hormone and antral follicle count levels after surgery

	Endometrioma (n=36)	Dermoid cyst (n=32)	p
AMH (ng/mL)	1.47 ± 0.55	2.17 ± 0.56	0.001
AFC	2.16 ± 0.94	3.40 ± 0.87	0.001

AMH: Anti-mullerian hormone, AFC: Antral Follicle count

Table 3. The comparison of the decreases in anti-mullerian hormone and antral follicle count levels between the groups after surgery

	Endometrioma (n=36)	Dermoid cyst (n=32)	p
AMH (ng/mL)	0.56 ± 0.26	0.42 ± 0.27	0.033
AFC	1.88 ± 0.85	1.53 ± 0.56	0.044

AMH: Anti-mullerian hormone, AFC: Antral follicle count

Table 1. Demographic parameters, anti-mullerian hormone and antral follicle count levels before surgery

	Endometrioma (n=36)	Dermoid cyst (n=32)	p
Age (years)	30.13 ± 4.61	28.7 ± 3.94	0.176
BMI (kg/m ²)	25.1 ± 2.3	23.9 ± 2.2	0.092
Cyst size (mm)	52.2 ± 14.3	48.3 ± 12.3	0.176
AMH (ng/mL)	2.04 ± 0.68	2.60 ± 0.57	0.001
AFC	4.05 ± 1.24	4.93 ± 0.94	0.002

BMI: Body mass index, AMH: Anti-mullerian hormone, AFC: Antral follicle count

patients with endometrioma and dermoid cyst and reported lower AMH and AFC levels in patients with endometrioma. In our study, we also found lower serum AMH values in patients with endometrioma than in patients with dermoid cysts before surgery. In addition, we found a negative correlation between AMH levels and endometrioma cyst size.

The formation of endometriotic cysts could be destructive for the ovaries via structural tissue hazards or through direct damage to the ovarian cortex and follicles. Larger endometriomas may have more negative effects on the ovaries due to the larger contact area with the ovarian surface, and patients with large endometriomas experience longer-term destructive effects of endometrioma than those with smaller ones.

Many studies have investigated the effects of surgery on ovarian

reserve in patients with endometrioma. Somigliana et al. ⁽¹⁰⁾ calculated serum AMH levels before and after surgery and reported that surgical excision of endometriomas causes damage to the ovarian reserve. Chang et al. ⁽²⁶⁾ calculated serum AMH levels before surgery and at one week, one month, and three months after surgery and showed lower AMH levels in the first week and first month after surgery; however, they also reported that AMH levels were restored at three months after surgery. By contrast, in the present study, the AMH level was 1.50 ng/mL at the third postoperative month compared with the preoperative level of 2.23 ng/mL, so it appears that the preoperative AMH levels in the present study were still very low. Interestingly, in a meta-analysis, Muzii et al. ⁽⁸⁾ showed no significant reduction in AFC levels after endometrioma surgery and reported that laparoscopic excision of an endometrioma may be considered safer for the ovarian reserve than was previously thought. However, they evaluated the ovarian reserve by assessing only AFC levels, they did not calculate the serum AMH levels.

In the present study, we found a significant decrease in AMH and AFC levels of the unilateral operated ovary at three months after surgery. Laparoscopic excision of dermoid cysts could also reduce the ovarian reserve after surgery. Yan et al. ⁽¹²⁾ showed a significant decrease in the AFC levels of the operated ovary after surgical excision of dermoid cysts, but they also reported that this decrease in ovarian function was comparable with the effects of the cyst. In the present study, we found significant decreases in the postoperative AFC and serum AMH levels of the ovary operated for dermoid cysts. The use of laparoscopic stripping to excise endometriomas and dermoid cysts was responsible for these decreases because this technique may involve excessive removal of the ovarian tissue and follicular loss ⁽²⁷⁾.

Recently, some studies compared the influence of laparoscopic stripping on ovarian reserve between endometrioma and non-endometriotic cysts. Cagnacci et al. ⁽¹⁵⁾ compared the effects of laparoscopic cystectomy on ovarian reserve between 28 patients with endometrioma and 43 patients with non-endometriotic benign cysts. They found a similar decrease in rates in the postoperative AFC and ovarian volume levels between the endometrioma and non-endometriotic groups, and they reported that the decline in postoperative ovarian reserve was independent of the histologic type and diameter of the removed cyst. However, they did not assess serum AMH levels, and interestingly, preoperative AFC levels were lower in the non-endometriotic group than in the endometrioma group. Lind et al. ⁽¹³⁾ investigated changes in preoperative and postoperative serum AMH levels between patients with benign ovarian cysts, endometriomas, and dermoid cysts. They found a decrease in AMH levels from 3.0 ng/mL to 2.5 ng/mL in patients with dermoid cysts and a decrease from 2.0 ng/mL to 0.8 ng/mL in patients with endometrioma. As shown by their results, the decrease in serum AMH levels in patients with endometrioma seems more destructive after surgery. In a retrospective analysis

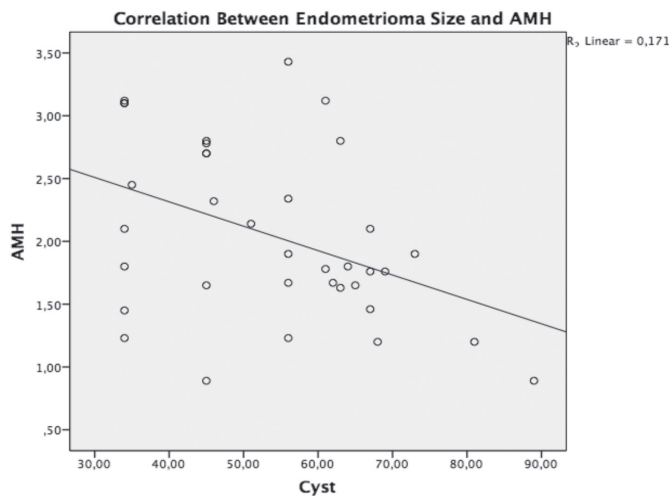


Figure 1. The correlation between endometrioma size and anti-mullerian hormone

AMH: Anti-mullerian hormone

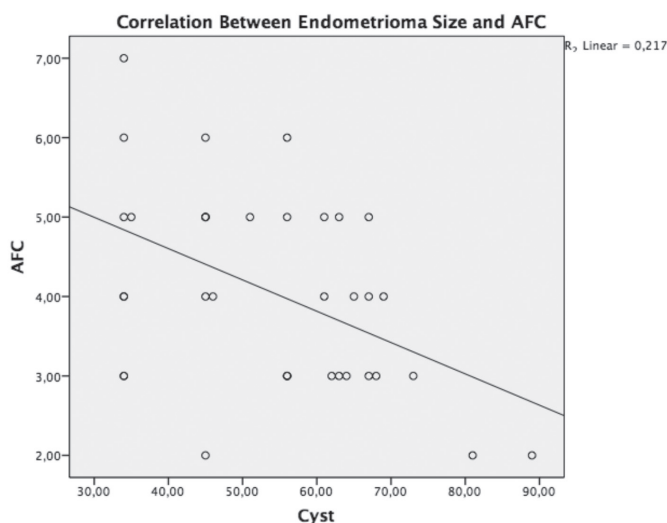


Figure 2. The correlation between endometrioma size and antral follicle counts

AFC: Antral follicle counts

of follicular loss after laparoscopic surgery for endometrioma compared with benign non-endometriotic benign ovarian cyst, Dogan et al. ⁽¹⁴⁾ reported higher functional follicular loss in patients with endometrioma according to pathologic assessments of cyst specimens. In our study, we found a significantly higher decrease in serum AMH levels and operated side AFC levels after surgery in patients with endometrioma than in patients with dermoid cysts. This difference may be attributed to laparoscopic stripping because endometriomas could be more adjacent and adherent to the ovaries than the dermoid cysts, and stripping could damage the ovaries of patients with endometrioma.

This study has limitations that should be acknowledged. First, the study included a small number of patients in each group. Second, there was no healthy control group, so we could not know the natural reduction rate in AMH levels after three months among this age group. Finally, we could not determine the effects of dermoid cyst on ovarian reserve before surgery because there was no healthy control group.

Conclusion

This study has the following main findings: (1) endometriomas are related to lower ovarian reserve when compared with dermoid cysts before surgery; (2) there is a negative correlation between endometrioma size and serum AMH and AFC levels; (3) laparoscopic stripping has destructive effects on serum AMH levels and operated side AFC levels after surgery for both patients with endometrioma and dermoid cysts; and (4) laparoscopic excision of endometrioma has more destructive effects on ovarian reserve than dermoid cysts. Further studies should be performed to show the effects of this decrease on fertility and new laparoscopic cyst excision techniques should be investigated to cause less damage to ovarian reserve for patients with endometrioma.

Ethics

Ethics Committee Approval: The study was reviewed by the Ethics Committee of İstanbul Medeniyet University Faculty of Medicine (approval number: 01435-2019) and was conducted in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: C.K., A.T., Design: C.K., Data Collection or Processing: C.K., Analysis or Interpretation: S.D., Literature Search: S.D., Writing: E.Ç.

Conflict of Interest: The authors report no conflict of interest.

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References

1. Gelbaya TA, Nardo LG. Evidence-based management of endometrioma. *Reproductive Biomedicine Online* 2011;23:15-24.
2. Kim MJ, Kim NY, Lee DY, Yoon BK, Choi D. Clinical characteristics of ovarian teratoma: age-focused retrospective analysis of 580 cases. *Am J Obstet Gynecol* 2011;205:e1-e4.
3. Chapron C, Fauconnier A, Goffinet F, Breart G, Dubuisson J. Laparoscopic surgery is not inherently dangerous for patients presenting with benign gynaecologic pathology. Results of a meta-analysis. *Human Reproduction* 2002;17:1334-42.
4. Medeiros LR, Rosa DD, Bozzetti MC, Fachel JM, Furness S, Garry R, et al. Laparoscopy versus laparotomy for benign ovarian tumour. *Cochrane Database of Systematic Reviews* 2009.
5. Hart RJ, Hickey M, Maouris P, Buckett W. Excisional surgery versus ablative surgery for ovarian endometriomata. *Cochrane Database of Syst Rev* 2008.
6. Maheshwari A, Fowler P, Bhattacharya S. Assessment of ovarian reserve—should we perform tests of ovarian reserve routinely? *Human Reproduction* 2006;21:2729-35.
7. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Arsenio AC, et al. Anti-müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update* 2009;16:113-30.
8. Muzii L, Di Tucci C, Di Felicianantonio M, Marchetti C, Perniola G, Panici PB. The effect of surgery for endometrioma on ovarian reserve evaluated by antral follicle count: a systematic review and meta-analysis. *Hum Reprod* 2014;29:2190-8.
9. Shah DK, Mejia RB, Lebovic DI. Effect of surgery for endometrioma on ovarian function. *J Minim Invasive Gynecol* 2014;21:203-9.
10. Somigliana E, Berlanda N, Benaglia L, Viganò P, Vercellini P, Fedele L. Surgical excision of endometriomas and ovarian reserve: a systematic review on serum antimüllerian hormone level modifications. *Fertil Steril* 2012;98:1531-8.
11. Goodman LR, Goldberg JM, Flyckt RL, Gupta M, Harwalker J, Falcone T. Effect of surgery on ovarian reserve in women with endometriomas, endometriosis and controls. *Am J Obstet Gynecol* 2016;215:589.e1-e6.
12. Yan L, Li M, Zhang B-Q, Xu XX, Xu Z, Han T, et al. Effect of ovarian dermoid cyst excision on ovarian reserve and response: Insights from in vitro fertilization. *Gynecology and Minimally Invasive Therapy* 2016;5:161-5.
13. Lind T, Hammarström M, Lampic C, Rodriguez-Wallberg K. Anti-müllerian hormone reduction after ovarian cyst surgery is dependent on the histological cyst type and preoperative anti-müllerian hormone levels. *Acta Obstet Gynecol Scand* 2015;94:183-90.
14. Dogan E, Ulukus EC, Okyay E, Ertugrul C, Saygili U, Koyuncuoglu M. Retrospective analysis of follicle loss after laparoscopic excision of endometrioma compared with benign nonendometriotic ovarian cysts. *Int J Gynecol Obstet* 2011;114:124-7.
15. Cagnacci A, Bellafronte M, Xholli A, Palma F, Carbone MM, Di Carlo C, et al. Impact of laparoscopic cystectomy of endometriotic and non-endometriotic cysts on ovarian volume, antral follicle count (AFC) and ovarian doppler velocimetry. *Gynecol Endocrinol* 2016;32:298-301.
16. Guerriero S, Ajossa S, Mais V, Risalvato A, Lai MP, Melis GB. The diagnosis of endometriomas using colour Doppler energy imaging. *Hum Reprod* 1998;13:1691-5.
17. Moore J, Copley S, Morris J, Lindsell D, Golding S, Kennedy S. A systematic review of the accuracy of ultrasound in the diagnosis of endometriosis. *Ultrasound Obstet Gynecol* 2002;20:630-4.

18. Patel MD, Feldstein VA, Chen DC, Lipson SD, Filly RA. Endometriomas: diagnostic performance of US. *Radiology* 1999;210:739-45.
19. Mais V, Guerriero S, Ajossa S, Angiolucci M, Paoletti AM, Melis GB. Transvaginal ultrasonography in the diagnosis of cystic teratoma. *Obstet Gynecol* 1995;85:48-52.
20. Patel MD, Feldstein VA, Lipson SD, Chen DC, Filly RA. Cystic teratomas of the ovary: diagnostic value of sonography. *AJR American journal of roentgenology* 1998;171:1061-5.
21. Hudelist G, English J, Thomas A, Tinelli A, Singer C, Keckstein J. Diagnostic accuracy of transvaginal ultrasound for non-invasive diagnosis of bowel endometriosis: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2011;37:257-63.
22. Streuli I, de Ziegler D, Gayet V, Santulli P, Bijaoui G, de Mouzon J, et al. In women with endometriosis anti-Müllerian hormone levels are decreased only in those with previous endometrioma surgery. *Hum Reprod* 2012;27:3294-303.
23. Uncu G, Kasapoglu I, Ozerkan K, Seyhan A, Oral Yilmaztepe A, Ata B. Prospective assessment of the impact of endometriomas and their removal on ovarian reserve and determinants of the rate of decline in ovarian reserve. *Hum Reprod* 2013;28:2140-5.
24. Pacchiarotti A, Frati P, Milazzo GN, Catalano A, Gentile V, Moscarini M. Evaluation of serum anti-Mullerian hormone levels to assess the ovarian reserve in women with severe endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2014;172:62-4.
25. Kim JY, Jee BC, Suh CS, Kim SH. Preoperative serum anti-mullerian hormone level in women with ovarian endometrioma and mature cystic teratoma. *Yonsei Med J* 2013;54:921-6.
26. Chang HJ, Han SH, Lee JR, Jee BC, Lee BI, Suh CS, et al. Impact of laparoscopic cystectomy on ovarian reserve: serial changes of serum anti-Müllerian hormone levels. *Fertility and Sterility* 2010;94:343-9.
27. Muzii L, Bianchi A, Crocè C, Mancini N, Panici PB. Laparoscopic excision of ovarian cysts: is the stripping technique a tissue-sparing procedure? *Fertil Steril* 2002;77:609-14.



Surgical anatomy of the pectineal ligament during pectopexy surgery: The relevance to the major vascular structures

Pektopeksi cerrahisinde kullanılan pektineal ligamanın anatomisi: Majör vasküler yapılarla ilişkisi

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Abstract

Objective: During pectopexy surgery, the prolapsed uterus or the vaginal apex is fixed to the pectineal ligament. The anatomic structures found in the lateral part of the prevesical and paravaginal space above the obturator fossa, raise the importance of the surgical steps required to prevent complications. This study was conducted to evaluate the proximity of vascular structures to the pectineal ligament.

Materials and Methods: The distances between the surgical suturing area during pectopexy surgery and the external iliac vein, pubic anastomotic vessel (corona mortis) and obturator canal were measured bilaterally in seven fresh female cadavers.

Results: The total length of the pectineal ligament was 5.9±0.76 cm on the left and 6.5±1.14 cm on the right side; the midpoint of the pectineal ligament was 2.8±0.52 cm on the left and 3.6±0.47 cm on the right side. From the midpoint of the left pectineal ligament, the mean distance to the left external iliac vein was 1.04±0.23 cm, to the left corona mortis it was 2.15±0.48 cm, and to the left obturator canal it was 3.12±0.95 cm. From the midpoint of the right pectineal ligament, the mean distance to the right external iliac vein was 1.25±0.43 cm, to the right corona mortis it was 2.37±0.63 cm, and to the right obturator canal it was 3.57±0.93 cm.

Conclusion: The anatomic findings of the study confirmed that the pectineal ligament was in close association with main vessels. The external iliac vein was measured as the closest anatomic structure to the pectineal ligament. Surgeons must be careful to minimize life-threatening complications because of the proximity of the pectineal ligament to main vessels.

Keywords: Cadaveric study, major vessels, pectineal ligament, pectopexy, pelvic organ prolapse, vascular anatomy

PRECIS: The findings of the study confirmed that the pectineal ligament was in close association with main vessels, the external iliac vein was measured as the closest structure.

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Öz

Amaç: Pektopleksi cerrahisi sırasında, prolapse olan uterus veya vajinal apeks pektineal ligamana sabitlenir. Obturator fossa üzerinde prevesikal ve paravajinal boşluğun lateral kısmında bulunan anatomik yapılar, komplikasyonları önlemek için yapılacak cerrahi adımların önemini arttırmaktadır. Bu çalışma, vasküler yapıların pektineal ligamana yakınlığını değerlendirmek için yapılmıştır.

Gereç ve Yöntemler: Pektopleksi cerrahisi sırasında cerrahi sütür alanı ile eksternal iliak ven, pubik anastomotik damarlar (korona mortis) ve obturator kanal arasındaki mesafeler 7 taze kadın kadavradaki iki taraflı olarak ölçülmüştür.

Bulgular: Pektineal ligamanın toplam uzunluğu solda $5,9 \pm 0,76$ cm ve sağ tarafta $6,5 \pm 1,14$ cm idi. Pektineal ligamanın orta noktası solda $2,8 \pm 0,52$ cm ve sağ tarafta $3,6 \pm 0,47$ cm idi. Sol pektineal ligamanın orta noktasından, sol eksternal iliak vene ortalama mesafe $1,04 \pm 0,23$ cm, sol korona mortise $2,15 \pm 0,48$ cm ve sol obturator kanala ortalama mesafe $3,12 \pm 0,95$ cm idi. Sağ pektineal ligamanın orta noktasından sağ eksternal iliak vene ortalama mesafe $1,25 \pm 0,43$ cm, sağ korona mortise $2,37 \pm 0,63$ cm ve sağ obturator kanala ortalama $3,57 \pm 0,93$ cm idi.

Sonuç: Çalışmanın anatomik bulguları pektineal ligamanın ana damarlarla yakın ilişki içinde olduğunu doğrulamıştır. Eksternal iliak ven, pektineal ligamana en yakın anatomik yapı olarak ölçülmüştür. Cerrahlar, pektineal ligamanın ana damarlara yakınlığı nedeniyle hayatı tehdit eden komplikasyonları en aza indirmeye dikkat etmelidir.

Anahtar Kelimeler: Kadavra çalışması, majör damarlar, pektineal ligaman, pektopleksi, pelvik organ prolapsus, vasküler anatomi

Introduction

There are many ligaments and delicate structures in the pelvis to which attention must be paid in surgical procedures. The importance of these anatomic structures emerges with newly documented surgical procedures and clinical anatomy studies. The pectineal ligament is one of the key anatomic structures in the pelvis, and is used in both abdominal and gynecologic surgeries. During procedures such as inguinal hernia repair, pelvic organ prolapse (POP), and stress urinary incontinence (SUI), the pectineal ligament is used as a surgical anchoring point⁽¹⁾. Detailed anatomic knowledge of the pectineal ligament and relevant anatomic landmarks improves surgical anatomy practice and facilitates surgery.

Pectopexy surgery, which is performed for POP, was first described by Banerjee and Noé⁽²⁾. During this surgical procedure, the prolapsed uterus or the vaginal apex is fixed to the pectineal ligament with a mesh. The anatomic structures found in this area, the lateral part of the prevesical and paravaginal space above the obturator fossa, raise the importance of surgical steps performed during the procedure to prevent probable complications⁽³⁾. This cadaveric study was conducted to evaluate the proximity of anatomic landmarks and vascular structures to the pectineal ligament related with the pectopexy surgery.

Clinical anatomy of the pectineal ligament

The pectineal ligament, which is also known as Cooper's ligament, was first described by Sir Astley Cooper as the ligamentous extension lying over the iliopectineal line⁽⁴⁾. Complete knowledge of the inguinal region is crucial to understand the anatomy of the pectineal ligament. The inguinal ligament, which is the anterior border of the femoral canal, lies between the anterior superior iliac spine and pubic tubercle. The posterolateral reflection of this ligament from the pubic tubercle forms the lacunar ligament, which is the medial border of the femoral canal. From the pectineal attachment of the lacunar ligament, the fibrous connective tissue called the pectineal ligament, which is a ligamentous extension, lies laterally through the iliopectineal line below the superior pubic ramus. The medial part of pectineal ligament close to the pubic tubercle is the thickest section and it becomes thinner while extending laterally⁽⁵⁾.

The pectineal ligament is primarily found on the lateral part of the prevesical and paravaginal space, forming the posterior border of the femoral canal, and has close proximity to the external iliac vessels, which lie on the superolateral part of the pectineal ligament. On the other hand, the pubic vein or arterial anastomosis between the inferior epigastric artery and obturator artery (corona mortis) is also adjacent to the pectineal ligament. At the inferolateral part of the pectineal ligament, the obturator nerve and obturator vessels are found. This obturator region consists of many vascular variations and anastomoses that the surgeon should be careful of during operations regarding the iliopectineal line and pectineal ligament.

Materials and Methods

This study did not involve any cadaver of or tissue from the recently dead, other than bequeathed cadavers and tissue obtained in the normal course of necropsy, and therefore did not require ethical board approval and informed consent. All the cadavers were paid the greatest respect during the dissection for educational purposes. A total of seven fresh frozen female cadavers were dissected. A full pelvic dissection was performed to obtain a clear vision of the anatomic landmarks, at the anatomy laboratory of Bahcesehir University Department of Anatomy. The dissections and measurements of the anatomic structures were performed by senior surgeons and anatomists (E.Ç., O.D., I.S.). The distance between the midpoint of the pectineal ligament, which is the surgical suturing area during pectopexy surgery, and the external iliac vein, pubic anastomotic vessel (corona mortis) and obturator canal was measured bilaterally (Figure 1 demonstrates how the measurements were performed and the distance between the lateral portion of the middle of pectineal ligament and vascular structures). Statistical analyses were performed using a standardized computer-based calculating system. Descriptive analysis was made and reported as mean $\pm$ standard deviation (SD).

Technique of cadaveric dissection

A midline vertical incision extending from the xiphoid process to the pubic symphysis circumferential around the umbilicus was performed to enter the abdominal cavity. The second and

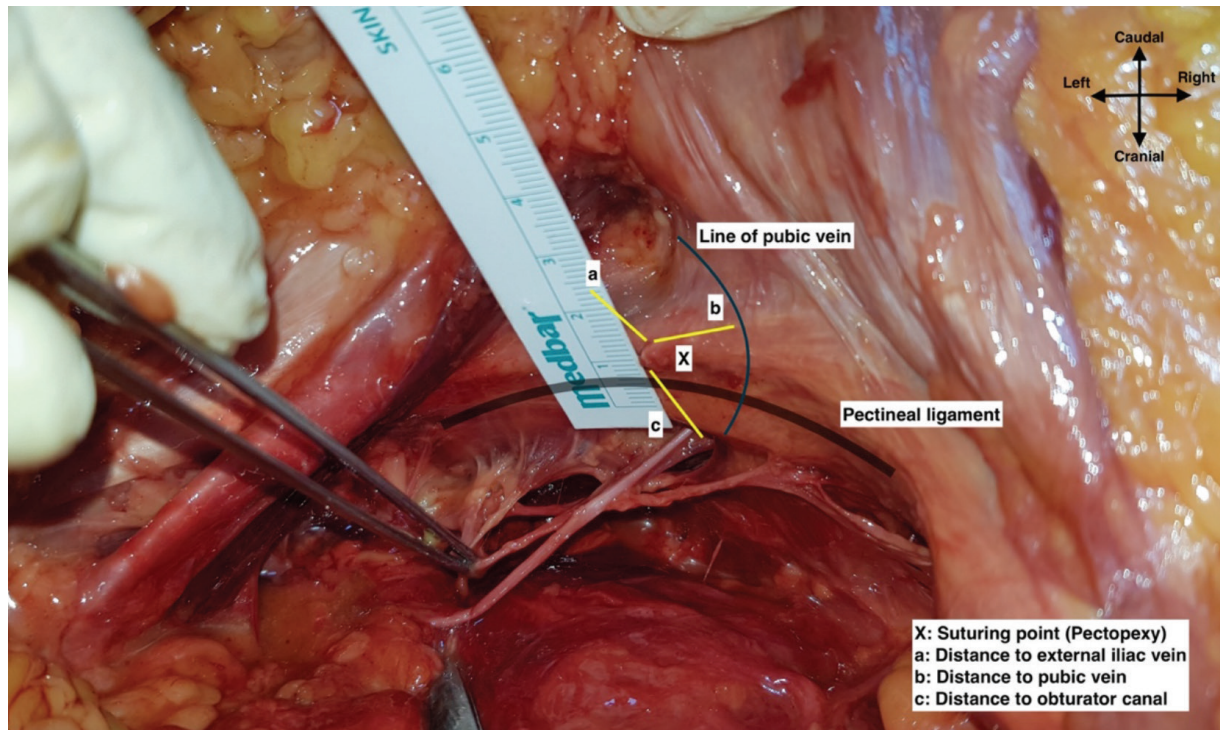


Figure 1. How the measurements were performed and the distance between the lateral portion of the middle of pectineal ligament and vascular structures, left pelvic side wall (here, the anterior abdomen was retracted excessively to take a clear photo, measurements were performed in the classic anatomic position as in surgery)

third incision were performed bilaterally following the costal margin from the xiphoid process to the midaxillary line and from the pubic symphysis to the anterior superior iliac spine, respectively, to maintain a full exposure of the abdominal cavity. The lateral parietal peritoneum covering the pelvic side wall was cut 2 cm cranial to the round ligament of the uterus and the retroperitoneal area was accessed. The psoas major muscle was exposed and on the medial part of the psoas major muscle the reflection of external iliac vessels was identified, attached to the psoas major muscle. The anterior and posterior leaf of the broad ligament was cut caudally and cranially, respectively, by preserving the round ligament of uterus. The paravesical space lateral to the bladder was developed and the obliterated umbilical artery was found and secured. The lateral part of the obliterated umbilical artery was identified in the obturator space. The median umbilical ligament was identified and cut at the level of pubic symphysis, the peritoneum covering the urinary bladder was reflected posteriorly and a blunt dissection was performed between the urinary bladder and the pubic symphysis to develop the prevesical space (retropubic space, Retzius space).

Afterwards, all fatty-lymphatic tissue covering the external iliac vessels and obturator fossa was removed with a meticulous dissection to obtain a straight measurement. Following the pathway of round ligament to the abdominal wall, the inguinal canal was identified and the inguinal ligament was exposed at the base of the rectus abdominis muscle. At this point, the inferior

epigastric vessels were dissected and secured. On the medial part of the inguinal ligament, the aponeurotic expansion to the pectineal line is called the lacunar ligament. Here, the pubic anastomotic vessels known as corona mortis were dissected and secured. Antero-medial to the lacunar ligament, the pubic tubercle was palpated, and the laterally extending ligamentous structure over the periosteum of the pectineal line, which is called the pectineal ligament, was identified. The origin of the pectineal ligament was defined as its attachment point to the pubic tubercle. Figure 2 demonstrates the pelvic anatomy and anatomic landmarks, after cadaveric dissection.

Results

The mean age of the cadavers was 74 ± 10.32 (range, 58-88) years. The mean Body Mass index (BMI) of the cadavers was 22.14 ± 4.31 kg/m² (Table 1). None of the cadavers had a history of pelvic surgery. After the origin of the pectineal ligament was determined, the measurements were obtained (Table 2). The total length of the pectineal ligament was 5.9 ± 0.76 cm on the left and 6.5 ± 1.14 cm on the right side; the midpoint of the pectineal ligament was 2.8 ± 0.52 cm on the left and 3.6 ± 0.47 cm on the right side. Figure 3 demonstrates the mean length of pectineal ligament. From the midpoint of the left pectineal ligament, the mean distance to the left external iliac vein was 1.04 ± 0.23 cm, the mean distance to the left corona mortis was 2.15 ± 0.48 cm, and the mean distance to the left obturator canal was 3.12 ± 0.95 cm. From the midpoint of the right pectineal

ligament, the mean distance to the right external iliac vein was 1.25 ± 0.43 cm, the mean distance to the right corona mortis was 2.37 ± 0.63 cm, and the mean distance to the right obturator canal was 3.57 ± 0.93 cm.

Discussion

Surgical anatomy of the pectineal ligament is especially important for urogynecology practice regarding POP and SUI operations. The anatomic location of the pectineal ligament

Table 1. The demographic data of the cadavers

Demographic characteristics	Values n (%)
Total cadaver count (n)	7
Average age $\pm$ SD (y)	74 ± 10.32
Race	
Caucasian	7 (100%)
Black	0 (0%)
Cause of death	
Neurologic	2 (28.5%)
Cardiopulmonary	4 (57.1%)
Cancer	1 (14.2%)

n: Number, y: Year, SD: Standard deviation

Table 2. The distance of the pectineal ligament to the relevant anatomic landmarks

Anatomic landmark	Length (cm)	Min (cm)	Max (cm)
Total length of the pectineal ligament			
Left	5.9 ± 0.76	4.9	7.0
Right	6.5 ± 1.14	5.1	8.1
The midpoint of the pectineal ligament from the pubic tubercle			
Left	2.8 ± 0.52	2.1	3.6
Right	3.6 ± 0.47	3.0	4.3
The distance to the external iliac vein			
Left	1.04 ± 0.23	0.7	1.4
Right	1.25 ± 0.43	0.9	2.1
The distance to the corona mortis			
Left	2.15 ± 0.48	1.6	2.8
Right	2.37 ± 0.67	1.5	3.7
The distance to the obturator canal			
Left	3.12 ± 0.95	1.4	4.1
Right	3.57 ± 0.93	2.4	5.3

min: Minimum, max: Maximum

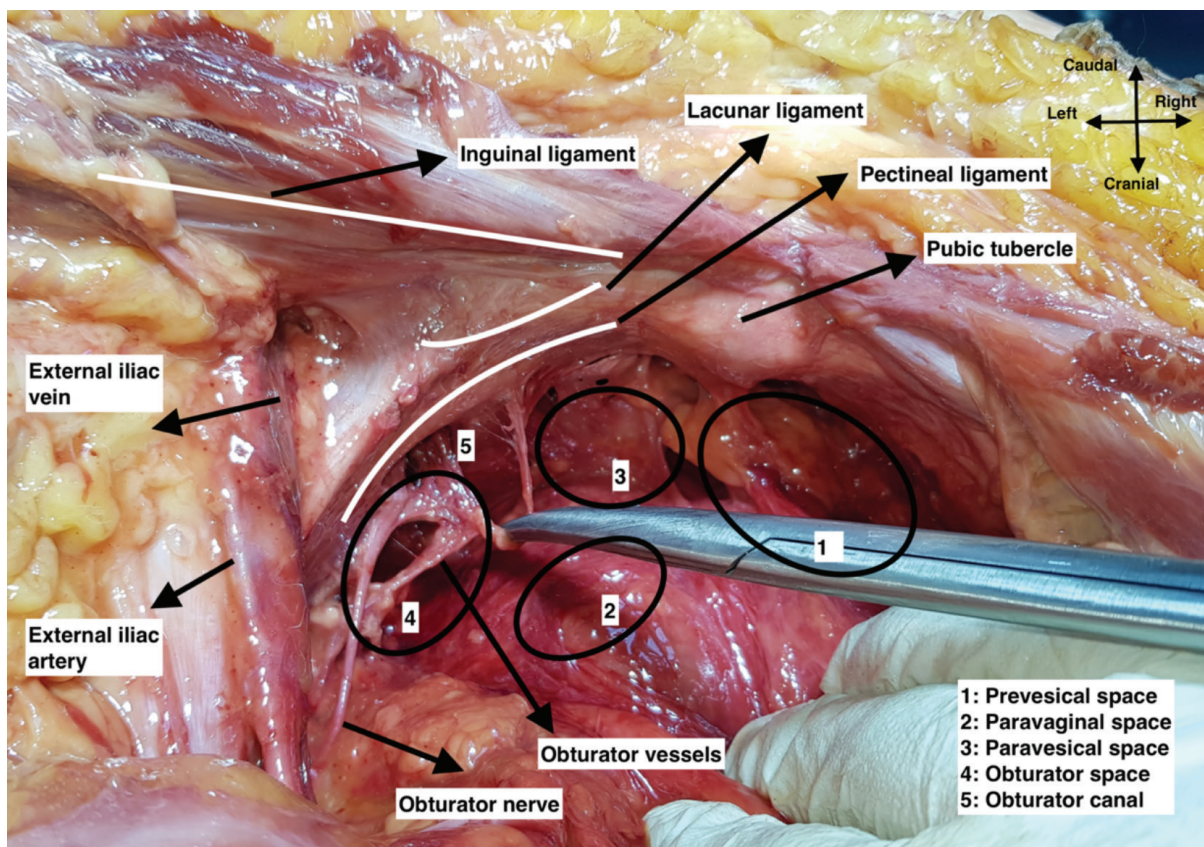


Figure 2. Pelvic anatomy and anatomic landmarks in a cadaver, left pelvic side wall

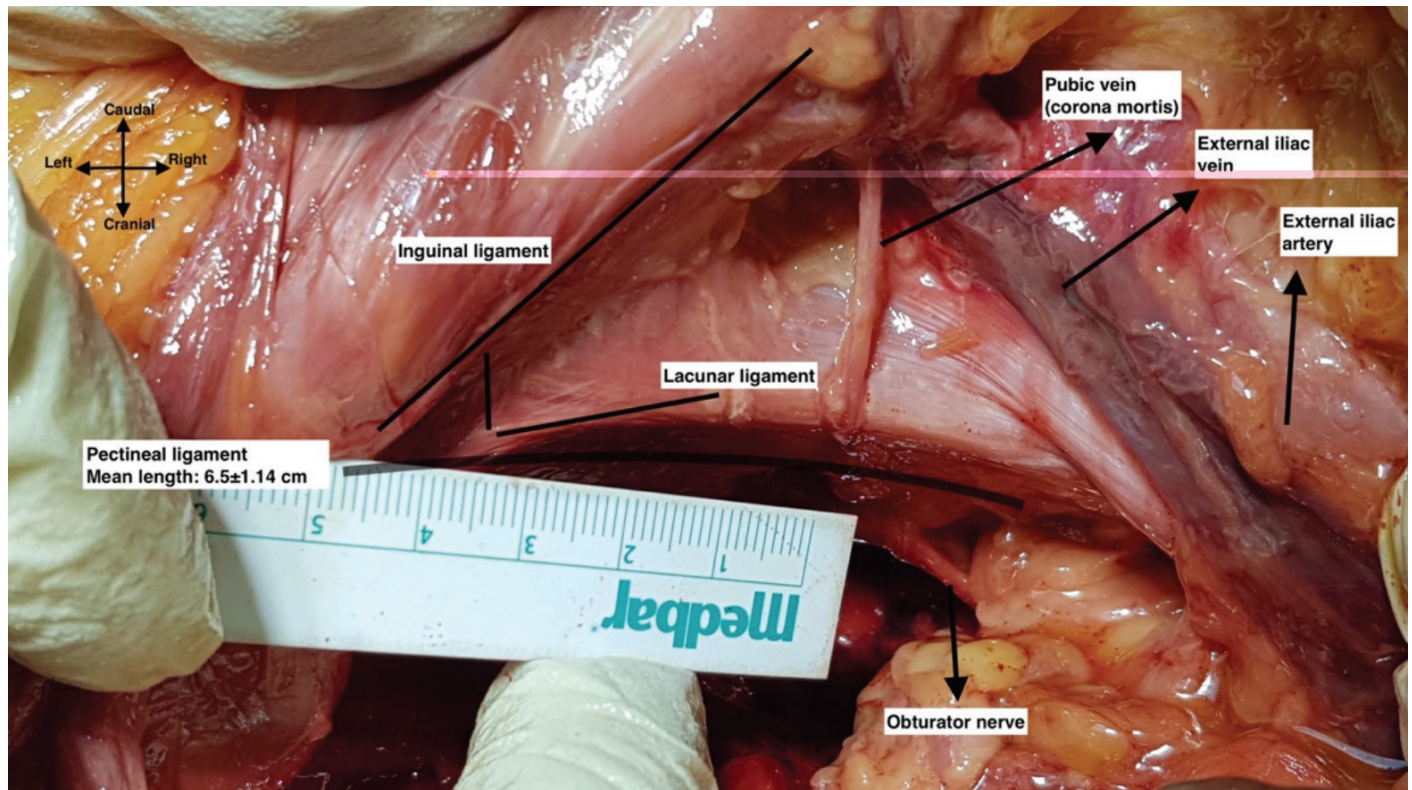


Figure 3. Anatomy and the mean length of pectineal ligament, right pelvic side wall

may lead to some complications during surgery. In this respect, knowing the proximity of relevant anatomic landmarks to the pectineal ligament may improve the surgical outcomes and decrease probable complications. In this study, the surgical dissection of seven cadavers revealed that the closest anatomic structure to the pectineal ligament was the external iliac vein on both sides of the pelvis; 1.04 ± 0.23 cm and 1.25 ± 0.43 cm on the left and right side of pelvis, respectively. Additionally, the pubic vascular anastomosis called the corona mortis was also quite close to the surgical anchoring point; 2.15 ± 0.48 cm and 2.37 ± 0.63 cm on the left and right side of pelvis, respectively. However, the obturator canal had no close relation with the pectineal ligament; 3.12 ± 0.95 cm and 3.57 ± 0.93 cm on the left and right side of pelvis, respectively.

There is a controversy on the origin and texture of the pectineal ligament because the anatomy of the inguinal region has a complex architecture with regard to the contributing ligaments and attachments. The debate on the pectineal ligament may be considered as an option for further studies; however, in the literature, it has been shown that the aponeurosis and fascia of pectineus muscle, attachments of the inguinal ligament and iliopubic tract, transversalis fascia, and fibers from the psoas minor muscle and periosteum over the pectineal line all appeared to be a part of the pectineal ligament⁽⁶⁻¹⁰⁾. Rouviere⁽¹¹⁾ stated that the pectineal ligament was the thickening of the fascia of the pectineus muscle, and therefore it had a strong

structure. Additionally, the anatomic and histologic findings of Faure et al.⁽¹⁾ in a cadaveric dissection study also showed that the pectineal ligament was the thickening of the pectineus fascia, rather than thickening of the periosteum.

On the other hand, classically, it is known that the pectineal ligament lies from the pubic tubercle to the iliopubic eminence, attaches to the periosteum of the superior pubic ramus, and covers the pectineal line of the pubis while extending dorsally. In this study, we measured the total length of pectineal ligament as 5.9 ± 0.76 cm and 6.5 ± 0.14 cm on the left and right side, respectively, and the midpoint of the pectineal ligament was 2.8 ± 0.52 cm and 3.6 ± 0.47 cm far from the pubic tubercle on the left and right side, respectively. Faure et al.⁽¹⁾ found similar results that the average total length was 53 (range, 25-65) mm, and the midpoint of the pectineal ligament was 3-4 cm away from the medial insertion. On the far lateral portion, the pectineal ligament becomes thinner and fuses with the transversalis fascia at the part of iliopubic eminence; however, the medial portion of the pectineal line, which corresponds to the midpoint of pectineal ligament, just below the lacunar ligament, constitutes a strong band⁽¹²⁾. Due to the remarkable strength of the pectineal ligament, its importance has been emphasized in many surgical studies.

Pectopexy surgery was suggested as a novel technique in POP surgery. During pectopexy surgery, the lateral portion of the middle part of pectineal ligament is used as the suturing

point; however, it is well known that the inferomedial part of the pectineal ligament is used in Burch's colposuspension procedures, which are performed for SUI^(2,13). Topographic anatomy yields that the risk of gross vessel injury may increase by proceeding towards the lateral part in pectopexy surgery, and our study demonstrated the closest anatomic structure was the external iliac vein.

Sacrocolpopexy and sacrouteropexy procedures are commonly performed for apical POP, in which the prolapsed cervix or vaginal vault is suspended to the sacrum with a mesh. Performing this procedure needs a great deal of experience and it represents a high learning curve. Because of the close anatomical structures which are present at the operation field; small intestines, sigmoid colon, ureter and presacral vessels are in risk of injury. Kale et al.⁽¹⁴⁾ conducted a case series study and applied pectopexy to seven patients with apical prolapse and reported no intraoperative and postoperative complications. During the pectopexy surgery, the pectineal ligament represents a distant space from the intestines, sigmoid colon, presacral vessels, and ureter, thus they stated that the pectineal ligament maintains a safe surgical field in pectopexy surgery. Moreover, Kale et al.⁽¹⁴⁾ offered this technique as a feasible procedure because the surgeon uses a wide area in the pelvis and the strong nature of pectineal ligament would decrease the postoperative recurrence rates. Our study did not demonstrate the exact anatomic distance to these structures; however, our anatomic dissections revealed that the small intestines and sigmoid colon were far away from the operation field unless an adhesion was detected. Presacral vessels are not in the surgical field and medial traction of the posterior leaf of the broad ligament will separate the ureter from the surgical area, so the risk of injury to these anatomic structures is less than with sacrocolpopexy procedures.

Noe et al.⁽¹⁵⁾ analyzed the results of the pectopexy and sacrocolpopexy procedures that were performed to 43 and 40 patients, respectively; they found that the duration of surgery and intraoperative blood loss was lower in the pectopexy group. Also, there were no severe intraoperative complications in either group such as bleeding, neural, vascular or intestinal injury. Banerjee and Noé⁽²⁾ compared 12 pectopexy cases with 242 sacrocolpopexy cases, there was no severe bleeding, neural, vascular or intestinal injury in the pectopexy group; however, the rate of bladder and intestinal injury was 0.7% and 1.2%, respectively, in the sacrocolpopexy group. Joshi et al.⁽¹⁶⁾ retrospectively evaluated consecutive pectineal ligament hysteropexy procedures. The results of 176 open and 18 laparoscopic cases demonstrated no intraoperative complications. In this respect, pectopexy surgery has a decreased risk of injury compared with major abdomino-pelvic organs; however, the risk of external iliac vein injury is increased for pectopexy procedures because of the close proximity. Despite this anatomic relevance, no injury has been reported in the literature for pectopexy surgery.

The anastomotic vessel between the external iliac and obturator vessels is called the pubic vein or artery, historically known as the corona mortis, which represents a danger zone, especially during femoral hernia repair operation. Nevertheless, when the retroperitoneum is accessed through the pelvic cavity, the risk of injury to the corona mortis is not frequent and is easily controlled⁽¹⁷⁾. The pubic anastomotic vessel is mostly detected as a vein and it lies behind the superior pubic ramus. The distance from the pubic symphysis varies between 40 and 96 mm⁽¹⁸⁾. We found the distance between the pectineal ligament and the corona mortis as 2.15 ± 0.48 cm and 2.37 ± 0.63 cm, on the left and right side, respectively. Surgeons should be careful not to injure the corona mortis, which is close to the pectineal ligament suturing point.

The obturator neuro-vascular bundle passes through the obturator canal and our study also demonstrated the distance of pectineal ligament to the obturator canal. The obturator artery and vein mostly lie posterior to the obturator nerve in the deep pelvic side wall within the fatty-lymphoid tissue. Previously, the length between the middle of the ichiopubic ramus to the obturator canal was analyzed and the researchers found the distance as 4.4 cm; this is especially important during retro-pubic anti-incontinence surgical procedures^(19,20). Nonetheless, surgeons must know the limitations and risks of new procedures they are going to perform. The distance of the midpoint of the pectineal ligament to the obturator canal was found as 3.12 ± 0.95 cm and 3.57 ± 0.93 cm, on the left and right side, respectively. From this point, the pectineal ligament is not close to the obturator canal, which is in the obturator space and surrounded by a fatty-lymphoid tissue.

In the English literature, we found no studies measuring the distance between the pectineal ligament, corona mortis, and other relevant vascular structures. In this respect, this study may make an important contribution to the current literature about apical prolapse surgery. The postmortem vasoconstriction and shrinkage of tissues will change the exact distance of surgical planes, but we used fresh frozen cadavers to optimize the measurements and decrease the lacking points in defining tissue planes.

As a conclusion, the pectineal ligament has a close relation with the main vessels in the pelvis. Indeed, positioning the suture to the lateral part of the midpoint of pectineal ligament increases the proximity with the external iliac vein, which was measured as the closest anatomic structure to the pectineal ligament in terms of pectopexy procedures. Pelvic surgeons must be careful and gentle, and retract the external iliac vein from the suturing point of pectineal ligament while performing pectopexy procedures.

Ethics

Ethics Committee Approval: This study did not involve any cadaver of or tissue from the recently dead, other than bequeathed cadavers and tissue obtained in the normal course of necropsy, and therefore did not require ethical board approval.

Informed Consent: It wasn't obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Ç.P., O.D., R.N.B., Design: Ç.P., O.D., R.N.B., Data Collection or Processing: M.S.M., M.Y., A.E.K., Analysis or Interpretation: O.D., Writing: Ç.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Faure JP, Hauet T, Scepti M, Chansigaud JP, Kamina P, Richer JP. The pectineal ligament: anatomical study and surgical applications. *Surg Radiol Anat* 2001;23:237-42.
2. Banerjee C, Noe KG. Laparoscopic pectopexy: a new technique of prolapse surgery for obese patients. *Arch Gynecol Obstet* 2011;284:631-5.
3. Selcuk I, Ersak B, Tatar I, Gungor T, Huri E. Basic clinical retroperitoneal anatomy for pelvic surgeons. *Turk J Obstet Gynecol*. 2018;15:259-69.
4. Cooper A. The anatomy and surgical treatment of inguinal congenital hernia. *JT Cox London*. 1804.
5. Loukas M, Benninger B., Tubbs RS. Peritoneal cavity. In: Marios Loukas, Brion Benninger, R. Shane Tubbs, editor. *Gray's Clinical Photographic Dissector of the Human Body*. 2nd ed: Elsevier; 2018;480.
6. Charpy A. Le pli de l'aîne. *Arch Med Toulouse* 17 337-3611910.
7. Testut L, Latarjet A. *Traité d'Anatomie Humaine*. 8ème éd, Doin, Paris, pp 935-9951928.
8. Bardeleben K von. *Handbuch der anatomie der menschen. Muskeln der Stammes*. P Eisler, Iena, Bd 2, p 6411912.
9. McVay CB, Anson BJ. Aponeurotic and fascial continuities in the abdomen, pelvis and thigh. *Anat Record* 1940;76:213-31.
10. Richer JP., Faure JP, Carretier M., Barbier J. The Ligaments of Cooper and Thomson. In: Bendavid R., Abrahamson J., Arregui ME., Flament JB., Phillips EH. (eds) *Abdominal Wall Hernias*. Springer, New York, NY2001.
11. Rouviere H. *Anatomie Humaine, descriptive et topographique*. Tome II, Masson, Paris, pp 92-1031974.
12. Standring S. *Gray's Anatomy: The Anatomical Basis of Clinical Practice*. 41st ed. ed: Elsevier; 2016.
13. Miklos JR, Kohli N. Laparoscopic paravaginal repair plus burch colposuspension: review and descriptive technique. *Urology*. 2000;56:64-9.
14. Kale A, Biler A, Terzi H, Usta T, Kale E. Laparoscopic pectopexy: initial experience of single center with a new technique for apical prolapse surgery. *Int Braz J Urol* 2017;43:903-9.
15. Noe KG, Spuntrup C, Anapolski M. Laparoscopic pectopexy: a randomised comparative clinical trial of standard laparoscopic sacral colpo-cervicopexy to the new laparoscopic pectopexy. Short-term postoperative results. *Arch Gynecol Obstet*. 2013;287:275-80.
16. Joshi VM, Otiv SR, Dagade VB, Borse M, Majumder RN, Shrivastava M, et al. Pectineal Ligament Hysteropexy for Uterine Prolapse in Premenopausal Women by Open and Laparoscopic Approach in Indian Urban and Rural Centers. *Female Pelvic Med Reconstr Surg*. 2015;21:215-9.
17. Selcuk I, Tatar I, Firat A, Huri E, Gungor T. Is corona mortis a historical myth? A perspective from a gynecologic oncologist. *J Turk Ger Gynecol Assoc*. 2018;19:171-2.
18. Darmanis S, Lewis A, Mansoor A, Bircher M. Corona mortis: an anatomical study with clinical implications in approaches to the pelvis and acetabulum. *Clin Anat*. 2007;20:433-9.
19. Kendir S, Akkaya T, Comert A, Sayin M, Tatlisumak E, Elhan A, et al. The location of the obturator nerve: a three-dimensional description of the obturator canal. *Surg Radiol Anat*. 2008;30:495-501.
20. Whiteside JL, Walters MD. Anatomy of the obturator region: relations to a trans-obturator sling. *Int Urogynecol J Pelvic Floor Dysfunct*. 2004;15:223-6.



Evaluation of second-trimester maternal serum betatrophin levels and lipid and carbohydrate metabolism parameters in patients with gestational diabetes mellitus

Gestasyonel diabetes mellituslu hastalarda ikinci trimester maternal serum betatropin düzeyleri ve lipid ve karbonhidrat metabolizması parametrelerinin değerlendirilmesi

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Abstract

Objective: We investigated the role of betatrophin in the etiopathogenesis of gestational diabetes mellitus (GDM) and its association with lipid and carbohydrate metabolism in patients with GDM and normoglycemic pregnant women.

Materials and Methods: A total of 60 patients [30 pregnant women with GDM (study group) and 30 healthy age-, body mass index-, and gestational age-matched pregnant women (control group)] were included in this study. Serum betatrophin, fasting glucose, insulin, glycated hemoglobin A1c (HbA1c), and C-peptide levels, as well as lipid parameters, were measured.

Results: Serum betatrophin, fasting glucose, HbA1c, insulin, and C-peptide levels were significantly higher in the GDM group than in the control group ($p<0.001$, $p=0.009$, $p=0.013$, $p<0.001$, and $p<0.001$, respectively). Levels of triglycerides and very-low-density lipoprotein cholesterol were significantly higher in the GDM group ($p=0.020$ and $p=0.020$, respectively), but total cholesterol and LDL cholesterol levels were similar in the two groups ($p=0.810$ and $p=0.273$, respectively). Betatrophin levels in the GDM group were correlated positively with insulin levels ($r=0.336$, $p=0.009$) and the homeostatic model assessment of insulin resistance (HOMA-IR) score ($r=0.269$, $p=0.038$), and negatively with the C-peptide levels ($r=-0.399$, $p=0.002$); they were not correlated with any other glucose or lipid parameters. Multivariate stepwise linear regression analysis demonstrated that insulin levels ($\beta=0.134$, $p=0.013$) and the HOMA-IR score ($\beta=0.112$, $p=0.017$) were associated independently with serum betatrophin levels.

Conclusion: These results demonstrate that serum betatrophin levels were significantly higher in pregnant women with GDM than in normoglycemic pregnant women. The levels of betatrophin were correlated significantly with insulin resistance parameters, which is a key feature of GDM pathophysiology.

Keywords: Betatrophin, gestational diabetes mellitus, insulin resistance

PRECIS: In this study, we compared serum levels of betatrophin in women with and without gestational diabetes mellitus, and investigated the relationships between betatrophin and lipid and glucose metabolism parameters.

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Öz

Amaç: Bu çalışmada betatrophinin gestasyonel diabetes mellitus (GDM) etiopatogenezindeki rolünü ve GDM ile komplike ve normoglisemik gebe kadınlarda betatrophinin lipid ve karbonhidrat metabolizması ile ilişkisini araştırdık.

Gereç ve Yöntemler: Çalışmaya toplam 60 hasta [GDM tanısı konmuş 30 gebe (çalışma grubu) ve 30 sağlıklı yaş, vücut kitle indeksi- ve gebelik haftası eşleştirilmiş gebe (kontrol grubu)] alındı. Serum betatrophin, açlık glukozu, insülin, glikolize hemoglobin A1c (HbA1c), C-peptid düzeyleri ve lipid parametreleri ölçüldü.

Bulgular: Serum betatrophin, açlık glukozu, HbA1c, insülin ve C-peptid düzeyleri GDM grubunda kontrol grubundan anlamlı olarak daha yüksekti (sırasıyla; $p<0,001$, $p=0,009$, $p=0,013$, $p<0,001$ ve $p<0,001$). GDM grubundaki betatrophin düzeyleri ile insülin seviyeleri ($r=0,336$, $p=0,009$) ve insülin direnci homeostatik model değerlendirmesi (HOMA-IR) skorunun ($r=0,269$, $p=0,038$) pozitif ve C -peptid seviyesi ile negatif olarak korelasyon gösterdiği bulundu ($r=-0,399$, $p=0,002$). Bununla birlikte betatrophinin diğer glukoz veya lipid parametreleri ile korele olmadığı saptandı. Çok değişkenli kademeli doğrusal regresyon analizi, insülin seviyelerinin ($\beta=0,134$, $p=0,013$) ve HOMA-IR skorunun ($\beta=0,112$, $p=0,017$) serum betatrophin seviyeleri ile bağımsız olarak ilişkili olduğunu gösterdi.

Sonuç: Bu sonuçlar GDM'li gebelerde serum betatrophin düzeylerinin normoglisemik gebelere göre anlamlı derecede yüksek olduğunu göstermektedir. Betatrophin seviyeleri, GDM patofizyolojisinin kilit bir özelliği olan insülin direnci parametreleri ile anlamlı şekilde korele idi.

Anahtar Kelimeler: Betatrophin, gestasyonel diabetes mellitus, insülin direnci

Introduction

Gestational diabetes mellitus (GDM) is defined as impaired carbohydrate tolerance characterized by severe hyperglycaemia that is recognized for the first time during pregnancy⁽¹⁾. GDM affects approximately 7% of all pregnancies; the rate varies from 1-28% depending on the diagnostic criteria and the study population⁽²⁾. GDM is associated with increased maternal, foetal, and neonatal risks, including preterm birth, hypertensive disease of pregnancy, macrosomia, polyhydramnios, foetal death, operative delivery, caesarean delivery, birth trauma, hypoglycaemia, hyperbilirubinemia, and respiratory distress syndrome⁽³⁾. GDM treatment is associated with significant reductions in primary outcomes from severe complications, such as perinatal death, shoulder dystocia, birth trauma, macrosomia, and preeclampsia⁽⁴⁾. Thus, screening for GDM is recommended for all pregnant women at 24-28 weeks' gestation, using a laboratory-based screening test and blood glucose levels when the diabetogenic effects of pregnancy are evident⁽⁵⁾.

Betatrophin is a recently identified circulating endocrine hormone that is secreted primarily by the liver and adipose tissue and plays an essential role in glucose homeostasis by promoting beta-cell proliferation⁽⁶⁾. This hormone, also known as lipasin, hepatocellular carcinoma-associated protein TD26, angiopoietin-like protein 8, and refeeding-induced fat and liver protein, also plays an important role in lipid metabolism by inhibiting lipoprotein lipase and reducing triglyceride clearance⁽⁷⁾. Studies have demonstrated that the expression of betatrophin is induced by insulin, food intake, and cold exposure, but is suppressed by starvation^(8,9). Some researchers noted that overexpression of betatrophin improved glucose tolerance by promoting β -cell proliferation and insulin production, whereas others failed to find any association between the expression of betatrophin and β -cell growth^(10,11). Increasing evidence suggests an association between altered betatrophin levels and type 2 DM or obesity, but the correlation between betatrophin expression and GDM is controversial. In addition, the effect of the betatrophin level on glucose and lipid metabolism has been controversial in several studies. Therefore, in this study,

we compared serum levels of betatrophin in women with and without GDM, and investigated the relationships between betatrophin and lipid and glucose metabolism.

Materials and Methods

This study was approved by the Local Ethics Committee of Fırat University, and informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki (approval no: 97132852/050.01.04). Sixty participants [30 pregnant women with GDM (study group) and 30 healthy age-, gestational age- and body mass index (BMI)-matched pregnant women (control group)] were enrolled in this prospective case-control study from the Fırat University Faculty of Medicine, Department of Obstetrics and Gynaecology between January 2017 and January 2018.

The inclusion criteria were maternal age 18-39 years, viable singleton pregnancy, admission for GDM screening at 24-28 weeks' gestation, BMI <35 kg/m², and an unremarkable medical or obstetric history. Exclusion criteria were the presence of any congenital malformation or chromosomal abnormality, foetal death, multiple pregnancies, maternal polycystic ovary syndrome, pregestational DM, family history of DM in a first-degree relative, hypertensive disease during pregnancy, chronic maternal disease (chronic hypertension, dyslipidaemia, chronic renal failure, pulmonary or cardiac disease, and malignancy), the use of any medication that interferes with lipid or glucose metabolism, and smoking or alcohol consumption. Gestational age was determined from the first day of the last menstrual period and confirmed by first trimester or early second-trimester ultrasonography.

GDM screening was performed in all participants between 24 and 28 gestational weeks using the 75-g oral glucose tolerance test (OGTT), as defined by the International Association of Diabetes and Pregnancy Study group one-step diagnostic approach⁽¹²⁾. After fasting for 8-10 h, the patients were requested to drink 75 g anhydrous glucose dissolved in 300 mL water within 5 min, followed by a measurement of venous plasma glucose concentrations 1 and 2 h after ingestion. The diagnosis of GDM was based on a single serum glucose level that met or exceeded the cut-off values (fasting glucose, 92 mg/dL; 1 h

value, 180 mg/dL; 2 h value, 153 mg/dL). Blood samples were centrifuged at 3500 rpm for 5 min to separate sera. Fasting plasma glucose levels and fasting serum levels of insulin and C-peptide were used to evaluate pancreatic β -cell function; we also measured serum levels of lipid metabolism parameters [total cholesterol; low-density-lipoprotein (LDL), high-density-lipoprotein (HDL), and very-low-density-lipoprotein (VLDL) cholesterol; and triglycerides]. Betatrophin levels and other biochemical parameters were measured on the day of OGTT screening. Glucose, lipid, and glycated hemoglobin A1c (HbA1c) levels were analyzed on an Olympus AU 2700 autoanalyzer (Olympus Optical Co., Tokyo, Japan). The homeostatic model assessment of insulin resistance (HOMA-IR) score, calculated by the formula defined by Matthews et al.⁽¹³⁾, was used to measure insulin resistance [fasting insulin level (μ U/mL) $\times$ fasting glucose level (mmol/L)/22.5]. BMI was measured during OGTT screening using the calculation: weight (kg)/height (m)².

Serum levels of betatrophin were determined using an enzyme-linked immunosorbent assay (ELISA; catalogue no. E-EL-H2206; Elabscience, Houston, TX, USA). Absorbance at 450 nm was recorded using an ELX800 ELISA reader. An automated model ELX50 washer was used to wash the plates. The assay results are expressed in pg/mL (range: 125-800 pg/mL).

Statistical Analysis

A power analysis (PASS 11; NCSS, LLC, Kaysville, UT, USA; www.ncss.com) suggested that at least 29 subjects should be included in each group if the greatest between-group difference in the betatrophin level was 11.12 ng/mL, with a standard deviation (SD) of 4.3 ng/mL, type I error of 0.05, and type II error of 0.10⁽¹⁴⁾. We recorded age, gravidity, parity, BMI, gestational age at screening, and perinatal outcomes of the patient and control groups. The Statistical Package for the Social Sciences version 22.0 software (SPSS Inc., Chicago, IL, USA) was used for the data analysis. Data are reported as means $\pm$ SDs, medians (ranges), or percentages. The normality of data distribution was checked using the Shapiro-Wilk test. Student's t-test was employed to compare biochemical parameters between the control and study groups. The Mann-Whitney U test was used to compare data when dependent variables were not normally distributed. The chi-square test and Fisher's exact test were used to compare categorical variables between the groups, as appropriate. Univariate correlations were analyzed using a non-parametric Spearman's correlation test. Multivariate stepwise linear regression was used to detect independent relationships between the metabolic parameters and serum betatrophin levels. p values <0.05 were considered to be significant.

Results

The maternal characteristics and perinatal outcomes of the study and control groups are summarized in Table 1. Patients

were matched in terms of age, BMI, and gestational age at screening, and these parameters did not differ significantly between the groups. Gravidity, parity, and perinatal outcomes were also similar in the two groups.

As expected, serum fasting glucose, insulin, and HbA1c levels, as well as HOMA-IR scores, were significantly higher in pregnant women with GDM than in controls (p=0.009, p<0.001, p=0.013, and p<0.001, respectively). Total cholesterol and LDL cholesterol levels were similar in the two groups (p=0.810 and p=0.273, respectively). VLDL cholesterol and triglyceride levels were significantly higher in the GDM group than in the control group (both p=0.020). Glucose and lipid metabolism parameters are summarized in Table 2.

Serum betatrophin levels were significantly higher in patients with GDM than in the control group (p<0.001; Figure 1). No significant correlation was evident between betatrophin levels and glucose or lipid metabolism parameters in the control group. However, significant correlations were detected between the betatrophin levels and insulin levels (r=0.336, p=0.009), C-peptide levels (r=-0.399, p=0.002), and HOMA-IR scores (r=0.269, p=0.038) in the GDM group; they were not correlated with any other glucose or lipid parameter. The results of correlation analyses for the GDM and control groups are

Table 1. Maternal characteristics and birth outcomes of the study and control groups

	Gestational DM (n=30)	Control (n=30)	p value
Age (years)*	34.50 $\pm$ 5.05	32.93 $\pm$ 5.34	0.248 ^t
BMI (kg/m ²)*	24.73 $\pm$ 5.21	22.90 $\pm$ 2.26	0.493 ^t
Gravidity**	4 (2-11)	3 (1-7)	0.324 ^u
Parity**	2 (1-9)	1 (0-5)	0.527 ^u
Gestational age at screening (weeks)**	25.0 (24-28)	24.0 (24-26)	0.752 ^u
Gestational age at birth (weeks)*	38.90 $\pm$ 1.86	39.20 $\pm$ 1.10	0.152 ^t
Mode of delivery***	-	-	0.493 ^f
Vaginal	9 (30)	12 (40)	-
Caesarean section	21 (70)	18 (60)	-
Birthweight (g)*	3396.00 $\pm$ 590.90	3249.00 $\pm$ 269.12	0.128 ^t
Sex***	-	-	0.210 ^x
Male	12 (40)	16 (53.3)	-
Female	18 (60)	14 (46.7)	-

*Mean $\pm$ standard deviation, **median (range), ***n (%).

Continuous data are expressed as mean $\pm$ standard deviation or the median (interquartile range) and categorical data are expressed as n (%). The differences among groups were assessed using the ^tt-test or ^uMann-Whitney U test, as appropriate. Categorical variables were assessed using the ^xPearson chi-square test and ^fFisher's exact test, as appropriate. BMI, body mass index; DM, diabetes mellitus

summarized in Table 3. Multivariate stepwise linear regression analysis revealed that the insulin levels ($\beta=0.134$, $p=0.013$) and HOMA-IR values ($\beta=0.112$, $p=0.017$) were independently related factors associated with serum betatrophin levels.

Discussion

The data presented here demonstrate that serum circulating betatrophin levels were significantly higher among pregnant

Table 2. Carbohydrate and lipid metabolism parameter levels

	Gestational DM (n=30)	Control (n=30)	p
Glucose (mg/dL)*	90.03±19.89	79.56±7.54	0.009
HbA1c (%)*	4.93±0.47	4.63±0.45	0.013
Insulin (µU/mL)*	84.85±2.89	13.83±6.69	<0.001
C-peptide (ng/mL)*	1.70±1.18	0.61±0.33	<0.001
Total cholesterol (mg/dL)*	228.56±40.40	225.70±51.14	0.810
HDL cholesterol (mg/dL)*	61.96±15.95	71.00±17.07	0.039
LDL cholesterol (mg/dL)*	140.65±37.71	129.79±38.31	0.273
VLDL cholesterol (mg/dL)*	45.33±16.22	36.33±12.60	0.020
Triglycerides (mg/dL)*	226.56±81.17	181.60±62.92	0.020
HOMA-IR score*	3.03±1.63	0.95±0.58	<0.001

*Mean ± standard deviation.

Student's t-test was used to compare biochemical parameters between the control and study groups. Significant p values are shown in bold.

HbA1c: Hemoglobin A1c, HDL: High-density-lipoprotein, LDL: Low-density-lipoprotein, VLDL: Very-low-density-lipoprotein, HOMA-IR: Homeostatic model of assessment of insulin resistance, DM: Diabetes mellitus

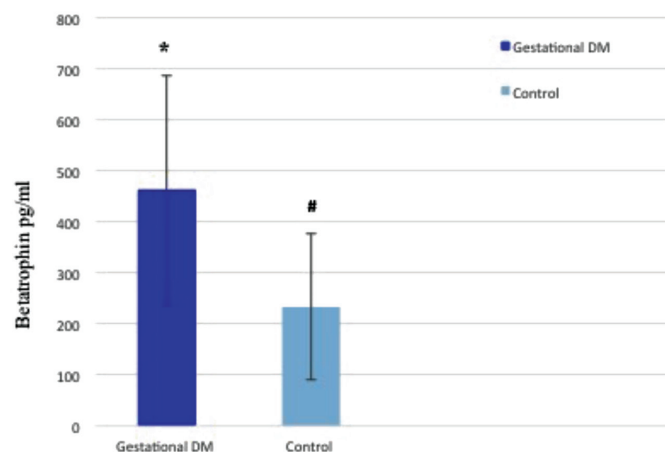


Figure 1. Differences in betatrophin levels between groups. Values are means ± standard deviations (pg/mL). * and # indicate significant differences ($p<0.001$). Gestational diabetes mellitus: 463.19±224.64, control: 233.13±143.63, $p<0.001$.

DM: Diabetes mellitus

women with GDM than among normoglycemic (control) pregnant women. In recent years, changes in various hepatocyte- and adipocyte-derived factors, such as adiponectin, resistin, leptin, and adipocyte fatty acid-binding protein, have been reported as mediators for the regulation of pregestational DM and GDM⁽¹⁵⁾. In addition, betatrophin has been identified as a new adipokine/hepatokine, and has been claimed to have important roles in promoting β -cell proliferation and β -cell mass expansion in animal and human studies^(16,17). Sun et al.⁽¹⁸⁾ demonstrated that the transplantation of betatrophin-expressing adipose-derived mesenchymal stem cells induced β -cell proliferation in mice with streptozotocin (STZ)-induced diabetes⁽¹⁸⁾. They showed that betatrophin overexpression induced pancreatic islet proliferation, β -cell-specific transcription factor expression, and insulin production by islet cells under glucose stimulation. In contrast, Gusarova et al.⁽¹⁹⁾ failed to show growth of beta cells in mice in response to targeted ANGPTL8 overexpression. Jiao et al.⁽²⁰⁾ demonstrated that betatrophin stimulated β -cell replication in an experimental S961-induced insulin resistance model in mice, but they did not increase human β -cell DNA replication in the transplanted setting⁽²⁰⁾. These inconsistent results of increased betatrophin may be due to the experimental models used (i.e., STZ-induced diabetes vs. S961-induced insulin resistance). In particular, the S961-induced insulin resistance model may be effective for shorter-duration experiments. Moreover, the proliferative

Table 3. Correlations between the betatrophin level and clinical and biochemical parameters in the GDM and control groups in weeks 24-28 of pregnancy

Variable	Control group		GDM group	
	r	p	r	p
Age	-0.251	0.182	-0.073	0.702
BMI at weeks 24-28	0.159	0.164	0.140	0.194
Fasting glucose level	-0.157	0.408	-0.303	0.104
HbA1C level	0.031	0.869	-0.056	0.770
Insulin level	0.176	0.352	0.336*	0.009
C-peptide level	0.279	0.059	-0.399*	0.002
HOMA-IR score	0.119	0.531	0.269*	0.038
Triglyceride level	0.194	0.303	-0.063	0.742
Total cholesterol level	-0.124	0.514	-0.111	0.561
HDL cholesterol level	0.094	0.622	-0.149	0.433
LDL cholesterol level	-0.109	0.566	-0.213	0.259
VLDL cholesterol level	0.194	0.305	-0.063	0.742

*Significant ($p<0.05$). Correlation analyses were performed by using non-parametric Spearman's correlation test. Significant p values are shown in bold.

BMI: Body mass index, HbA1c: glycated hemoglobin A1C, HDL: High-density-lipoprotein, LDL: Low-density-lipoprotein, VLDL: Very-low-density-lipoprotein, HOMA-IR: Homeostatic model of assessment of insulin resistance, GDM: Gestational diabetes mellitus.

potential of pancreatic islet cells can vary among mouse age groups, which may have biased the outcomes. Although the mechanism underlying the effect of betatrophin on glucose homeostasis has been attributed to increased β -cell proliferation, the precise impact of betatrophin on insulin secretion remains unclear, and further studies are needed to elucidate the exact mechanism.

In view of the frequency of GDM, several animal and human studies have been conducted to identify potential pathophysiological factors for insulin resistance in women with GDM. As a novel biomarker of glucose and lipid metabolism, betatrophin has been suggested to have a regulatory role in insulin resistance in patients with GDM⁽²¹⁾. The results of the present study also reveal that insulin levels and HOMA-IR scores are associated independently with serum betatrophin levels. Chen et al.⁽²²⁾ reported a significant increase in the betatrophin level in patients with type 2 DM, and showed significant correlations between the betatrophin level and insulin resistance indices, including the HOMA-IR score. Kong et al.⁽²³⁾ conducted a meta-analysis to evaluate the association between circulating betatrophin levels and GDM, and indicated that circulating betatrophin was evident in patients with GDM, especially in those with BMIs ≥ 28 kg/m² during the third trimester. They proposed that a higher tendency for insulin resistance in the third trimester of pregnancy and in obese patients may have contributed to these results, obtained from a subgroup analysis. Wawrusiewicz-Kurylonek et al.⁽²⁴⁾ showed increased maternal circulating betatrophin levels in patients with GDM, and the levels were about five times higher in cord blood than in maternal serum. They noted a negative correlation between maternal betatrophin and serum C-peptide concentrations, and suggested that decreased insulin secretion capability altered the betatrophin level.

Lipid metabolism significantly changes through pregnancy, due mainly to an increase in adipose tissue followed by increased lipolysis and hypercholesterolemia⁽²⁵⁾. Changes in oestrogen and progesterone have been proposed to contribute significantly to this physiologic hyperlipidaemia. However, the association between increased adiposity and circulating betatrophin in maternal serum has not been clearly defined⁽²⁶⁾. In this study, we observed no correlation between the serum betatrophin levels and lipid parameters in the GDM or control group. Similarly, Erol et al.⁽²⁷⁾ evaluated circulating betatrophin levels and metabolic parameters in women with GDM and found no correlation between betatrophin levels and lipid parameters, including triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels. By contrast, Fenzl et al.⁽²⁸⁾ demonstrated a significant association between betatrophin levels and atherogenic lipid profiles in patients with morbid obesity or type 2 DM. These conflicting results may be attributed to differences in study design, sample size, or the immunoassay kits used for laboratory assessment. Further studies with larger samples are needed before more definitive conclusions can be made.

Study Limitations

The current study has several limitations, such as the examination of a small sample from a single centre. However, the number of patients participating in the study was sufficient to analyse the serum betatrophin level and metabolic parameters. The change in betatrophin during the course of pregnancy was not determined because we were only able to analyse second-trimester serum betatrophin levels. The main strength of the study was the prospective cohort design with strict inclusion and exclusion criteria, which minimised the effects of potential confounding factors. A further strength was that the study and control groups were well matched in terms of baseline characteristics.

Conclusion

This study demonstrated that serum betatrophin levels were significantly higher in pregnant women with GDM than in normoglycemic pregnant women. The level of betatrophin was correlated significantly with insulin resistance parameters, which is a key feature of GDM pathophysiology. These findings may help to clarify the pathophysiology of GDM and may be useful for the prediction of GDM development during the second trimester.

Acknowledgments

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Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee of Firat University, and informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki (approval no: 97132852/050.01.04).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ç.K., Concept: H.B., Design: E.Ç.K., Data Collection or Processing: R.M., A.A., Analysis or Interpretation: M.F.G., F.G.B., Literature Search: H.B., E.Ç.K., Writing: F.G.B., R.M., M.F.G., H.B., E.Ç.K., A.A.

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References

1. American Diabetes Association. Classification and diagnosis of diabetes. *Diabetes Care*, 2015; 38 Suppl: S8-S16.
2. Jiwani A, Marseille E, Lohse N, Damm P, Hod M, Kahn JG. Gestational diabetes mellitus: results from a survey of country prevalence and practices. *J Matern Fetal Neonatal Med*. 2012;25:600-10.
3. Feldman RK, Tieu RS, Yasumura L. Gestational diabetes screening: the International Association of the Diabetes and Pregnancy Study

- Groups compared with Carpenter-Coustan Screening. *Obstet Gynecol* 2016; 127:10-7.
4. Negrato CA, Mattar R, Gomes MB. Adverse pregnancy outcomes in women with diabetes. *Diabetol Metab Syndr* 2012;4:41.
 5. ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus. *Obstet Gynecol*. 2018;131(2):e49-e64.
 6. Ren G, Kim JY, Smas CM. Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism. *Am J Physiol Endocrinol Metab* 2012;303:E334-E351.
 7. Zhang R. Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. *Biochem Biophys Res Commun* 2012;424:786-92.
 8. Fu Z, Berhane F, Fite A, Seyoum B, Abou-Samra AB, Zhang R. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. *Sci Rep* 2014; 23:5013.
 9. Quagliarini F, Wang Y, Kozlitina J, Grishin NV, Hyde R, Boerwinkle E, et al. Atypical angiopoietin-like protein that regulates ANGPTL3. *Proc Natl Acad Sci U S A* 2012;109:19751-6.
 10. Hu H, Sun W, Yu S, Hong X, Qian W, Tang B, et al. Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients. *Diabetes Care* 2014;37: 2718-22.
 11. Gómez-Ambrosi J, Pascual E, Catalán V, Rodríguez A, Ramírez B, Silva C, et al. Circulating betatrophin concentrations are decreased in human obesity and type 2 diabetes. *J Clin Endocrinol Metab* 2014;99:E2004-E2009.
 12. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010;33:676-82.
 13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-9.
 14. Trebotic LK, Klimek P, Thomas A, Fenzl A, Leitner K, Springer S, et al. Circulating betatrophin is strongly increased in pregnancy and gestational diabetes mellitus. *PLoS One* 2015;10:e0136701.
 15. Miehle K, Stepan H, Fasshauer M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clin Endocrinol (Oxf)* 2012;76:2-11.
 16. Yi P, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic β cell proliferation. *Cell* 2013;153:747-58.
 17. Espes D, Lau J, Carlsson PO. Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes. *Diabetologia* 2014;57:50-3.
 18. Sun LL, Liu TJ, Li L, Tang W, Zou JJ, Chen XF, et al. Transplantation of betatrophin-expressing adipose-derived mesenchymal stem cells induces β -cell proliferation in diabetic mice. *Int J Mol Med*. 2017;39:936-48.
 19. Gusarova V, Alexa CA, Na E, Stevis PE, Xin Y, Bonner-Weir S, et al. ANGPTL8/betatrophin does not control pancreatic beta cell expansion. *Cell* 2014;159:691-6.
 20. Jiao Y, Le Lay J, Yu M, Naji A, Kaestner KH. Elevated mouse hepatic betatrophin expression does not increase human β -cell replication in the transplant setting. *Diabetes* 2014;63:1283-8.
 21. Xu J, Lin Y, Zhou H, Zhao L, Xiang G. The correlation between circulating betatrophin and insulin resistance in general population: a meta-analysis. *Horm Metab Res* 2017;49:760-71.
 22. Chen X, Lu P, He W, Zhang J, Liu L, Yang Y, et al. Circulating betatrophin levels are increased in patients with type 2 diabetes and associated with insulin resistance. *J Clin Endocrinol Metab* 2015;100:96-100.
 23. Kong FJ, Ma LL, Li G, Chen YX, Zhou JQ. Circulating betatrophin levels and gestational diabetes mellitus: a systematic review and meta-analysis. *PLoS One* 2017;12:e0169941.
 24. Wawrusiewicz-Kurylonek N, Telejko B, Kuzmicki M, Sobota A, Lipinska D, Pliszka J, et al. Increased maternal and cord blood betatrophin in gestational diabetes. *PLoS One* 2015;10:e0131171.
 25. Herrera E, Ortega-Senovilla H. Maternal lipid metabolism during normal pregnancy and its implications to fetal development. *Clin Lipidol* 2010;5:899-911.
 26. Mankuta D, Elami-Suzin M, Elhayani A, Vinker S. Lipid profile in consecutive pregnancies. *Lipids Health Dis* 2010;9:58.
 27. Erol O, Ellidağ HY, Ayık H, Özel MK, Derbent AU, Yılmaz N. Evaluation of circulating betatrophin levels in gestational diabetes mellitus. *Gynecol Endocrinol* 2015;31:652-6.
 28. Fenzl A, Itariu BK, Kosi L, Fritzer-Szekeres M, Kautzky-Willer A, Stulnig TM, et al. Circulating betatrophin correlates with atherogenic lipid profiles but not with glucose and insulin levels in insulin-resistant individuals. *Diabetologia* 2014;57:1204-8.



Association between polymorphic markers human leucocyte antigen-G and tumour necrosis factor alpha and susceptibility to recurrent miscarriages among Bulgarian women

Polimorfik belirteçler insan lökosit antijen-G ve tümör nekroz faktörü alfa arasındaki ilişki ve Bulgar kadınlar arasında tekrarlayan düşüklere yatkınlık

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Abstract

Objective: To analyze the role of 14 base pair (bp) insertion (ins)/deletion (del) and tumour necrosis factor alpha (TNF- α) G/A polymorphisms as risk factors for spontaneous miscarriage in patients with two or more unsuccessful pregnancies and a group of control women with at least two normal live births.

Materials and Methods: To investigate the role of these mutations, 50 patients with two or more idiopathic recurrent miscarriages and 50 normal fertile women were tested for the presence of human leucocyte antigen-G (HLA-G) 14 bp ins/del and TNF- α -308 G/A variants. The frequencies of the studied polymorphisms were compared between the two groups.

Results: Individuals with a history of miscarriages had a significantly higher prevalence of 14 bp insertion alleles compared with control patients ($p=0.04$). There was also a two times higher relative risk for miscarriage among carriers of this variant. No statistical difference in allele frequencies of the TNF- α -308 G/A polymorphism was established between controls and study patients ($p=0.78$).

Conclusion: The 14 bp ins HLA-G variant could be associated with a higher risk for unsuccessful pregnancy according to the results from the study. There is no association between the studied TNF- α -308 GA polymorphism and rate of spontaneous abortions.

Keywords: HLA-G, insertion, deletion, TNF-alpha, GA variant, recurrent miscarriage

Öz

Amaç: İki veya daha fazla başarısız gebeliği olan hastalarda ve en az iki normal canlı doğum yapan kontrol grubu kadınlarında, spontan düşüklere için risk faktörü olarak, 14 baz çifti (bp) insersiyonlar/delesyon (del) ve tümör nekroz faktörü alfa (TNF- α) G/A polimorfizmlerinin rolünü analiz etmektir.

Gereç ve Yöntemler: Bu mutasyonların rolünü araştırmak için, iki veya daha fazla idiopatik tekrarlayan düşüklere olan 50 hasta ve 50 normal doğurgan kadın, insan lökosit antijen-G (HLA-G) 14 bp ins/del ve TNF- α -308 G varyantlarının varlığı açısından test edildi. İncelenen polimorfizmlerin frekansları iki grup arasında karşılaştırıldı.

Bulgular: Düşük öyküsü olan bireylerde, kontrol hastalarına kıyasla, 14 bp insersiyon alellerinin prevalansı anlamlı olarak daha yüksekti ($p=0,04$). Ayrıca, bu varyantın taşıyıcıları arasında rölâtif düşük yapma riski iki kat daha fazladır. Kontroller ve çalışma grubu hastaları arasında TNF- α -308 G/A polimorfizminin alel frekanslarında istatistiksel fark saptanmadı ($p=0,78$).

Sonuç: On dört bp ins HLA-G varyantı, çalışma sonuçlarına göre başarısız gebelik için daha yüksek bir riskle ilişkili olabilir. İncelenen TNF- α -308 G/A polimorfizmi ile spontan düşük oranı arasında bir ilişki yoktur.

Anahtar Kelimeler: HLA-G, insersiyon, delesyon, TNF-alfa, GA varyantı, tekrarlayan düşük

PRECIS: We have investigated the role of HLA-G and TNF Alpha variants as risk factors for recurrent miscarriages among Bulgarian women with fertility issues.

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Introduction

Recurrent miscarriage is described as three or more consecutive pregnancy losses⁽¹⁾. It is estimated that unsuccessful pregnancies make up around 30% of all conceptions⁽²⁾. There are various factors for miscarriage such as anatomic, endocrine, immunologic, and genetic. Nevertheless, the reasons often remain unknown⁽³⁾.

The fetus could be defined as a semi-allograft due to the presence of paternal human leucocyte antigen (HLA) class I molecules in the trophoblast, which invade the decidua basalis⁽⁴⁾. In order to prevent a potential miscarriage, the maternal immune system must accept the fetus and immunologic tolerance should be established⁽⁵⁾. HLA-G and tumour necrosis factor-alpha (TNF- α) molecules are both involved in this immunomodulation by regulating the functions of natural killer (NK) cells and the cytotoxic T-lymphocytes^(6,7).

The expression of HLA-G was first reported in the fetal trophoblast cells of the placenta⁽⁸⁾ and it has immunosuppressive functions; it inhibits proliferation and impairs the functions of T cells and NK cells, and induces the apoptosis of activated CD8+ T cells⁽⁹⁾. Also, HLA-G binds to the killer cell immunoglobulin-like receptor 2L4 (KIR2DL4) found on the surface of the decidual NK cells, and assists the remodeling of spiral arteries. This allows enough blood flow to the fetus and its normal development⁽¹⁰⁾.

However, there are several different polymorphisms found in the HLA-G locus that could have an impact on these functions. One of them is the insertion/deletion (ins/del) of a 14 base pair (bp) polymorphism in exon 8⁽¹¹⁾. Although the ins leads to a more stable RNA transcript, in individuals who are homozygous for the 14 bp ins polymorphism, there are decreased levels of soluble HLA-G⁽¹²⁾. In people who have the del, there are higher levels of this molecule in the plasma, which could explain the possible role of the insertion polymorphism as a risk factor for recurrent miscarriage^(13,14). TNF- α is a proinflammatory cytokine, whose levels might be increased in people carrying the AA polymorphism at position -308 in the TNF- α promoter region compared with carriers of the normal GG variant.⁽¹⁵⁾ This cytokine is considered a key factor for thrombotic events at the maternal uteroplacental blood vessel barrier because of activation of vascular endothelial cell procoagulant in animal models⁽¹⁶⁾. The TNF- α -308 AA polymorphism causes an increased production of the TNF- α cytokine in blood, it might influence the blood flow to the fetus in carriers, and could be associated with pregnancy loss⁽¹⁷⁾.

The aim of the present study was to analyze the role of 14 bp ins/del and TNF- α G/A polymorphisms as risk factors for spontaneous miscarriage in patients with two or more unsuccessful pregnancies, and a group of control women with at least two normal live births.

Materials and Methods

Participants

A total of 100 patients were divided into two groups and analyzed between October 2018 and July 2019 in the Laboratory of Medical Genetics in Varna, Bulgaria. Informed consent was obtained from all participants in the study prior to the analysis. The first group consisted of 50 patients with 2 or more idiopathic recurrent miscarriages during the first trimester. The second group included 50 normal fertile women without a previous history of miscarriage. All of the case subjects were without anatomic, microbial, viral, or endocrine diseases, which could explain the miscarriages.

The study was approved by the Ethics Committee of University Hospital St Marina, Varna, Bulgaria Laboratory of Medical Genetics on March 31st, 2016 (approval number: 78, date: 25.10.2018). Informed consent was obtained from all participants.

Methods

Genomic DNA samples from the individuals were extracted from peripheral blood and genotyped using polymerase chain reaction (PCR) and gel electrophoresis. They were tested for the presence of HLA-G 14 bp ins/del and TNF- α -308 G/A polymorphisms. The HLA-G region was amplified by using a forward primer 5'GTGATGGGCTGTTTAAAGTGTACCC-3' and a reverse primer 5'-GGAAGGAATGCAGTTCAGCATGA-3'. PCR was performed in a 20 μ L reaction, containing 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Estonia), primer mix, and the patient's DNA. The cycling conditions used were as follows: initial denaturation at 95 °C for 12 minutes, 35 cycles of 94 °C for 30 seconds, 63 °C for 40 seconds, 72 °C for 2 minutes, followed by extension at 72 °C for 7 minutes. All PCR products were evaluated using gel electrophoresis on 2% agarose gels containing ethidium bromide and then visualized under ultraviolet light (Figure 1).

The TNF- α polymorphism was amplified using touch-down real-time PCR using forward 5'TAGGTTTTGAGGGGCAAGG3' and reverse 5'TAGGTTTTGAGGGGCAAGA3' primers. PCR was performed in 20 μ L reactions, containing 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Estonia), primer mix, and the patient's DNA. The cycling conditions were initial denaturation at 95 °C for 10 minutes, 5 cycles of 94 °C for 20 seconds, 67 °C for 30 seconds, 72 °C for 45 seconds, 35 cycles of 94 °C for 20 seconds, 63 °C for 30 seconds, 72 °C for 45 seconds, followed by extension at 72 °C for 7 minutes.

Statistical Analysis

Differences between the two groups were analyzed using the Statistical Package for the Social Sciences software version 23 (IBM, USA) using odds ratio (OR), chi-square, Fisher's and Kruskal-Wallis tests. ORs were calculated with a confidence interval of 95%. A difference was considered significant at a

$p < 0.05$.

Results

The median age of the women with recurrent miscarriages was 35.0 years (25% percentile - 31.75; 75% percentile - 39.00). All were nulliparous, the mean miscarriage rate was 2.70 [standard deviation = 1.147]. The median age in the control group was 33.00 years (25% percentile - 30.75; 75% percentile - 36.00). Nine women (18.0%) from the case group were homozygotes for the wild type (del/del), 15 women (30.0%) were homozygotes for the mutant type (ins/ins), and 26 participants (52.0%) were heterozygotes (del/ins). The frequencies for the control group were 19 (38.0%), seven (14.0%), and 24 (48.0%) women, respectively (Figure 2).

The investigated polymorphism of the HLA-G gene showed a



Figure 1. Gel electrophoresis for the detection of the 14 base pair insertion/deletion polymorphism.

Lane 1: No template control, lanes 2,3,6: Insertion, lanes 4,5: Deletion, lane 7-9: heterozygote, M: 1 kb DNA ladder

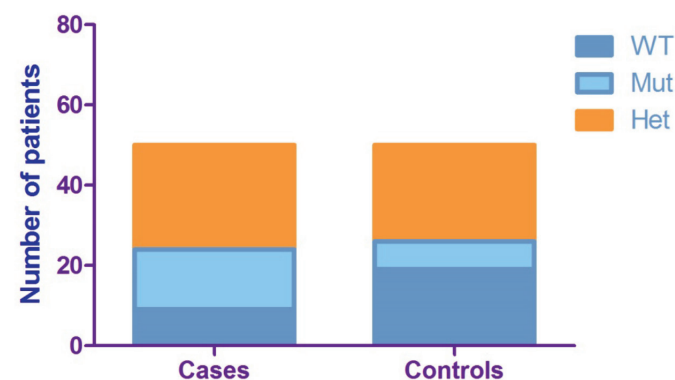


Figure 2. Prevalence distribution of the genotypes of the participants for the 14 base pair insertion/deletion variant in the HLA-G gene.

WT: Wild type, homozygotes for the deletion allele, Mut: Homozygotes for the insertion allele, Het: Heterozygotes for the insertion/deletion allele

statistically significant difference ($p=0.04$) compared with the control group (chi-square, degrees of freedom (df): 6.56). The OR was 4.52 and carriers of the ins polymorphism had a 2.12 times higher relative risk for a miscarriage compared with those with the del.

The women were also divided into two other subgroups, depending on their number of miscarriages – two or less and more than two, together with the corresponding HLA-G genotype in order to estimate the role of this polymorphism as an additional risk factor. In the first group, there were two homozygotes for the del, 11 homozygotes for the ins, and 15 heterozygotes. In the second group, there were seven homozygotes for the del, four homozygotes for the ins, and 11 heterozygotes. After applying the chi-square test, there was no statistical difference between the groups (chi-square, df 6.03, $p=0.05$).

The Kruskal-Wallis test was used to check if the HLA-G ins was associated with multiple miscarriages by comparing the number of spontaneous abortions and the genotypes of patients with recurrent miscarriages. The lowest number of unsuccessful pregnancies was two and the highest was seven. After applying this test, the p value was 0.13 and the null hypothesis was retained (Figure 3).

There were no significant differences in allele frequencies of TNF- α -308 G/A polymorphism between controls and study patients (chi-square, df: 0.08; $p=0.78$); eight (16.0%) AG heterozygotes and 42 (84.0%) GG homozygotes from the study group, and seven (14.0%) AG heterozygotes and 43 (86.0%) GG homozygotes from the control group. No homozygotes for the mutant allele AA were found in either group.

Fisher's test was performed in order to evaluate the cumulative effect of having a mutated allele for both polymorphisms. The

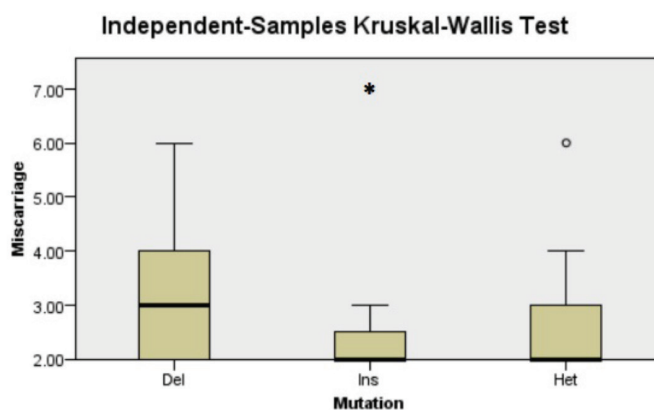


Figure 3. Kruskal-Wallis test for the comparison of the distribution of miscarriage and the different categories of mutation among the participants with recurrent miscarriages.

Del: Homozygous for the deletion allele, Ins: Homozygotes for the insertion allele, Het: Heterozygote carriers; *: the highest number of miscarriages for the group of homozygotes for the insertion allele; o: the highest number of miscarriages for the group of heterozygotes

number of patients from the RSA group and control group who were homozygous for the 14 bp ins and heterozygote GA carriers was two and one, respectively. There were four women who were heterozygotes for both variants from the first group, and five from the control group. No statistically significant difference was found between the two groups ($p=0.999$).

Discussion

HLA-G molecules modulate the immune system by inhibiting the activity of cytotoxic T lymphocytes and NK cells, causing apoptosis of activated CD8+ T and CD8+ NK cells, as well as by inhibiting the proliferation of allogenic CD4+ T cells⁽¹⁸⁾. HLA-G could also inhibit the transcription processes in the NK cells, thus protecting the extravillous trophoblast⁽¹⁸⁾.

All these functions of HLA-G illustrate its crucial role in the modulation of the maternal immune response, ensuring tolerance towards the semi-allogenic fetus in order to avoid miscarriage⁽¹⁹⁾.

However, the 14 bp ins variant in the HLA-G gene will lead to alternative splicing and to the lack of 92 bps from the 3'untranslated region⁽²⁰⁾. Despite the fact that this ins increases the stability of the RNA transcript⁽¹²⁾ in individuals who are homozygous for the 14 bp ins, there are lower concentrations of soluble HLA-G in serum compared with people who are homozygous for the 14 bp del^(14,21). This might interfere with maintaining pregnancy because low serum concentrations of HLA-G are considered a prognostic marker for increased risk of miscarriage and poor possibility of successful implantation of the embryo after in vitro fertilization^(19,22).

According to our results, the 14 bp ins variant is more common among women with recurrent miscarriages. Moreover, women who have this polymorphism have an approximately two times higher relative risk for a miscarriage, compared with controls. These findings correlate with another study, which concluded that the group of women with recurrent miscarriages showed a higher frequency of the ins allele in HLA-G, both in single and double copies⁽²³⁾. Another research group found that the total number of ins alleles was higher among participants with fertility issues, but the number of heterozygotes was the highest⁽²⁴⁾.

We also applied the Kruskal-Wallis test in order to estimate if carrying the HLA-G ins allele would increase the number of miscarriages, but according to the results, this variant had no impact on the amount of spontaneous abortions. In addition, after comparing the role of the HLA-G 14 bp ins variant as a risk factor for more than two miscarriages, there was no statistical difference. One limitation of the study is that the sample size was small. If more people were included, a statistical difference might have been established because the p value was close to the level of significance for both tests. However, the 14 bp ins polymorphism could be considered as a risk factor for a pregnancy loss itself, regardless of the number of miscarriages.

Even though the mechanisms for this are not clear, in a study conducted in Denmark, there was a correlation between the 14 bp ins/del polymorphism and fetoplacental growth. The authors concluded that mothers who were homozygous for the 14 bp del gave birth to babies with higher birthweight compared with the children of mothers homozygous for the 14 bp ins⁽²⁵⁾. However, the exact mechanisms of the protective effect of the HLA-G del remain to be determined.

The TNF- α -308 GA variant is also considered as a risk factor for miscarriages. In vitro experiments showed that this substitution resulted in higher activity of the transcription and increased levels of TNF- α in lipopolysaccharide-stimulated whole blood cell cultures⁽²⁶⁾. People who are carriers of the TNF- α -308 GA genotype also have a higher plasma concentration of TNF- α compared with those with the GG genotype⁽²⁷⁾. However, during pregnancy there is normally an increased production of Th2 or immunosuppressor cytokines such as interleukin 4 (IL-4), IL-10, and the levels of the proinflammatory cytokines IL-2 and TNF- α are decreased⁽²⁸⁾. Also, because TNF- α could activate NK cells in animal models⁽²⁹⁾ and blood clotting by increasing the expression of prothrombinase fibrinogen-like protein 2 fgl2,⁽³⁰⁾ it was assumed that the TNF- α -308 GA variant could be a risk factor for recurrent abortions.

Nevertheless, we found no statistical difference between the two studied groups for the TNF- α -308 GA polymorphism. The distribution of the mutated alleles was similar between the two groups. This is in agreement with the results from another study among 132 women with recurrent miscarriages, which concluded that TNF- α -238, but not the TNF- α -308 GA variant could have a potential impact⁽³¹⁾. The role of the studied polymorphism in TNF- α was excluded by another research group, which stated that there were higher levels of TNF- α during early pregnancy in women with recurrent miscarriages, but this was due to other variants in the TNF- α gene and not to the TNF- α -308 GA polymorphism⁽³²⁾.

Moreover, according to our results, mutations in both locuses of the HLA-G and TNF- α gene did not differ between the two groups and no cumulative effect was observed.

Study Limitations

Further studies are needed to define other factors that increase the risk of recurrent miscarriages. Confirmation of the data on a larger sample size could provide a better insight into the possible protective effect of del/del homozygotes for the HLA-G polymorphism.

Conclusion

Recurrent miscarriages may be due to various etiologic factors, but the 14 bp ins HLA-G variant could be associated with a higher risk for unsuccessful pregnancy according to the results from our study. There is no association between the studied TNF- α -308 GA polymorphism and the rate of spontaneous abortions among Bulgarian women. However, the 14 bp ins

variant could be included in the test panel for women with recurrent miscarriages.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University Hospital St Marina, Varna, Bulgaria Laboratory of Medical Genetics on March 31st, 2016 (approval number: of 2016/36-266).

Informed Consent: Informed consent was obtained from all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: T.C., Design: T.C., Data Collection or Processing: M.H., Analysis or Interpretation: M.H., Literature Search: M.L., Writing: L.A.

Conflict of Interest: The authors declare no conflict of interest.

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References

1. Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PloS one* 2010;5:e10287.
2. Page JM, Silver RM. Genetic causes of recurrent pregnancy loss. *Clinical obstetrics and gynecology* 2016;59:498-508.
3. ASRM PCot. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertility and sterility* 2012;98:1103-11.
4. Tilburgs T, Scherjon SA, van der Mast BJ, Haasnoot GW, Voort-Maarschalk MV-v, Roelen DL, et al. Fetal-maternal HLA-C mismatch is associated with decidual T cell activation and induction of functional T regulatory cells. *Journal of reproductive immunology* 2009;82:148-57.
5. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. *Nature immunology* 2006;7:241-6.
6. Liu S, Diao L, Huang C, Li Y, Zeng Y, Kwak-Kim JY. The role of decidual immune cells on human pregnancy. *Journal of reproductive immunology* 2017;124:44-53.
7. Elahi MM, Asotra K, Matata BM, Mastana SS. Tumor necrosis factor alpha- 308 gene locus promoter polymorphism: an analysis of association with health and disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 2009;1792:163-72.
8. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 1990;248:220-3.
9. Rizzo R, Vercammen M, van de Velde H, Horn PA, Rebmann V. The importance of HLA-G expression in embryos, trophoblast cells, and embryonic stem cells. *Cellular and molecular life sciences* 2011;68:341-52.
10. Rajagopalan S. HLA-G-mediated NK cell senescence promotes vascular remodeling: implications for reproduction. *Cellular & molecular immunology* 2014;11:460-6.
11. Shankarkumar U, Shankarkumar A, Chedda Z, Ghosh K. Role of 14-bp deletion/insertion polymorphism in exon 8 of the HLA-G gene in recurrent spontaneous abortion patients. *Journal of human reproductive sciences* 2011;4:143-6.
12. Rousseau P, Le Discorde M, Mouillot G, Marcou C, Carosella ED, Moreau P. The 14 bp deletion-insertion polymorphism in the 3' UT region of the HLA-G gene influences HLA-G mRNA stability. *Human immunology* 2003;64:1005-10.
13. Donadi EA, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. *Cellular and molecular life sciences* 2011;68:369-95.
14. de Almeida BS, Muniz YCN, Prompt AH, Castelli EC, Mendes-Junior CT, Donadi EA. Genetic association between HLA-G 14-bp polymorphism and diseases: a systematic review and meta-analysis. *Human immunology* 2018;79:724-35.
15. Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langemans JA, et al. TNF- α promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. *Journal of neuroimmunology* 1997;72:149-53.
16. Clark DA, Ding J-W, Yu G, Levy GA, Gorczynski RM. Fgl2 prothrombinase expression in mouse trophoblast and decidua triggers abortion but may be countered by OX-2. *Molecular human reproduction* 2001;7:185-94.
17. Li H-H, Xu X-H, Tong J, Zhang K-Y, Zhang C, Chen Z-J. Association of TNF- α genetic polymorphisms with recurrent pregnancy loss risk: a systematic review and meta-analysis. *Reproductive Biology and Endocrinology* 2016;14:6.
18. LeMaout J, Zafaranloo K, Le Danff C, Carosella ED. HLA-G up-regulates ILT2, ILT3, ILT4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. *The FASEB journal* 2005;19:662-4.
19. Ferreira LM, Meissner TB, Tilburgs T, Strominger JL. HLA-G: At the interface of maternal-fetal tolerance. *Trends in immunology* 2017;38:272-86.
20. Rizzo R, Bortolotti D, R Baricordi O, Fainardi E. New insights into HLA-G and inflammatory diseases. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)*. 2012;11:448-63.
21. Hviid TVF, Hylenius S, Rørbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. *Immunogenetics* 2003;55:63-79.
22. Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, et al. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *European journal of immunology* 2002;32:311-5.
23. Amodio G, Canti V, Maggio L, Rosa S, Castiglioni MT, Rovere-Querini P, et al. Association of genetic variants in the 3' UTR of HLA-G with recurrent pregnancy loss. *Human immunology* 2016;77:886-91.
24. Arjmand F, Samadi M. Association of 14-bp insertion/deletion polymorphism of HLA-G gene with idiopathic recurrent miscarriages in infertility center patients in Yazd, Iran. *Journal of immunotoxicology* 2016;13:249-54.
25. Hviid TVF. HLA-G genotype is associated with fetoplacental growth. *Human immunology* 2004;65:586-93.
26. Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID. Association of the TNF- α - 308 G/A promoter polymorphism with insulin resistance in obesity. *Obesity research* 2002;10:401-7.
27. Jiménez-Morales S, Velázquez-Cruz R, Ramírez-Bello J, Bonilla-González E, Romero-Hidalgo S, Escamilla-Guerrero G, et al. Tumor necrosis factor- α is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. *Human immunology* 2009;70:251-6.

28. Marzi M, Vigano A, Trabattoni D, Villa M, Salvaggio A, Clerici E, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clinical & Experimental Immunology* 1996;106:127-33.
29. Clark DA, Chaouat G, Arck PC, Mittrucker HW, Levy GA. Cutting edge: Cytokine-dependent abortion in CBA $\times$ DBA/2 mice is mediated by the procoagulant fgl2 prothrombinase. *The Journal of Immunology* 1998;160:545-9.
30. Knackstedt M, Zenclussen A, Hertwig K, Hagen E, Dudenhausen J, Clark D, et al. Th1 cytokines and the prothrombinase fgl2 in stress-triggered and inflammatory abortion. *American Journal of Reproductive Immunology* 2003;49:210-20.
31. Liu C, Wang J, Zhou S, Wang B, Ma X. Association between-238 but not-308 polymorphism of Tumor necrosis factor alpha (TNF-alpha) v and unexplained recurrent spontaneous abortion (URSA) in Chinese population. *Reproductive Biology and Endocrinology* 2010;8:114.
32. Piosik ZM, Goegebeur Y, Klitkou L, Steffensen R, Christiansen OB. Plasma TNF- α levels are higher in early pregnancy in patients with secondary compared with primary recurrent miscarriage. *American Journal of Reproductive Immunology* 2013;70:347-58.



Pregnancy associated plasma protein A: An indicator of adverse obstetric outcomes in a South India population

Gebeliğe bağlı plazma proteini A: Güney Hindistan nüfusunda olumsuz obstetrik sonuçların bir göstergesi

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Abstract

Objective: First trimester aneuploidy screening (FTAS) has become an integral part of antenatal care in most of centers in India. The serum markers used for FTAS are pregnancy-associated plasma protein A (PAPP-A) and beta human chorionic gonadotropin. In the present study, we aimed to assess the role of PAPP-A in specific adverse fetal maternal events. To analyze pregnancy outcomes with low maternal PAPP-A ($\leq 5^{\text{th}}$ percentile) at the FTAS screening test in southern India, and them compared with a control group of $>5^{\text{th}}$ percentile value.

Materials and Methods: A total of 1800 consecutive pregnancies in the first trimester were followed up with PAPP-A levels. The study group consisted 108 subjects, which was compared with a matched control group of 288 subjects. The outcomes considered were spontaneous abortions, fetal anomalies, preterm delivery (PTD), hypertension in pregnancy, intrauterine growth restriction, gestational diabetes, mode of delivery, and birthweight.

Results: For our grouped data, the 5^{th} percentile value for PAPP-A was 0.49 multiple of medians, (incidence-6%). The incidence of fetal major anomalies was higher in the study group [odds ratio (OR): 1.87]. The incidence of minor anomalies, gestational diabetes, and hypertensive disorders was higher in the study group but not statistically significant. The total rate of PTDs (OR:2.1), small-for-gestation-age fetuses (OR:2.3), and low birthweight babies (OR-2.12) was significantly higher in the study group. We found positive likelihood ratio of 1.4 for PTD, 2 for $<5^{\text{th}}$ percentile birthweight, and 1.7 for $<10^{\text{th}}$ centile birthweight.

Conclusion: Low PAPP-A pregnancies are at risk of various obstetric complications. Hence, such a pregnancy should have closer surveillance. Further research work on intervention strategy is needed.

Keywords: Pregnancy-associated plasma protein a, dual marker test, first trimester aneuploidy screening, adverse pregnancy outcomes, growth restriction of fetus

Öz

Amaç: İlk trimester anöploidi taraması (FTAS), Hindistan'daki merkezlerin çoğunda doğum öncesi bakımın ayrılmaz bir parçası haline gelmiştir. FTAS için kullanılan serum belirteçleri hamilelikle ilişkili plazma protein A (PAPP-A) ve beta insan koryonik gonadotropindir. Bu çalışmada spesifik olumsuz fetal maternal olaylarda PAPP-A'nın rolünü değerlendirmeyi amaçladık.

Güney Hindistan'da FTAS tarama testinde düşük maternal PAPP-A (≤ 5 persentil) ile gebelik sonuçlarını analiz etmek ve bunları >5 persentil değerine sahip bir kontrol grubu ile karşılaştırmak.

Gereç ve Yöntemler: İlk trimesterde toplam 1800 ardışık gebelik PAPP-A düzeyleri ile takip edildi. Çalışma grubu 108 denekten oluşmaktaydı ve 288 denekle eşleşen kontrol grubu ile karşılaştırıldı. Değerlendirilen sonuçlar spontan düşükler, fetal anomaliler, erken doğum (ED), gebelikte hipertansiyon, intrauterin büyüme kısıtlaması, gestasyonel diyabet, doğum şekli ve doğum ağırlığı idi.

Bulgular: Gruplandırılmış verilerimiz için, PAPP-A için 5. persentil değeri 0.49 medyanların katları idi (insidans: %6). Fetal majör anormali insidansı çalışma grubunda daha yüksekti [olasılık oranı (OR): 1,87]. Küçük anomaliler, gestasyonel diyabet ve hipertansif bozuklukların görülme sıklığı çalışma grubunda daha yüksekti ancak istatistiksel olarak anlamlı değildi. ED'lerin (OR: 2,1), gebelik yaşı için küçük fetüslerin (OR: 2,3) ve düşük doğum ağırlıklı

PRECIS: Using low pregnancy-associated plasma protein A levels in the first trimester of pregnancy, we analyzed the risk of adverse pregnancy outcomes in a South Indian population.

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Öz

bebeklerin (OR: 2,12) toplam oranı çalışma grubunda anlamlı olarak daha yüksekti. Pozitif OR ED için 1,4, <5. persentil doğum kilosu için 2 ve <10. centil doğum kilosu için 1,7 idi.

Sonuç: Düşük PAPP-A gebelikleri çeşitli obstetrik komplikasyon riski altındadır. Bu nedenle, böyle bir hamileliğin daha yakından izlenmesi gerekir. Müdahale stratejisi üzerinde daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Gebeliğe bağlı plazma proteini a, ikili markör testi, ilk trimester anöploidi taraması, olumsuz gebelik sonuçları, fetüsün büyüme kısıtlaması

Introduction

Pregnancy-associated plasma protein-A (PAPP-A) is a glycoprotein that is secreted by the syncytial trophoblast and decidua, and appears in circulation soon after the blastocyst implantation⁽¹⁾. PAPP-A has metalloprotease activity, which cleaves insulin-like growth factor binding protein and releases insulin-like growth factor (IGF). Free IGF, which is ~1% in circulation, has a role in cell multiplication, differentiation, and invasion of trophoblastic cells; hence, it is important for placental development. IGF also regulates the uptake of glucose and amino acid, thus fetal growth^(2,3). Accordingly, PAPP-A is a part of the system that controls IGF activity⁽⁴⁾. There is growing evidence that low PAPP-A levels in the first trimester are associated with adverse fetal and maternal outcomes such as spontaneous abortion (SA) preterm delivery (PTD), fetal growth restriction (FGR), preeclampsia (PE), and stillbirth, apart from chromosomal aneuploidy⁽⁵⁻⁷⁾.

Low PAPP-A is defined as a maternal serum concentration less than the 5th percentile. The landmark randomized clinical trial FASTER found that the PAPP-A of <5th percentile was associated with FGR [odds ratio (OR)-3.2], SA (OR-2.5), PTD (OR-1.9), birthweight below the 5th percentile (OR-2.8), PE (OR-1.5), and placental abruption (OR-1.8)⁽⁸⁾. Other cohort studies have confirmed similar findings^(9,10).

The aim of the present study was to obtain the 5th percentile value in our grouped data, and to analyze the risk of adverse pregnancy outcomes in ≤5th percentile in the PAPP-A group in reference to the >5th percentile group.

Materials and Methods

The study was approved by the Manipal Ethics Committee, Manipal Academy of Higher Education (approval number: MUEC/03/2015). This prospective study was conducted at Dr. TMA Pai Hospital, Udupi, affiliated to Manipal University, Manipal from November 2015 to 2018. Informed consent was obtained after briefing the participants about the study in Kannada and English languages. Gestational age was based on the last menstrual period with previous regular cycles and corresponding scans between 7-10 weeks of gestation or on measured crown-rump length (CRL) at 7-10 weeks of gestation. A disparity in CRL of up to 5 days was considered acceptable. We excluded multiple pregnancies, patients with renal diseases, chronic hypertension, insulin-dependent diabetes mellitus, cardiac disorders, and chromosomal abnormalities. All singleton pregnancies undergoing first trimester aneuploidy screening

were enrolled in the study. Information regarding age; weight; height; first trimester events such as threatened abortion, and previous history of fetal aneuploidy were obtained. The smoking status was also determined, even though it was uncommon.

Three to four milliliters of venous blood sample was collected. The serum was separated and analyzed at the biochemistry department of Kasturba Hospital, Manipal. The test was conducted using an automatic analyzer with an electrochemiluminescence assay on a Cobase 601 analyzer, which provided marker concentration. Risk calculation was performed using the SSWD Lab version 5.0 software, and values are given as multiple of medians (MoM).

The percentile values for PAPP-A were obtained from 1800 enrolled pregnancies for FTAS. All women with serum PAPP-A of less than the 5th percentile were enrolled as cases, and women with normal (>5th percentiles) PAPP-A were taken as controls. The fetal aneuploidy risk of 1:250 or higher was taken as high risk for chromosomal abnormality and hence, invasive test counseling was offered. All the confirmed chromosomal abnormalities were counseled regarding the outcome of the fetus and excluded from the study. The rest of the women were followed as per the hospital protocols. Woman detected with lethal anomalies at the second trimester scan were given the option for termination of pregnancy (TOP). Pregnancy outcome information was obtained for SA, fetal anomalies, PTD, hypertension in pregnancy (PIH), PE, gestational diabetes (GDM), small-for-gestational-age (SGA) babies, delivery mode, and low birth weight babies (LBW).

SA was defined as loss of pregnancy before 20 weeks of gestation or a fetus weighing <500 g. PTD was defined as a delivery before 37 completed weeks. SGA was defined as birth weight less than -2 SD below the gestational age. The reference for percentile birth weight was taken from Callen's book of ultrasound.⁽¹¹⁾ PIH was determined as a blood pressure of more than 140/90 mm Hg after 20 weeks of pregnancy in two recordings 6 hours apart, and without significant proteinuria. PE was determined as hypertension with proteinuria >300 mg/24 hours or any sign of end-organ damage⁽¹²⁾. LBW was defined as a birth weight of less than 2.5 kg⁽¹³⁾.

Statistical Analysis

Statistical analysis was performed using The Statistical Package for the Social Sciences (SPSS) software version 20, and p values <.05 were considered statistically significant after application of the chi-square test. Student's t-test was used for the comparison of means. Fisher's exact test was used when appropriate.

OR with 95% confidence intervals was calculated for several outcomes. The performance characteristics (sensitivity, specificity negative and positive predictive values, likelihood ratio) were calculated. Receiver operating characteristics curves (ROC) were constructed and the area under the curve was calculated for certain pregnancy complications.

Results

A total of 1800 pregnant women attending the antenatal clinics underwent first trimester fetal aneuploidy screening and the 1st, 3rd, 5th, 10th, 50th, 90th, 95th, and 99th percentile values for PAPP-A obtained in present cohort were 0.23, 0.33, 0.49, 0.61, 1.26, 2.4, 3.13, and 5.1 MoM. The 5th percentile of PAPP-A values corresponded to 0.49 MoM in the study population. The incidence of low PAPP-A was 6%. A total of 113 pregnancies with a PAPP-A value ≤ 0.49 constituted the study group. Amongst them, one had aneuploidy fetus, one had a SA at the 15th week of gestation, seven had a TOP in view of lethal anomaly, five had pre-gestational diabetes, and five were lost to follow-up, which were excluded for delivery outcome analysis. Accordingly, 94 patients were followed until delivery and then analyzed. Two hundred eighty-two (1:3) pregnant women were enrolled in the control group with normal PAPP-A values; every 5th pregnancy from a cohort of 1687 subjects was taken to form the control group. The consort flow diagram (Figure 1) depicts the subject enrolment. We observed five fetuses with cardiac anomalies (transposition of the great vessels n=1, atrio-ventricular wall defect (n=2, double-outlet

right ventricle (n=1, hypoplastic left heart (n=1), one fetus with a urogenital abnormality, and one pregnancy with severe oligohydramnios at 18 weeks of gestation in the study group. We had a total of six pregnancies with an increased risk of fetal aneuploidy and prenatal diagnosis revealed one fetus had Patau syndrome. There were five lethal anomalies in the control group, three involving the central nervous system (agenesis of the corpus callosum, severe hydrocephalus, vermian agenesis), one cardiac anomaly (double-outlet right ventricle), and one with a renal anomaly. Accordingly, 288 patients were enrolled in the control group to obtain 282 controls for analysis, because there was one second trimester abortion.

The characteristics of both groups are presented in Table 1. Parameters such as age, weight, weight gain in pregnancy, and sampling period were comparable in both groups. The mean body mass index (BMI) was 22.9 ± 3.91 kg/m² for the study group, whereas it was 21.7 ± 3.87 kg/m² in the control group, which was found to be significantly higher. There were 170 (60%) nulliparous patients in the control group and 45 (49%) in the study group, the difference was not statistically significant. The mean CRL was 58.36 ± 5.5 mm in the study group and 60.0 ± 6.4 mm in the control group ($p=0.051$). Table 2 compares the obstetrics complications with ORs for various pregnancy complications. The incidence of threatened abortion (10.6% vs. 11.3%) and premature rupture of membrane (7.4% vs. 8.5%) was not significantly different between the study and control groups. We found a higher incidence of minor birth defects (6.4% vs. 4.3%) in the study

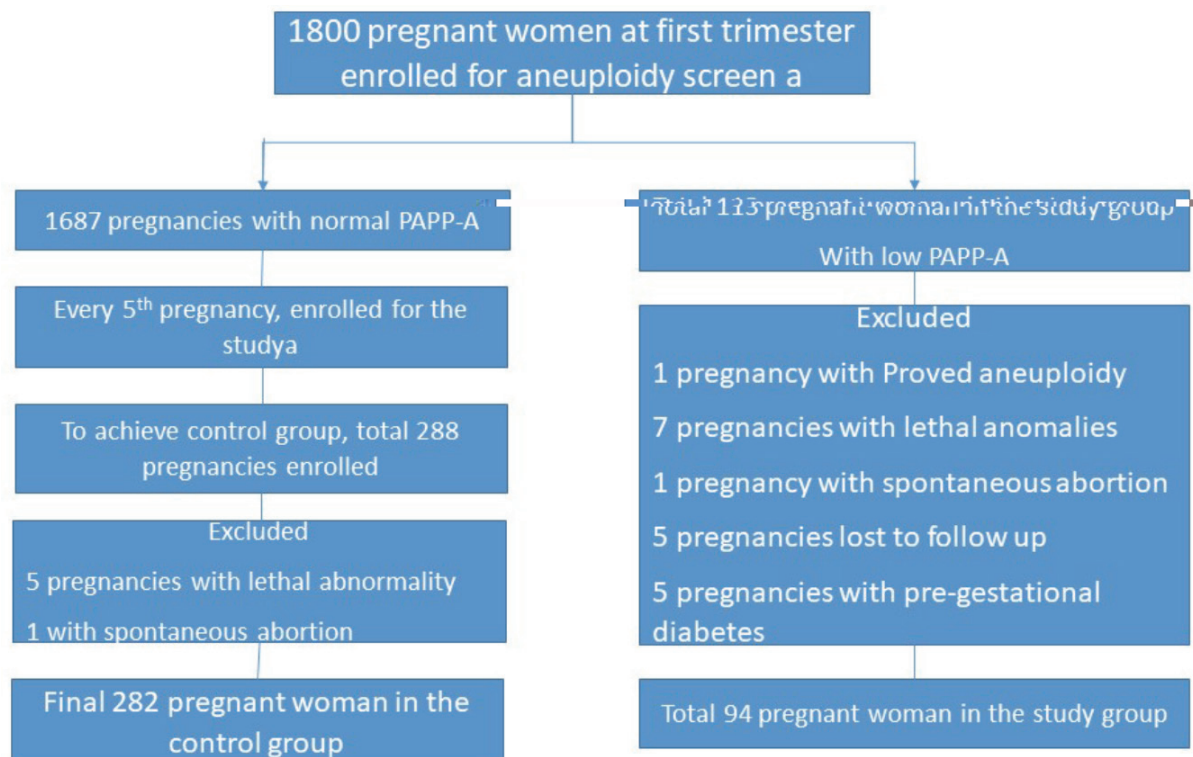


Figure 1. Consort diagram about enrolling subjects and sample size in the different group

group, which was not statistically significant. Table 3 mentions the association of low PAPP-A with pregnancy outcomes. Table 4a and 4b depicts the predictive value of PAPP-A for various complications. ROC analysis suggested further predictive ability.

Discussion

We took percentile values for the study, another way is to fix arbitrary absolute values for the formation of the study group and analysis. In our study, the cut-off value was 0.49 MoM, which is in concordance with study by Patil et al.⁽¹⁴⁾ Low PAPP-A is considered between 0.3-0.5 MoM in different

studies.⁽¹⁵⁻¹⁷⁾ The incidence of low PAPP-A (<5th percentile) was 6% in our study, the study by Yaron et al.⁽¹⁶⁾, reported 15.4%, such a variation may be due to different populations and patient profiles. The incidence was 5.4% in a study by Cooper et al.⁽¹⁸⁾, with an absolute cut-off for PAPP-A <0.4 MOM.

We observed a higher incidence of SA (1.1% vs. 0.7%) in the study group, which was not statistically significant. The study by Barrett et al.⁽¹⁵⁾ observed increased pregnancy loss and Kaijomaa et al.⁽¹⁹⁾ reported an OR of 7.7 for SA in the low PAPP-A group, suggesting a strong risk factor. An abortion has diverse etiology and one important reason is depleted hormonal and nutritional support to a growing fetus. Here, the low amount of PAPP-A could be one of the reasons for suboptimal placental growth and function leading to the above-mentioned insufficiency.^(10,17) Another reason could be undetected chromosomal abnormalities becoming naturally aborted in this case.

We observed a higher number of congenital lethal abnormalities in targeted scans in the study group (OR: 3.7); such increased chromosomal/non-chromosomal birth defects were observed by Barrett et al.⁽¹⁵⁾ (RR: 2.2), even after excluding pregnancies with chromosomal aneuploidy. All 12 patients with major birth defects underwent TOP and fetal autopsy; fetal chromosomal status was known for only a few fetuses. There was no statistically significant difference between the groups for minor anomalies, a similar observation was documented by Barrett et al.⁽¹⁵⁾ We observed cardiac defects as a major birth defect in the study group, and mainly nervous system abnormalities in the control group. Such an observation is not present in the available literature, hence, this could be coincidence. However, further studies are required to know the type of fetal malformations in women with low PAPP-A.

After excluding all birth defects and chromosomal abnormalities, a significant increase risk of PTD, SGA

Table 1. Characteristics of study population in subgroups

	Study group n=94	Control group n=282	p
Maternal age (years $\pm$ SD)	28.7 $\pm$ 3.72	27.8 1 $\pm$ 3.74	0.14
BMI I (kg/m ² $\pm$ SD)	22.9 $\pm$ 3.91	21.7 $\pm$ 3.87	0.04
Pre-pregnancy weight (kg $\pm$ SD)	54.79 $\pm$ 10.46	52.59 $\pm$ 10.39	0.19
Weight gain in pregnancy (kg $\pm$ SD)	10.28 $\pm$ 3.84	11.04 $\pm$ 4.13	0.17
Gravidae	1.75 $\pm$ 0.74	1.60 $\pm$ 0.82	0.20
Gestational weeks at FTS (SD)	12.30 $\pm$ 0.50	12.30 $\pm$ 0.43	0.39
Crown rump length (mm $\pm$ SD)	58.36 $\pm$ 5.40	60.06 $\pm$ 6.46	0.05
β hCG value (MoM $\pm$ SD)	0.91 $\pm$ 0.57	1.03 $\pm$ 0.85	0.58

SD: Standard deviation, BMI: Body Mass index, FTS: First trimester combined screening, β hCG: Beta human chorionic gonadotropin Mom: Multiple of medians

Table 2. Complications during pregnancy in both the groups and predictive ability of marker

	Study group n=94 (%)	Control group, n=282 (%)	p	Odds ratio (95% confidence interval)
Structural lethal abnormality*	7 (7.1%)*	5 (1.8%)*	0.2	3.7 (1.21-11.0)
Preterm delivery	23 (24.7%)	38 (13.5%)	0.01	2.15 (1.17-3.77)
PIH/PE	14 (14.9%)	26 (9.2%)	0.12	1.72 (0.85-3.45)
Oligohydramnios	15 (16%)	33 (11.7%)	0.28	1.43 (0.74-2.77)
SGA				
<10 th centile	31 (33%)	49 (17.4%)	0.001	2.34 (1.37-3.97)
<5 th centile	22 (23.4%)	27 (9.6%)	0.001	2.88 (1.55-5.36)
Gestational diabetes	15 (16%)	26 (9.2%)	0.08	1.87 (0.94-3.70)
Low birth weight	32 (34%)	55 (19.6%)	0.004	2.12 (1.26-3.56)
Composite outcome**	57 (60.6%)	111 (39.4%)	0.001	2.37 (1.41-3.85)

*Study group consist of 108 subjects and control group 288 subjects, **Structural anomalies not included, PIH: Pregnancy induced hypertension, PE: preeclampsia, SGA: Small for gestational age

Table 3. Outcome of pregnancy in both groups

	Study group n=94 (%)	Control group n=282 (%)	p
Mean gestational age at delivery	37.6±1.51	38.3±1.27	0.001
Mean weight at birth	2.63±0.52	2.95±0.47	0.001
Caesarean delivery	42 (44.7%)	90 (31.1%)	0.03
Stillbirth	1 (1.1%)	0	0.85

Table 4a. Performance characteristics of low PAPP-A for selected outcomes

Variable	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio
Major anomalies	53.8	76	2.2	0.61
Gestational diabetes	36.6	76.4	1.5	0.82
Hypertension in pregnancy	37.7	77.7	1.6	0.80
Preterm delivery	35.0	76	1.4	0.85
Small for gestation age				
<5 th percentile	44.9	78	2.0	0.71
<10 th percentile	38.8	78.7	1.72	0.79
Low birth weight	36.8	78.5	1.7	0.8
PAPP-A: Pregnancy-associated plasma protein A				

Table 4b. Performance characteristics of low PAPP-A for selected outcomes

Outcome	AUC	p	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
Gestational diabetes	0.590	0.060	0.502	0.678
Hypertension in pregnancy	0.608	0.025	0.516	0.701
Preterm delivery	0.628	0.002	0.550	0.705
Small for gestation age				
<5 th percentile	0.710	<0.001	0.637	0.782
<10 th percentile	0.671	<0.001	0.607	0.735
Low birth weight	0.655	<0.001	0.591	0.720

PAPP-A: Pregnancy-associated plasma protein A AUC: Area under the curve

and LBW was found in the study group. The maximum risk increase was about 3 folds for SGA (OR: 2.88). These findings dovetail with other studies^(8,10,15). However, there is a report mentioning its non-association with both PTD and IUGR⁽²⁰⁾. The study by Pummara et al.⁽³⁾ suggested that <10th PAPP-A percentile was associated with idiopathic preterm and concluded that such pregnancies should be labelled as high risk for preterm delivery. A systemic review and meta-analysis by Morris et al.⁽¹⁰⁾ also confirmed a moderate association of low PAPP-A with SGA and PTD with poor the predictive ability. We found a positive likelihood ratio (LR) of 1.4 for PTD, 2 for <5th percentile birth weight, and 1.7 for <10th percentile birthweight. A similar positive LR of 1.84 for PTD, 2.65 for <5th percentile birth weight, and 1.96 for <10th percentile birth weight was revealed by Morris et al.⁽¹⁰⁾ Our findings are suggestive of low sensitivity but high negative predictive value for the prediction of adverse events, which are in concordance with the FASTER trial⁽⁸⁾. We had one stillbirth of 900 g at 30 weeks of gestation in the study group, which was not statistically significant. The incidence of PIH was higher in the study group (14.9% vs. 9.2%); however, it was not statistically significant. A similar positive but strong correlation was found in various studies^(7,17). PIH can be explained by defective placentation and inflammatory cascade activation resulting in different complications. The study by Beneventi et al.⁽²¹⁾ suggested that first trimester PAPP-A levels were significantly lower among the pregnancies subsequently affected by gestational diabetes. We also noticed a higher incidence of GDM (16% vs. 9.2%), which was not statistically significant. A study by Petry et al.⁽⁴⁾ suggested the possibility of a link between low PAPP-A concentrations and glucose levels due to the effect of IGF on insulin sensitivity.

We believe that the root cause for the increased incidence of PTD is placental insufficiency, IUGR/SGA, and LBW. The placental syncytio-trophoblasts produces PAPP-A, which acts as one of the proteins for the prevention of the recognition of the fetus by the maternal immune system, helps in angiogenesis, placental cellular hyperplasia, and maternal vascular system invasion by trophoblasts. It also helps in matrix mineralization; therefore, low PAPP-A is linked to the rejection of fetus and unhealthy placentation resulting in a spectrum of complications. Low PAPP-A has been accepted as a marker for pregnancy complications, and it is recommended to follow such patients for growth disorders⁽²²⁾.

Limitations and strengths: this study provides useful information to physicians that can help them in the management of high-risk pregnancy with normal karyotype and low PAPP-A. The PAPP-A values cannot be extrapolated because it represents the Southern region of India. The higher BMI in the study group can be a confounding factor. Due to the small sample size, only few events could be registered for certain pregnancy outcomes.

Conclusion

PAPP-A levels can be used to differentiate pregnancy as high risk for adverse obstetric outcomes. Low PAPP-A has a modest association with adverse pregnancy outcomes in the absence of chromosomal abnormalities. The awareness of low values can help in improving fetal outcomes by closer surveillance for adverse events. However, it has limited use due to its lower predictive value. Further research on the development of a prediction model and formation of a preventive strategy for different obstetric adverse events is needed.

Ethics

Ethics Committee Approval: The study was approved by the Manipal Ethics Committee, Manipal Academy of Higher Education (approval number: MUEC/03/2015).

Informed Consent: Informed consent was taken from all participants.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Concept: R.G.B., S.B., Design: R.G.B., Data Collection or Processing: A.A., P.N., K.H.S, Analysis or Interpretation: A.A., P.N., K.H.S, Literature Search: A.A., P.N., K.H.S, Writing: A.A., P.N., K.H.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Fialova L, Malbohan IM. Pregnancy-associated plasma protein A (PAPP-A): theoretical and clinical aspects. *Bratisl Lek Listy* 2002;103:194-205.
2. Wells G, Bleicher K, Han X, McShane M, Chan YF, Bartlett A, et al. Maternal diabetes, Large-for-Gestational-Age Births, and First Trimester Pregnancy-Associated Plasma Protein-A. *J Clin Endocrinol Metab* 2015 Jun;100:2372-9.
3. Pummara P, Tongsong T, Wanapirak C, Sirichotiyakul S, Luewan S. Association of first-trimester pregnancy-associated plasma protein A levels and idiopathic preterm delivery: A population-based screening study. *Taiwan J Obstet Gynecol*. 2016;55:72-5.
4. Petry CJ, Ong KK, Hughes IA, Acerini CL, Frystyk J, Dunger DB. Early Pregnancy-Associated Plasma Protein A Concentrations Are Associated With Third Trimester Insulin Sensitivity. *J Clin Endocrinol Metab* 2017;102:2000-8.
5. Kavak ZN, Basgul A, Elter K, Uygur M, Gokaslan H. The efficacy of first-trimester PAPP-A and free beta hCG levels for predicting adverse pregnancy outcome. *J Perinat Med* 2006;34:145-8.
6. Gagnon A, Wilson RD. Obstetrical complications associated with abnormal maternal serum markers analytes. *J Obstet Gynaecol Can* 2008;30:918-32.
7. Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* 2000;107:1265-70.
8. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *Am J Obstet Gynecol* 2004;191:1446-51.
9. Gupta S, Goyal M, Verma D, Sharma A, Bharadwaj N, Kabra M, et al. Adverse pregnancy outcome in patients with low pregnancy-associated plasma protein-A: The Indian Experience. *J Obstet Gynaecol Res* 2015;41:1003-8.
10. Morris RK, Bilagi A, Devani P, Kilby MD. Association of serum PAPP-A levels in first trimester with small for gestational age and adverse pregnancy outcomes: systematic review and meta-analysis. *Prenat Diagn* 2017;37:253-65.
11. Norton ME. *Callen's Ultrasonography in Obstetrics and Gynecology* E-Book. Elsevier Health Sciences; 2016.
12. Kwik M, Morris J. Association between first trimester maternal serum pregnancy associated plasma protein-A and adverse pregnancy outcome. *Aust N Z J Obstet Gynaecol*. 2003;43:438-42.
13. Huynh L, Kingdom J, Akhtar S. Low pregnancy-associated plasma protein A level in the first trimester. *Can Fam Physician* 2014;60:899-903.
14. Patil M, Panchanadikar TM, Wagh G. Variation of papp-a level in the first trimester of pregnancy and its clinical outcome. *J Obstet Gynaecol India* 2014;64:116-9.
15. Barrett SL, Bower C, Hadlow NC. Use of the combined first-trimester screen result and low PAPP-A to predict risk of adverse fetal outcomes. *Prenat Diagn* 2008;28:28-35.
16. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. *Prenat Diagn* 2002;22:778-82.
17. Malik J, Rai P, Das A, Das S. Pregnancy-associated plasma protein A - a level in first trimester and its impact on pregnancy outcome. 2016;2645
18. Cooper S, Johnson J, Metcalfe A, Pollard J, Simrose R, Connors G, et al. The predictive value of 18 and 22 week uterine artery Doppler in patients with low first trimester maternal serum PAPP-A. *Prenat Diagn* 2009;29:248-52.
19. Kaijomaa M, Ulander V, Hamalainen E, Alfthan H, Markkanen H, Heinonen S, et al. The risk of adverse pregnancy outcome among pregnancies with extremely low maternal PAPP-A. *Prenat Diagn* 2016;36:1115-20.
20. Morssink LP, Kornman LH, Hallahan TW, Kloosterman MD, Beekhuis JR, de Wolf BT, et al. Maternal serum levels of free beta-hCG and PAPP-A in the first trimester of pregnancy are not associated with subsequent fetal growth retardation or preterm delivery. *Prenat Diagn* 1998;18:147-52.
21. Beneventi F, Simonetta M, Lovati E, Albonico G, Tinelli C, Locatelli E, et al. First trimester pregnancy-associated plasma protein-A in pregnancies complicated by subsequent gestational diabetes. *Prenat Diagn* 2011;31:523-8.
22. McCowan LM, Figueras F, Anderson NH. Evidence-based national guidelines for the management of suspected fetal growth restriction: comparison, consensus, and controversy. *Am J Obstet Gynecol* 2018;218:855-68.



Female genital tract melanoma: Analysis from a regional cancer institute

Kadın genital sistem melanomu: Bölgesel bir kanser enstitüsünden analiz

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Abstract

Objective: Malignant melanoma of the genital tract comprises 3% of all melanomas afflicting females. They are characterized by poor prognosis with 5-year survival of 0-25% and high incidence for distant metastasis. This study was performed to assess various clinical features, treatment options, and the management of genital melanomas.

Materials and Methods: This was a retrospective analysis where records of patients with genital melanomas between 2005 to 2018 were reviewed to obtain demographic and clinical information, including age of diagnosis, presenting symptoms, performance status, pathology reports, treatment, follow-up, and survival.

Results: Between 2005 and 2018, 31 women were analyzed. The median age was 53.5 (range: 28.5-85) years. Vaginal bleeding was the most common presenting symptom (80.6%), followed by discharge (29%), mass in the vagina/perineum (19.3%), pain (16.1%), and difficulty in micturition (9.6%). The most common site of origin was the vagina (67.7%), followed by that vulva (19.3%) and cervix (12.9%). Tumor diameter was more than 3 cm in 74.2% (23/31). Out of 31 patients, only 16 opted for treatment. Four patients underwent surgery, 10 received primary chemotherapy, and two needed palliative radiotherapy for heavy bleeding. The median survival in the treatment group was 5 (range: 2.5-28) months, almost similar to patients not receiving any treatment (5 months, range: 2-11).

Conclusion: Genital melanoma are rare but aggressive tumors. Diagnosis is usually made with biopsy. No effective treatment strategy is yet available. However, surgery is the preferred first-line treatment, radiotherapy and chemotherapy have been used in adjuvant settings.

Keywords: Genital melanoma, chemotherapy, primary surgery

Öz

Amaç: Genital sistemin malign melanomu, kadınları etkileyen tüm melanomların %3'ünü oluşturmaktadır. Beş yıllık sağkalım %0-25 ve uzak metastaz insidansı ile kötü prognoz ile karakterizedir. Bu çalışma genital melanomların çeşitli klinik özelliklerini, tedavi seçeneklerini ve tedavi yöntemlerini değerlendirmek için yapıldı.

Gereç ve Yöntemler: 2005-2018 yılları arasında genital melanomlu hastaların kayıtlarının tanı yaşı, semptomlar, performans durumu, patoloji raporları, tedavi, takip ve sağkalım gibi demografik ve klinik bilgileri elde etmek için incelendiği retrospektif bir analizdir.

Bulgular: 2005-2018 arasında 31 kadın analiz edildi. Ortanca yaş 53.5 (dağılım: 28,5-85) idi. Vajinal kanama en sık başvuru semptomuydu (%80,6), bunu akıntı (%29), vajina/perineumdaki kitle (%19,3), ağrı (%16,1) ve işeme zorluğu (%9,6) izledi. En sık oluşma yeri vajinaydı (%67,7), bunu vulva (%19,3) ve serviks (%12,9) izledi. Tümör çapı %74,2'de (23/31) 3 cm'den fazlaydı. Otuz bir hastanın sadece 16'sı tedaviyi tercih etti. Dört hastaya cerrahi, 10 hastaya primer kemoterapi ve iki hastaya ağır kanama için palyatif radyoterapi uygulandı. Tedavi grubundaki ortalama sağkalım 5 (aralık: 2,5-28) aydı, bu da neredeyse hiç tedavi almayan hastalara (5 ay, aralık: 2-11) benzerdi.

Sonuç: Genital melanom nadir fakat agresif tümörlerdir. Tanı genellikle biyopsi ile konur. Henüz etkili bir tedavi stratejisi mevcut değildir. Bununla birlikte, ameliyat birinci basamak tedavi olarak tercih edilir, adjuvan ortamlarında radyoterapi ve kemoterapi kullanılmıştır.

Anahtar Kelimeler: Genital melanom, kemoterapi, primer cerrahi

PRECIS: A retrospective analysis was performed in patients of female genital tract melanoma.

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Introduction

Malignant melanoma of the female genital tract comprises 3% of all melanomas affecting females, and 18% of mucosal melanomas⁽¹⁾. Melanomas arising in the female genital tract primarily occur in the vulva and vagina (95% and 3%, respectively), with the urethra or cervix being rarer causes⁽¹⁾. The first case of primary malignant vaginal melanoma was reported in 1887, and the modern literature has noted about 500 cases globally. They are characterized by poor prognosis with an overall 5-year survival rate of 0-25%, difficult local control, and a high incidence of nodal and distant metastasis⁽²⁾. Being rare tumors with typically late diagnosis, unsatisfactory therapy, and a relative paucity of published data, we performed this study to assess various clinical features, available treatment options, and the management of genital melanomas.

Material and Methods

This was a retrospective data analysis of cases of genital malignant melanoma, which was approved by the local ethics committee in our regional cancer and research institute. After institutional review board (IRB) approval, we used institutional records to identify patients who were diagnosed as having genital malignant melanomas (vulva, vagina, cervix) between 1 January 1st, 2005, and December 31st, 2018. Clinical records were reviewed to obtain demographic and clinical information, including age at the time of diagnosis, presenting symptoms, Eastern Cooperative Oncology Group (ECOG) performance status, treatment, follow-up and survival. Surgical pathology reports were reviewed to obtain gross and histopathologic tumor characteristics. All histopathologic diagnoses were confirmed using immunohistochemistry by gynecologic pathologists at our institution.

Patients were classified by primary disease site: vulval, vaginal or cervical. Computed tomography/positron emission computed tomography (CT/PET-CT) and/or ultrasonography findings were collected at baseline and during the course of treatment as a part of the initial metastatic examinations or to detect and localize recurrence during follow-up. Patients were staged using the American Joint Committee on Cancer (AJCC) 7th edition guidelines for vulval melanoma. Cervical melanomas were staged as per the International Federation of Gynecology and Obstetrics (FIGO) staging of cervix cancer (2018). For vaginal melanomas, clinical staging system was used for standardization. Therapies were categorized into the following groups: surgery, chemotherapy, and radiotherapy (RT). When information on survival was not available in the medical records, an attempt was made to contact the patient and/or relatives. If such information could still not be obtained, data on the patient after their last contact were censored. Overall survival (OS) was calculated from the date of the surgical diagnosis.

Results

Patient characteristics

Between 2005 and 2018, 31 women presented to our regional cancer institute with a diagnosis of genital tract malignant melanoma. Patient characteristics are summarized in Table 1. The median age was 53.5 (range: 28.5-85) years. Vaginal bleeding was the most common presenting symptom (80.6%), followed by discharge per vaginam (29%), mass in the vagina/perineum (19.3%), pain (16.1%), and difficulty in micturition (9.6%). In our study, the most frequent site of origin was the vagina (67.7%), followed by the vulva (19.3%) and cervix (12.9%). The tumor diameter was more than 3 cm in 74.2% (23/31) and less than 3 cm in 25.8% (8/31). Thirteen (41.9%) patients in study group had lymph node metastasis and seven (29%) had distant metastasis.

Treatment

Out of the total 31 patients, 16 patients opted for treatment (Table 2). Four patients underwent surgery followed by adjuvant chemotherapy (CT) or RT. Primary CT was given in 10 patients and two patients received palliative RT for heavy bleeding. Fifteen patients refused treatment (Table 3). Of these, 8 (53.4%) were above age of 65 years. Tumor size was >3 cm in 12 patients (80%) and the majority were diagnosed as being in higher stages (>2b, 10/15, 66.6%). OS was less than 6 months in 11 cases (73.3%).

Characteristics of patients (n=4) who underwent surgery

Among patients with surgery (n=4), one had wide local excision (WLE) with partial urethral resection (2 cm) for melanoma in the lower one-third of the vagina (Figure 1), others had radical vulvectomy with bilateral inguofemoral lymphadenectomy for vulval melanoma, radical hysterectomy with partial vaginectomy (5 cm) for melanoma in the upper one-third of the posterior vagina, and radical hysterectomy with bilateral pelvic lymphadenectomy for cervical melanoma, respectively (Table 4). Three (75%) patients were aged less than 60 years. The median tumor size was 3.75 (range, 3-4) cm with a median depth of tumor invasion of 11 (8-15) mm. Both cases of vaginal melanoma were stage 1b, and cervical and vulval melanoma were found in stage 3c postoperatively with right internal iliac lymph node and left inguinal lymph node metastasis, respectively. The median free surgical margins were more than 17.5 (range, 15-20) mm.

As an adjuvant treatment, patients with lower one-third vaginal melanoma received external beam RT (EBRT, 50 Gy/25#), cervical melanoma received dacarbazine (400mg, 3 cycles) with tamoxifen, and vulval melanoma received temozolomide (200 mg/m², days 1 to 5) plus cisplatin (25 mg/m², days 1 to 3, three weekly for six cycles) with EBRT (50 Gy/25#). The patient with upper one-third vaginal melanoma had no adjuvant treatment but received temozolomide (100 mg, 5 cycles) during recurrence (rectum). She was alive at the

time of the last follow-up (28 months). Overall, the median time of recurrence was 9.5 (range, 7-13) months in these four patients.

Table 1. Patient characteristics (n=31)

Characteristics	Number (%)
Age (years), median (range)	53.5 (28.5-85)
ECOG performance status	
0	11 (35.4)
1	12 (38.7)
2	5 (16.1)
3	3 (9.6)
Presenting symptoms	
Bleeding per vaginum	25 (80.6)
Discharge per vaginum	9 (29)
Mass in perineum/vagina	6 (19.3)
Pain	5 (16.1)
Difficulty in micturition	3 (9.6)
Tumor location	
Vulva	6 (19.3)
Vagina	21 (67.7)
Cervix	4 (12.9)
Tumor size	
<3 cm	8 (25.8)
>3 cm	23 (74.2)
Stage	
Vulva (AJCC) -	
3c	3
4	3
Vagina	
1	6
2	9
3	6
Cervix (FIGO 2018)	
2b	1
3b	2
3c	1
Metastasis	
Local (lymph nodes)	13 (41.9)
Distant	9 (29)
AJCC: American Joint Committee on Cancer, FIGO: International Federation of Gynecology and Obstetrics	

Characteristics of patients who underwent primary chemotherapy

Among the patients who received primary chemotherapy (n=10), the majority were aged >65 years (7/10, 70%) with tumor size more than 3 cm (9/10, 90%). All of them were in advanced stages (>2b) with poor performance status in most patients (ECOG 2.3-7/10, 70%). The OS was less than 6 months in 40% of cases (Table 5).

Discussion

Vulvar melanomas account for less than 1% of all melanomas, but they represent 10% of all malignant tumors involving the vulva⁽³⁾. Although vulvar melanomas arise on the hairy skin of the vulva, because of its sun-shielded location and continuity with vaginal mucosa, it has been mostly described along with mucosal melanomas. Vulvar melanomas, arising from the outer, non-glabrous hair-bearing portion of the labia majora, may share common risk factors with cutaneous melanoma.

Table 2. Treatment administered (n=16)

Surgery	
Wide local excision	1
Vulvectomy	1
Radical hysterectomy	2
Chemotherapy	
Primary	10
Adjuvant	4
Second line	1
Radiotherapy	
Primary (palliative)	2
Adjuvant	3



Figure 1. Melanoma in lower 1/3 of vagina

Chronic inflammatory disease, viral infections, chemical irritants, and genetic factors have also been implicated as risk factors⁽⁴⁾. However, the exact pathogenesis of vulvovaginal or cervixmelanomas is relatively unknown. It is suggested that they arise from aberrantly located melanocytes in the vaginal

epithelium found in 3% of healthy women, and in the basal portion of the vaginal epidermis and cervical melanocytic cells as embryologic remnants of neural crest cells⁽⁵⁾. Diagnosis is determined by gynecologic examination, histologic results, and immunohistochemical staining.

Although vulvar melanomas are reported to be more frequent compared with vaginal/vulvovaginal melanomas, our study reported vaginal melanomas in 67.7% of patients, vulvar in 19.3%, and cervical in 12.9% of patients. In the literature, vaginal melanomas have been primarily found in the anterior wall (38%) of the vagina, mostly in the lower one-third (34%)⁽⁶⁾. Our study reported vaginal melanoma most commonly in upper half of the posterior vagina (57.1%), followed by the lower half of the anterior vagina (19%), upper half of the anterior vagina (14%), and lateral vagina (9.5%).

Genital melanomas mostly occur in older women, with a median age of 68 years in the vulva, and 60 years in the vagina and cervix^(7,8). The median age of patients in our study was less, 53.5 (range, 28.5-85) years. The most common presenting symptoms reported in literature are vaginal bleeding and discharge, presence of mass lesion, and less commonly, pain, pruritus or micturition difficulties. Our study also found vaginal bleeding to be the most common symptom (80.6%), followed by discharge (29%), mass lesion (19.3%), pain (16.1%), and difficulty in micturition (9.6%).

There is no universal staging system for genital melanomas. Current staging is performed as per the AJCC system for vulvar melanoma, which is based on tumor thickness and the status of regional lymph nodes⁽⁹⁾. By contrast, the clinical presentation

Table 3. Patients who did not receive treatment (n=15)

	n (%)
Age (median - 50 years)	
<65 yrs	7 (46.7)
>65 yrs	8 (53.4)
Size of lesion	
<3 cm	3 (20)
>3 cm	12 (80)
Stage	
1-2	5 (33.3)
>2	10 (66.6)
Performance status (ECOG)	
0,1	10 (66.6)
2,3	5 (33.3)
Overall survival	
<6 months	11 (73.3)
>6 months	4 (26.7)
ECOG: Eastern Cooperative Oncology Group	

Table 4. Characteristics of patients (n=4) who underwent surgery

No.	Age (years)	Location	Procedure	Tumor size (cm)	Depth of invasion (mm)	stage	Margins (mm)	Lymph node status
1	58	Upper 1/3 of vagina	Radical hysterectomy with partial vaginectomy	4	10	1b	>20 mm	Negative
2	42	Lower 1/3 of vagina	Wide local excision with partial urethral resection	3	12	1b	>15 mm	Negative
3	28	Cervix	Radical hysterectomy with bilateral pelvic lymphadenectomy	3.5	8	3c	>20 mm	Right internal iliac node metastasis
4	70	vulva	Radical vulvectomy with bilateral inguinofemoral lymphadenectomy	4	15	3c	>15 mm	left inguinal node metastasis

Table 4. Continued

Adjuvant treatment	Recurrence (months)	Site of recurrence	Status
Temozolamide at recurrence (100 mg, 5 cycles)	13	Rectum	Alive at last follow up (21 months)
EBRT (50Gy/25#)	7	Liver, brain	Expired at 11months
Dacarbazine (3 cycles, 400 mg) with tamoxifene	11	Vaginal vault	Expired at 32 months
EBRT (50 Gy/25#)	8	Lung	NA

Table 5. Characteristics of patients (n=10) who underwent primary chemotherapy

Age (years)	
<65	3
>65	7
Tumor size (cm)	
<3 cm	1
>3 cm	9
Stage	
1-2	0
>2	10
Performance status	
0.1	3
2.3	7
Overall survival	
<6 months	4
>6 months	6

and spread pattern of malignant melanoma of the cervix is similar to that of squamous cell carcinoma of the cervix, thus the FIGO staging system has been accepted by most researchers⁽⁸⁾. In the absence of a prognostic staging system for vaginal melanomas, clinical staging system was used for standardization⁽¹⁾.

Genital melanomas had been associated with poor prognosis, irrespective of the stage of tumor and treatment modality used. Keeping this in mind, the patients were counseled regarding disease prognosis and management options. As a result, only 16 patients opted for further treatment; four were offered surgery, 10 underwent primary chemotherapy, and two received palliative RT. Other contributing factors for refusing treatment were as follows: advanced disease stage (lesion >3 cm, 80%; stages >2b, 66%), low socioeconomic status and poor performance status (5/15, 33.3%). However, some patients may have taken the treatment outside our institute, which could not be accounted for because of the lack of a central registry of malignancies in our country.

Surgery is the main treatment option for genital tract melanoma. As expected, patients who were medically fit and had lesions amenable to surgical resection were planned for surgery in our institute. The literature on cutaneous melanoma supports a 10-mm margin for melanomas, 2 mm thick and less, and a 20-mm margin for melanomas more than 2 mm thick, which accounted for all patients in our study. Although we were able to achieve negative margins in our patients (median 17.5 mm), it required resection of the distal urethra (2 cm) in one patient due to the anatomic location of the lesion (lower one-third of the vagina). To completely resect the primary lesion with adequate margins,

the spectrum of surgery has ranged from conservative WLE to radical (vaginectomy, radical hysterectomy, radical vulvectomy, pelvic exenteration). Without a clear improvement in survival, more conservative surgery followed by adjuvant therapy has been considered for women with genital melanoma⁽¹⁾. Lymph node dissection is not recommended because as the rate of lymph node metastasis is low with no survival benefits⁽¹⁰⁾. However, in cases of clinically involved lymph nodes, lymphadenectomy can be performed. Our patient with vulval melanoma with left inguinal lymph node metastasis underwent inguinofemoral lymphadenectomy.

Attempts have been made to evaluate the benefits of adjuvant treatment after surgery. RT is recommended as a postoperative adjuvant therapy in patients with tumor size >3 cm or incomplete tumor resection⁽¹¹⁾. Temam et al.⁽¹²⁾ found a relative risk of recurrence of 0.4 (95% CI: 0.2-0.9) in patients with mucosal melanoma who received adjuvant radiation therapy after surgery, which did not translate to improvement in OS. Frumovitz et al.⁽¹³⁾ also found that although adjuvant RT might reduce the risk of local recurrence, there was no difference in recurrence rates or survival between patients who did and did not receive adjuvant RT because the risk of distant metastasis was not reduced. Two of our patients in the surgery group received adjuvant RT due to a large tumor size, suboptimal margins, and recurrence with distant metastasis at 7.5 months. RT is not recommended as a primary treatment but it was used in our study as a hemostatic agent in two patients for intractable bleeding per vaginum. Similarly, the role of CT is not well established in genital melanomas, but it has been used in adjuvant settings or in advanced cases, with palliative intent. In our study, 10 patients received primary CT and four were given CT in an adjuvant setting. The patients who received primary CT in our study were older (age >65 years, 70%), advanced stage (>2b, 100%), had larger tumor size (>3 cm, 90%) with poor performance status (ECOG 2.3-70%). Multiple cytotoxic agents including dacarbazine, temozolomide and platinum compounds were used, both as single agents and in combination. The median survival was 5 (range, 2.5-18) months, almost similar to that in patients who received no treatment (5 months, range, 2-11). Postoperative adjuvant immunotherapy using interferon alpha-2b has been used to prevent relapse and has been approved by the United States Food and Drug Administration for the treatment of metastasized melanoma. Topical treatment with 5% imiquimod has been reported for preventing relapse⁽¹⁴⁾. However, immunotherapy could not be used at our institute due to financial constraints. Our study has various limitations. First, almost half of study population refused treatment, irrespective of age, stage or size of lesion. It is possible that some of these may have received treatment elsewhere but could not be traced. This emphasizes the need for a central registry of malignancies in our country, but this is difficult to achieve in the foreseeable future. Second, there was a high rate of loss to follow-up, so accurate

information regarding recurrence or OS was unavailable. Third, the long observational study period could have added treatment heterogeneity. Although any meaningful statistical analysis could not be done in any of the treatment arm, this is usually not feasible in rare diseases due to small sample sizes.

Conclusion

Genital melanomas are rare but aggressive tumors that affect women in their 6th and 7th decade of life. It usually has a poor prognosis because it is often diagnosed at advanced stages with no effective treatment strategy available. Diagnosis is usually made with biopsy and immunohistochemical analysis. Primary surgery is the preferred first-line treatment. RT and chemotherapy have been used in the adjuvant setting with benefit.

Ethics

Ethics Committee Approval: Retrospective study.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.A., Concept: R.A., Design: C.P., B.P., Data Collection or Processing: G.P., S.P., Analysis or Interpretation: P.D. D.B., Literature Search: P.D., Writing: R.A.

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References

1. Mihajlovic M, Vlajkovic S, Jovanovic P, Stefanovic V. Primary mucosal melanomas: a comprehensive review. *Int J Clin Exp Pathol* 2012;5:739-53.
2. Baloglu A, Bezircioglu I, Cetinkaya B, Yavuzcan A. Primary malignant melanoma of the vagina. *Arch Gynecol Obstet* 2009;280:819-22.
3. Stang A, Streller B, Eisinger B, Jöckel KH. Population-based incidence rates of malignant melanoma of the vulva in Germany. *Gynecol Oncol* 2005;96:216-21.
4. Wechter ME, Gruber SB, Haefner HK, Lowe L, Schwartz JL, Reynolds KR, et al. Vulvar melanoma: a report of 20 cases and review of the literature. *J Am Acad Dermatol* 2004;50:554-62.
5. Gupta D, Malpica A, Deavers MT, Silva EG. Vaginal melanoma: a clinicopathologic and immunohistochemical study of 26 cases. *Am J Surg Pathol* 2002;26:1450-7.
6. Kühn F, Dieterich M, Klar E, Gerber B, Prinz C. Primary Malignant Vaginal Melanoma - Case Report and Review of the literature. *Geburtshilfe Frauenheilkd* 2012;72:740-73.
7. Xia L, Han D, Yang W, Li J, Chuang L, Wu X. Primary malignant melanoma of the vagina: a retrospective clinicopathologic study of 44 cases. *Int J Gynecol Cancer* 2014;24:149-55.
8. Yuan G, Wu L, Li B, An J. Primary malignant melanoma of the cervix: Report of 14 cases and review of literature. *Oncotarget* 2017;18:73162-67.
9. Patrick RJ, Fenske NA, Messina JL. Primary mucosal melanoma. *J Am Acad Dermatol* 2007;56:828-34.
10. Kalampokas E, Kalampokas T, Damaskos C. Primary Vaginal Melanoma, A Rare and Aggressive Entity. A Case Report and Review of the Literature. *In Vivo* 2017;31:133-9.
11. Piura B. Management of primary melanoma of the female urogenital tract. *Lancet Oncol* 2008;9:973-81.
12. Temam S, Mamelle G, Marandas P, Wibault P, Avril MF, Janot F, Julieron M, et al. Postoperative radiotherapy for primary mucosal melanoma of the head and neck. *Cancer* 2005;103:313-39.
13. Frumovitz M, Etchepareborda M, Sun CC, Soliman PT, Eifel PJ, Levenback CF, Ramirez PT. Primary malignant melanoma of the vagina. *Obstet Gynecol* 2010;116:1358-65.
14. Jack A, Boyes C, Aydin N, Alam K, Wallack M. The treatment of melanoma with an emphasis on immunotherapeutic strategies. *Surg Oncol* 2006;15:13-24.



Validation of the Turkish version of European Organization for Research and Treatment of Cancer QLQ-OV28 ovarian cancer specific quality of life questionnaire

Over kanserli hastalarda hayat kalitesini ölçen Avrupa Kanseri Araştırma ve Tedavi Örgütü QLQ-OV28 sorgu formunun Türk toplumuna uyarlanması ve olguların yaşam kalitesinin belirlenmesi

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Abstract

Objective: To examine reliability and validity of the European Organization for Research and Treatment of Cancer (EORTC) QLQ-OV28 questionnaire into the Turkish language according to the instructions provided by EORTC.

Materials and Methods: Ninety-seven patients who were diagnosed as having ovarian cancer and treated between January 2005 and June 2010 with an expected survival time of at least 3 months, were enrolled into the study. The exclusion criteria were diagnoses of any disease that could disrupt consciousness and concurrent malignancies. The EORTC QLQ-OV28 module was translated into Turkish by professional translators and physicians. The test-retest reliability of the Turkish version of the questionnaire was performed on 30 patients. Answers were scored according to the instructions provided by the EORTC. The total score was calculated as explained above and after scoring procedures, all subscale scores were linearly transformed to a 0-100 scale. All patients concomitantly completed the Spielberg State Trait Anxiety Inventory (STAI) and Beck Depression Inventory (BDI). Patients were analyzed in two groups: on-treatment and off-treatment groups consisted of patients who did and did not undergo chemotherapy or surgery within the last two months, respectively. The demographic data of all patients were recorded. EORTC QLQ-OV28 scores of both groups were compared. Correlations between EORTC QLQ-OV28 subscales and total score of BDI and STAI were analyzed.

Results: For test-retest reliability, Spearman's rho was 0.84 ($p < 0.001$). The on-treatment group scored statistically significantly higher than the off-treatment group in peripheral neuropathies, attitude to disease and treatment, sexual function and other chemotherapy adverse effect subscales of the questionnaire. Correlations between EORTC QLQ-OV28 subscales and the total scores of BDI and STAI of the groups were statistically significant, except the sexual function subscale.

Conclusion: The Turkish translated version of EORTC QLQ-OV28 module is a reliable, consistent, and a valid instrument for assessing the impact of treatment modalities on QoL among Turkish speaking women with ovarian cancer.

Keywords: Quality of life, ovarian cancer, EORTC

PRECIS: QoL of surviving patients has become an important goal of cancer research and Turkish translated version of EORTC QLQ-OV28 module can help physicians detect the physical and functional effects of disease, the treatment side effects and society/family reactions.

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Öz

Amaç: Over kanserli hastalarda hayat kalitesini ölçen EORTC QLQ-OV28 sorgu formunun Türk toplumuna uyarlanması.

Gereç ve Yöntemler: Ocak 2005- Ocak 2010 tarihleri arasında Over kanseri tanısı olan, tedavi edilmiş, beklenen sağkalım süresi 3 aydan uzun olan 97 hasta çalışmaya dahil edildi. Eşlik eden başka malignitesi olan ve bilişsel aktiviteyi etkileyecek ek hastalıkları olan hastalar dışlandı. EORTC QLQ-OV28 modülü profesyonel çevirmenler ve hekimler tarafından Türkçe'ye çevrildi. Test-retest güvenilirliği 30 hastadan oluşan pilot çalışma ile yapıldı. Cevaplar EORTC talimatları doğrultusunda puanlandırıldı. Total skorlama yapıldıktan, alt ölçek ve sorulara ait ham puanların hesaplanmasının ardından 0-100 skolasında linear transformasyon uygulandı. Tüm hastalardan eş zamanlı Spielberg State Trait Anxiety Inventory (STAI) and Beck Depression Inventory (BDI) formlarını doldurmaları istendi. Son iki ay içinde kemoterapi almış ya da opere edilmiş hastalar hala tedavi görmekte olan hasta grubu ve son iki ay içinde hiç tedavi almamış hastalar tedavisi bitmiş hasta grubu olarak ayrıldı.

Bulgular: Test-retest güvenilirliği için Spearman's rho 0,84 saptanmıştır ($p<0,001$). Halen tedavi görmekte olan hasta grubunun periferik nöropati, hastalık ve tedaviye karşı tavır, seksüel fonksiyon ve diğer kemoterapi yan etkileri alt ölçekleri skorları tedavisi bitmiş hasta grubunun skorlarından istatistiksel olarak anlamlı oranda yüksek bulunmuştur. EORTC QLQ-OV28 alt ölçekleri ve BDI ve STAI testlerinin total skorları arasındaki korelasyonlar seksüel fonksiyon alt ölçeği dışında istatistiksel olarak anlamlıdır.

Sonuç: Over kanserli hastalarda tedavi modalitelerinin hayat kalitesini üzerine etkilerini değerlendirmek için geliştirilen EORTC QLQ-OV28 sorgu formunun Türkçe versiyonunun tutarlı, güvenilir, uygun yapılandırılmış ve standardize edilmiş bir sorgulama formudur.

Anahtar Kelimeler: Hayat kalitesi, over kanseri, EORTC

Introduction

Ovarian cancer is a major gynecologic malignancy and the standard treatment is surgery followed by chemotherapy. Over the past 30 years, improvements in cure rates and length of survival have been observed. Five-year survival rates have been reported as high as 90% for patients diagnosed at stages of localized disease⁽¹⁾. However, although the advances in the treatment of ovarian cancer have significantly improved survival, there remains a cost in terms of unpleasant adverse effects, which reduce the patient's quality of life (QoL). This fact set another aspect in the cancer management beyond survival, and the QoL of surviving patients has become an important goal of cancer research. Inevitably, patient-reported QoL outcome instruments are now more important in cancer-related studies and in evaluating treatment results. An instrument that measures the QoL in these patients should cover the diagnosis of disease, the meaning of disease to the patient, the physical and functional effects of disease, the treatment adverse effects, and society/family reactions⁽²⁾. The European Organization for Research and Treatment of Cancer (EORTC) developed the EORTC QLQ-OV28, a symptom-specific questionnaire for use in ovarian cancer clinical trials to supplement the EORTC QLQ-C30, a cancer-specific core questionnaire⁽³⁾. The EORTC QLQ-OV28 module has been translated into many languages and validated in different populations⁽⁴⁾.

The current version of EORTC QLQ-OV28 module has been translated into 55 languages as of May 2019 around the world and has also been validated and tested in multicultural settings⁽⁵⁾. Although we obtained permission to translate the core questionnaire (OV28) from EORTC QOL Department in 2010, due to incomplete follow-up of permission process, an other Turkish translation of the module was accepted by EORTC QOL Department and is available for use. The current EORTC QLQ-OV28 Turkish module is only a translation without any clinical data and no published literature is available to examine various aspects of EORTC QLQ-OV28 module Turkish version translation and validation process⁽⁵⁾. Therefore, the aim of this study was to examine the reliability and validity

of this questionnaire into Turkish language according to the instructions provided by EORTC by evaluating the clinical data of 97 patients⁽⁶⁾.

Materials and Methods

Questionnaire

The EORTC QLQ-OV28 module consists of 28 questions containing symptom scales [abdominal/gastrointestinal symptoms (GI) OV 31-37, peripheral neuropathy (PN) OV 41-43, other chemotherapy adverse effects (CH) OV 38-40, 44-47, hormonal/menopausal (HM) OV 48-49, attitude to disease and treatment (AT) OV 52-54, body image (BI) OV 50-51, and Sexual Function scale (SEF) OV 55-58]. Higher scores mean a poorer QoL, whereas for the items of SEF, OV 55-58, higher scores mean better QoL. Therefore, scores of SEF 55-58 need to be reversed during calculations of total scores. Two professional English-Turkish translators, who were not familiar with the EORTC QLQ-OV28, worked independently to produce the Turkish version of the questionnaire. At the first meeting, a common draft of the Turkish version was produced with a list of alternatives for the controversial items and response choices. At the second meeting, between the two translators and Turkish physicians with experience of health and QoL terminology, some revisions were made and a second draft was produced. Ten symptomatic women were asked to self-complete the second draft and then they were interviewed for possible ambiguous questions. At the third meeting, the final Turkish version was completed.

The study was approved by the ethics committee of the medical faculty of İstanbul Zeynep Kamil Women and Child Diseases Training and Research Hospital (approval number: 2019/139). The institutional ethics committee approved the study and informed consent was obtained from each participant. The Turkish version of the full questionnaire is available from the first author on request.

Study population and data collection

Initially, a pilot study was conducted in order to evaluate

the internal consistency and the test-retest reliability of the Turkish version of the questionnaire. Women completed the final version at their first visit in the oncology outpatient clinic of Zeynep Kamil Hospital (a tertiary referral teaching institution, Istanbul, Turkey), before meeting a physician. Questionnaires were printed in large fonts (16 font size) in order to be read and self-completed also by patients with poor eyesight. When a patient could not read or write, a relative or an accompanying helper of that patient assisted them in completing the questionnaire, if available. If not, support personnel, not familiar with the concepts of oncology and QoL, provided nondirective assistance to such patients. To measure the test-retest reliability of the final version of the questionnaire, a two-weeks' test-retest analysis was used. Therefore, 30 women were asked to complete the questionnaire at their initial visit and repeat the procedure two weeks later in the same clinic. The responses of the two completed questionnaires were then analyzed using Spearman's correlation test.

After the pilot study, patients who were diagnosed as having ovarian cancer and treated between January 2005 and June 2010 in the same institution, aged between 18-75 years, and with an expected survival time of at least 3 months, were enrolled into the study. The exclusion criteria were diagnoses of any disease that could disrupt consciousness and concurrent malignancies. The participants completed the questionnaire as described above. Answers were scored according to the instructions provided by the EORTC. The total score was calculated as explained above, and after scoring procedures, all subscale scores were linearly transformed to a 0-100 scale.

All patients concomitantly completed the Spielberg State Trait Anxiety Inventory (STAI) and the Beck Depression Inventory (BDI)^(7,8). STAI is a 40-item instrument that differentiates between the temporary condition of 'state anxiety' and the more general and long-standing quality of 'trait anxiety.' The STAI has been adapted into more than 40 languages⁽⁷⁾. To evaluate if EORTC QLQ-OV28 is a predicting instrument also for depression, the correlation with BDI was also assessed. The BDI is a 21-question multiple-choice self-report inventory that is one of the most widely used instruments for measuring the severity of depression. The evaluation of the psychometric properties and cut-off points of the BDI in a Turkish adult population was reported by Kapci⁽⁸⁾.

Patients were prospectively registered into the study and divided into two groups. The on-treatment group consisted of patients who were under chemotherapy or had surgery within the last two months. Patients of the off-treatment group had not received any treatment within the previous two months. The demographic data of all patients were recorded. The EORTC QLQ-OV28 scores of both groups were compared. Correlations between EORTC QLQ-OV28 subscales and total scores of the BDI and STAI were analyzed.

Statistical analysis

The test-retest reliability was assessed by the Spearman's correlation and Wilcoxon's rank sum tests. A rho value of greater than 0.8 was considered as highly reliable⁽⁹⁾. Internal consistencies of the subscales were assessed using Cronbach's alpha coefficient and item to other scale correlations. The content/face validity, which indicates whether the questionnaire makes sense to patients and experts, and whether all the important and relevant domains were included, was assessed by an expert panel that included two urogynecologists and one psychometrician. The levels of the missing data were used as an indicator of inappropriate questions⁽¹⁰⁾. Criterion validity, which describes how well the questionnaire correlates with existing standards⁽¹⁰⁾, was assessed by comparing the EORTC QLQ-OV28 scores with the scores of STAI and BDI. Spearman's correlation coefficient was used for this purpose. All tests were performed using the Statistical Package for the Social Sciences (SPSS) for Windows 11.5. Values are given as percentage (%) or mean $\pm$ standard deviation (SD). Non-parametric tests were used for the analysis because of the non-normality of the data (Wilcoxon rank and Mann-Whitney U tests). The degree of statistical significance was set at <0.05 and all given p values were two-tailed.

Results

A total of 97 patients who met the inclusion criteria were enrolled in the study. The mean age of the patients was 52.6 ± 12.6 years. The on-treatment group comprised 39 patients and the off-treatment group consisted of 58 patients. The sociodemographic and clinical characteristics of the patients are detailed in Table 1.

The number of missing items was zero (0%). For the test-retest reliability, Spearman's rho was 0.84 ($p < 0.001$). The results of the internal consistency and item to other scale correlations were presented in Table 2.

Cronbach's alpha values of all scales were higher than 0.70 and these results showed a high level of internal consistency. The on-treatment group scored higher (poorer QoL) than the off-treatment group, and the differences were statistically significant only in the PN, AT, CH, SEF subscales (Table 3). However, when the non-conditional items of SEF subscale (OV-55 and OV-56) were analyzed separately, the Cronbach's alpha value was 0.93. The comparison of the scores of OV-55 and OV-56 of the on- and off-treatment groups were 96.15 ± 10.44 and 82.47 ± 19.60 ($p < 0.001$). OV57 and OV58, conditional items to be answered only by sexually active women, were not statistically analyzed because only 50% (29/58) and 13% (5/34) of the patients answered these questions in the off- and on-treatment groups, respectively.

The total BDI and STAI scores were 10.4 ± 8.8 and 77.2 ± 19.9 in both groups, respectively. Correlations between EORTC QLQ-OV28 subscales and the total scores of BDI and STAI of the groups are presented in Table 4, where all of the correlations were statistically significant except the SEF subscale.

Discussion

The results of this study show that the Turkish version of EORTC QLQ-OV28 has a high internal consistency and test-retest reliability. Overall, the on-treatment group showed poorer QoL than the off-treatment group. These results might be as a consequence of the heterogeneity of disease stage in the groups (high rates of advanced stages in the on-treatment

group and high rates of borderline and early stages in the off-treatment group).

Similar results were reported in a study providing the validated Taiwan Chinese version of the EORTC QLQ-OV28⁽⁴⁾. Indeed, chemotherapy and/or surgery have an immediate negative effect on QoL and improvements in physical and functional well-being are likely to be observed at later intervals relative to earlier intervals after treatment⁽¹¹⁾. Women previously treated for gynecologic cancer describe a range of psychosocial difficulties including depression and anxiety, and the most commonly described personal coping strategy is the use of

Table 1. Sociodemographic and clinical characteristics of the patients

Mean ± SD / n (%)		On-treatment (n=39)	Off-treatment (n=58)
Age (Years)		57.08±11.906	49.81±12.31
Marital status	Married	28 (71.8)	47 (81)
	Widowed	9 (23.1)	6 (10.3)
	Single	2 (5.1)	5 (6.8)
Education	Illiterate	7 (17.9)	5 (8.6)
	Primary	25 (64.1)	36 (62.1)
	Secondary	1 (2.4)	4 (6.8)
	College	3 (7.7)	8 (13.8)
	University	3 (7.7)	5 (8.6)
Disease stage	Borderline	0 (0.0)	15 (25.9)
	1A	4 (10.3)	10 (17.2)
	1B	0 (0.0)	2 (3.4)
	1C	6 (15.4)	12 (20.7)
	2A	2 (5.1)	0 (0.0)
	2B	0 (0.0)	1 (1.7)
	2C	5 (12.8)	2 (3.4)
	3A	2 (5.1)	2 (3.4)
	3B	1 (2.6)	1 (1.7)
	3C	15 (38.5)	12 (20.7)
Recurrence	4	4 (10.3)	1 (1.7)
Radiotherapy	Yes	4 (10.3)	4 (6.9)
	No	35 (89.7)	54 (93.1)
Chemotherapy	Yes	0 (0.0)	2 (3.4)
	No	39 (100)	56 (96.6)
Neoadjuvant	Yes	39 (100)	33 (56.9)
	No	0 (0.0)	25 (43.1)
Colostomy	Yes	5 (12.8)	2 (3.4)
	No	34 (87.2)	56 (96.6)
	Yes	2 (5.1)	0 (0.0)
	No	37 (94.9)	58 (100)

SD: Standard deviation

Table 2. Internal consistency and item to other scale correlation

	Cronbach's alpha	Item to other scale correlation
Abdominal/gastrointestinal symptoms OV 31-37	0.78	0.026-0.516
Peripheral Neuropathy OV 41-43	0.74	0.176-0.692
Other chemotherapy side effects OV 38-40, 44-47	0.72	0.300-0.693
Hormonal/menopausal OV 48,49	0.82	0.081-0.574
Body image OV 50,51	0.81	0.034-0.567
Attitude to disease and treatment OV 52-54	0.87	0.012-0.568
Sexual function OV 55-56*	0.93	0.012-0.300

*Conditional questions (OV 57,58) were excluded

Table 3: Comparison of quality of life scores of the European Organization for Research and Treatment of Cancer QLQ-OV28 between the groups (Mann-Whitney U test).

	On-treatment (n=39)	Off-treatment (n=58)	p
Abdominal/gastrointestinal symptoms OV 31-37	24.7±22.1	19.9±15.9	0.515
Peripheral neuropathy OV 41-43	45.6±28.3	31.4±28.2	0.010*
Other chemotherapy adverse effects (CH exclude) OV 38-40, 44-47	43.2±21.5	17.2±15.2	<0.001*
Hormonal/menopausal OV 48-49	45.3±31.9	35.3±32.6	0.094
Body image OV 50-51	26.1±32.3	20.7±26.7	0.451
Attitude to disease and treatment OV 52-54	58.4±29.0	34.1±32.4	<0.001*
Sexual function OV 55-56**	96.2±10.4	82.5±19.6	<0.001*

*p<0.05. **Conditional questions (OV 57,58) were excluded.

Table 4. Correlations of European Organization for Research and Treatment of Cancer QLQ-OV28 subscales and total score of BDI and STAI between groups (Spearman's correlation test).

	BDI total score data-set		STAI total score data-set	
	r	p	r	p
Abdominal/gastrointestinal symptoms OV 31-37	0.41	<0.001*	0.38	<0.001*
Peripheral neuropathy OV 41-43	0.49	<0.001*	0.39	<0.001*
Other chemotherapy adverse effects OV 38-40, 44-47	0.45	<0.001*	0.34	0.001*
Hormonal/menopausal OV 48,49	0.42	<0.001*	0.39	<0.001*
Body image OV 50,51	0.44	<0.001*	0.43	<0.001*
Attitude to disease and treatment OV 52-54	0.61	<0.001*	0.55	<0.001*
Sexual function OV 55-56**	0.11	0.278	-0.05	0.641
*p<0.05, **Conditional questions (OV 57,58) were excluded STAI: Spielberg State Trait Anxiety Inventory, BDI: Beck Depression Inventory				

positive thinking⁽¹²⁾. It is obvious that the severity and the discomfort of treatment-related symptoms may affect this attitude. In the present study, patients showed a significant correlation between their decreased QoL and depression and anxiety assessed by BDI and STAI questionnaires, respectively. The SEF subscale contains items pertaining to sexual function and two items (libido and extent of sexual activity) were asked of all patients and the remaining two questions were asked only to those who were sexually active. A significant number of women with gynecologic cancer are not sexually active. Furthermore, in the first phase of the development of the EORTC QLQ-OV28 module, items assessing sexual life had lower ratings than the other items⁽³⁾. In this study, especially in the on-treatment group, few women answered these items and, contrary to other subscales, significant differences and correlations were not observed in the SEF subscale. This finding was also observed in a study of validation of EORTC QLQ-OV28 into Taiwan Chinese and explained as a result of features of Chinese culture by the authors⁽⁴⁾. It should also be noted that in another randomized ovarian cancer study, the EORTC QLQ-OV28 module was designed without the use of these sexual function items⁽¹³⁾. Bajpai et al.⁽¹⁴⁾ also showed due to cultural taboos prevalent in India, people feel uncomfortable in talking about sexual behavior in general and a similar observation was noted when patients were asked to respond to question numbers 55-58 in the study of validation of EORTC QLQ-OV28 into Indian languages.

The inconsistency of these items with the module has led to the placement of this subscale at the end of the module, so that it

could be omitted without interfering with the other presented items. The decision about neglecting or omitting should be made cautiously because the separate analysis of these non-conditional items of sexual function showed a high consistency and significant difference between the groups in the present study.

Conclusion

Like the original questionnaire, the Turkish translated version of the EORTC QLQ-OV28 module is a reliable, consistent, and a valid instrument for assessing the impact of treatment modalities on QoL among Turkish-speaking women with ovarian cancer.

Ethics

Ethics Committee Approval: The study was approved by the ethics committee of the medical faculty of İstanbul Zeynep Kamil Women and Child Diseases Training and Research Hospital (approval number: 2019/139).

Informed Consent: Informed consent was obtained from each participant.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ç.C., Design: N.P.A., Ç.C., Data Collection or Processing: A.K., Analysis or Interpretation: A.K., Writing: Y.A.

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References

1. Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2007, National Cancer Institute. Bethesda, MD.
2. Anderson B, Lutgendorf S. Quality of life as an outcome measure in gynecologic malignancies. *Curr Opin Obstet Gynecol* 2000;12:21-6.
3. Greimel E, Bottomley A, Cull A, Waldenstrom AC, Arraras J, Chauvenet L, et al. An international field study of the reliability and validity of a disease-specific questionnaire module (the QLQ-OV28) in assessing the quality of life of patients with ovarian cancer. *Eur J Cancer* 2003;39:1402-8.
4. Chie W.-C., Lan, C.-Y., Chiang, C. and Chen, C.-A. Quality of life of patients with ovarian cancer in Taiwan: validation and application of the Taiwan Chinese version of the EORTC QLQ-OV28. *Psychooncology* 2010;19:782-5.
5. EORTC QLQ-OV28 module. <https://qol.eortc.org/questionnaire/qlq-ov28/> (Available from 20.05.2019)
6. Sprangers MA, Cull A, Groenvold M, Bjordal K, Blazeby J, Aaronson NK. The European Organization for Research and Treatment of Cancer approach to developing questionnaire modules: an update and overview. *EORTC Quality of Life Study Group. Qual Life Res.* 1998;7:291-300.
7. Ramanaiah NV, Franzen M, Schill T. A psychometric study of the State-Trait Anxiety Inventory. *J Pers Assess* 1983;47:531-5.

8. Kapci EG, Uslu R, Turkcapar H, Karaoglan A. Beck Depression Inventory II: evaluation of the psychometric properties and cut-off points in a Turkish adult population. *Depress Anxiety* 2008;25:E104-10.
9. Fayers P, Aaronson N, Bjordal K, Curan D, Groenvold M. EORTC QLQ-C30 Scoring Manual (2nd edn). RTC Quality of Life Study Group: Brussels, 1999
10. Khullar V. Outcome assessment. In: Bent AE, Ostergard DR, Cundiff GW, Swift SE, (eds.). *Ostergard's urogynecology and pelvic floor dysfunction*. 5th ed., Philadelphia: Lippincott, Williams & Wilkins. 2003;51-9.
11. von Gruenigen VE, Frasure HE, Jenison EL, Hopkins MP, Gil KM. Longitudinal assessment of quality of life and lifestyle in newly diagnosed ovarian cancer patients: the roles of surgery and chemotherapy. *Gynecol Oncol* 2006;103:120-6.
12. Steginga SK, Dunn J. Women's experiences following treatment for gynecologic cancer. *Oncol Nurs Forum*. 1997;24:1403-8.
13. Cull A, Howat S, Greimel E, Waldenstrom AC, Arraras J, Kudelka A, et al. Development of the European Organization of Research and Treatment of Cancer questionnaire module to assess the quality of life of ovarian cancer patients in clinical trials: a progress report. *Eur J Cancer* 2001;37:47-53.
14. Bajpai J, Panda PK, Kagwade S, et al. Translation and validation of European Organization for Research and Treatment for Cancer quality of life questionnaire-OV-28 module into Indian languages (Hindi and Marathi) to study quality of life of ovarian cancer patients from a tertiary care cancer center. *South Asian J Cancer*. 2018;7:37-41



Anatomic variations of the Uterine Artery. Review of the literature and their clinical significance

Uterin Arterin anatomik varyasyonları. Literatürün gözden geçirilmesi ve klinik önemi

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Abstract

Uterine arteries are the main vessels supplying blood to the uterus. Mainly, they originate from the anterior trunk of the internal iliac artery. Uterine arteries play an important role in pregnancy as well as transcatheter arterial embolization for postpartum hemorrhage and uterine fibroid management. This is a review of the English literature in the PubMed database of the anatomic variety on the origin of uterine arteries and their clinical significance. Eleven studies describe the origin of the uterine arteries and their variations in the literature. In six studies, the uterine artery emerged from internal iliac artery in the majority of the cases, either as a separate branch, or as a bifurcation with the inferior gluteal artery, or trifurcation with superior and inferior gluteal artery. In two studies, the inferior gluteal artery manifested as the main source of the uterine artery, whereas in three studies, the umbilical artery posed as its main origin. Internal iliac artery is described as the most common vascular origin of uterine artery. However, this review highlights that the main vessels of origin for uterine arteries are internal iliac, umbilical and inferior gluteal artery. Nevertheless, classification and further research for this peculiar anatomic structure is fundamental in the future.

Keywords: Uterine artery, branch, origin, anatomic variation

Öz

Uterin arterleri uterusu kan sağlayan ana damarlardır. Esas olarak, internal iliak arterin ön gövdesinden kaynaklanırlar. Uterin arterler hamilelikte postpartum kanama ve uterin fibroid yönetimi için transkateter arteriyel embolizasyon kadar önemli bir rol oynar. Bu, anatomik çeşitliliğin PubMed'in uterus arterlerinin kökeni ve klinik önemi üzerine veritabanında yapılan bir İngiliz literatürü derlemesidir. On bir çalışma uterus arterlerinin kökenini ve literatürdeki varyasyonlarını tanımlamaktadır. Altı çalışmada, uterus arteri olguların çoğunda, ayrı bir dal olarak veya alt gluteal arter ile bifurkasyon veya superior ve inferior gluteal arter ile trifurkasyon olarak internal iliak arterden çıkmaktaydı. İki çalışmada, inferior gluteal arter uterus arterinin ana kaynağı olarak ortaya çıkarken, üç çalışmada umbilikal arteri ana kaynağı olarak ortaya çıkmıştır. Internal iliak arter, uterus arterinin en yaygın vasküler orijini olarak tanımlanır. Bununla birlikte, bu derleme uterus arterleri için ana orijin damarlarının internal iliak, umbilikal ve inferior gluteal arter olduğunu vurgulamaktadır. Bununla birlikte, bu olağandışı anatomik yapı için sınıflandırma ve daha ileri araştırmalar gelecekte esastır.

Anahtar Kelimeler: Uterin arter, dal, orijin, anatomik varyasyon

Introduction

Uterine arteries are the main vessels supplying blood to the uterus. They impose a significant clinical role in multiple medical conditions, especially during pelvic and gynecologic surgery. Uterine arteries go through dramatic changes during pregnancy⁽¹⁾, increasing in volume and becoming more tortuous, playing an important role in perinatal outcomes⁽²⁾. Furthermore, uterine arteries are the target of embolization when dealing with fibroids and leiomyomata⁽³⁾ as well as uterine bleeding⁽⁴⁾, either because of pathologies mentioned or postpartum hemorrhage⁽⁵⁾. Uterine

artery location and origin is also important in pelvic surgery. High vascular ligation of the afore-mentioned artery is a necessary step during hysterectomy, myomectomy, and other and gynecologic oncology procedures⁽⁶⁾. However, uterine arteries demonstrate a plethora of anatomic variation mainly concerning their origin, raising a challenge for the surgeon. This is a review of the literature about uterine artery anatomic variations and their clinical value.

Clinical anatomy of uterine artery

The uterine artery traditionally arises from the internal iliac artery, anteriorly⁽⁷⁾. Partly the uterine artery passes, medially,

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through the base of the broad ligament of uterus before bifurcating at the isthmus level⁽⁸⁾. The ascending branch travels in parallel along the side of the uterus and fallopian tubes, following a U path and gives coil-shaped branches called the helicine branches. The ascending branch of the uterine artery anastomoses to the ovarian artery⁽⁹⁾. The descending part supplies the cervix and vagina⁽¹⁰⁾, anastomosing with the vaginal arteries and the inferior rectal arteries⁽¹¹⁾. The uterine artery crosses the ureter superiorly at the level of the lateral part of the uterine cervix below the isthmus part of the uterus, explaining why the ureter is at greater risk of injury during pelvic and gynecologic surgeries⁽¹²⁾.

For the current paper, we reviewed the English literature in the PubMed database for articles concerning variations in uterine artery anatomy, spanning years 1900 to 2019. The search included the keywords uterine artery or internal iliac artery or hypogastric artery and anatomy or anatomic variations. We revised the results maintaining only cadaveric, surgical and radiologic studies on the anatomy of the uterine arteries and their variations. We also went through the references of the papers found, in order to discover more bibliographic resources. Eleven studies describe the origin of the uterine artery and its variations in the literature. Article chronology ranges from 1918⁽¹³⁾ to 2019⁽¹⁴⁾, with more than half after 2010, indicating the increasing need for deeper understanding of uterine blood supply that has arisen after consolidation of uterine artery embolization for several uterine pathologies. Four studies were cadaveric^(13,15-17) and five studies were radiological, four of which used computed tomography (CT) angiography with 3D reconstruction^(14,18-20) and the fifth one included an evaluation of angiographies during uterine fibroid embolization⁽²¹⁾. One study uses surgical evaluation⁽²²⁾ and one study combines all methods⁽²³⁾. The combined results on the origin of uterine artery in our review are described in Table 1.

In six studies, the uterine artery emerged from internal iliac artery in the majority of their cases, either as a separate branch or as a bifurcation with inferior gluteal artery or trifurcation with superior and inferior gluteal artery. Kozlov et al.⁽¹⁵⁾ presented the internal iliac artery as the origin of the uterine artery in 60% of cases, followed by the umbilical artery as a separate branch, and via a common trunk in 27% and 1.8%, respectively. Albulescu et al.⁽¹⁹⁾ described the origin of the uterine artery as a separate branch from the internal iliac, as a bifurcation with the inferior gluteal, and as a trifurcation with superior and inferior gluteal arteries in 37%, 10%, and 29%, correspondingly. The remaining 24% arose from the inferior gluteal artery as a separate branch. Obimbo et al.⁽¹⁶⁾ demonstrated the uterine artery as the first branch of the internal iliac artery in 18.9% and second or third branch in 70.8% of cases. They also analyzed the course of uterine arteries in relation to the ureter and their branches. In 96.2% (102/106) of cases, the artery passed the ureter anteriorly, and in 3.8% (4/106 cases) it passed posteriorly. The uterine artery continues as a single vessel, bifurcates upon

reaching the uterus or trifurcates in 76.4% (81/106), 17.1% (18/106), and 6.7% (7/106), respectively. Naguib et al.⁽²⁰⁾ reported the uterine artery as a branch of the anterior division of internal iliac artery in 90% (86/95) of cases, as a branch of the posterior, main stem or bifurcation of internal iliac artery in 1%, 2%, and 1%, respectively. The uterine artery emerges via a common trunk with the obturator and internal pudendal in 5% and 1%, as well in this study. Roberts et al.⁽¹⁷⁾ described the uterine artery as a separate branch of the internal iliac, as a branch of the umbilical, inferior vesical, and internal pudendal in 27, 19, one, and one cases, respectively. Lipshutz⁽¹³⁾ also posed the internal iliac artery as the main origin of the uterine artery in 60 out of 67 cases, with the remaining four, two, and one originating from the superior vesical, internal pudendal, and obturator arteries, respectively.

In two studies, the inferior gluteal artery manifested as the main source of the uterine artery. Yunxiu et al.⁽¹⁴⁾ reported the uterine artery as a separate branch of the inferior gluteal artery in 64.3% (144/224) of cases. The uterine artery emerged independently from the internal iliac and via its trifurcation in 22.8% (51/224) of cases and 12.9% (29/224) of cases, respectively, in this study. Gomez-Jorge et al.⁽²¹⁾ described the uterine artery as a branch of the inferior gluteal, internal iliac artery or as a trifurcation with inferior and superior gluteal arteries in 51%, 43%, and 6%, respectively.

In three studies, the umbilical artery posed as the main origin of the uterine artery. Arfi et al.⁽¹⁸⁾ reported the uterine artery arose via a common trunk with the umbilical artery in 62.4% (54/86) of cases. In 25.6% (22/86) of cases, it was a branch of the internal iliac artery, in 9.3% (8/86) a branch of the superior gluteal artery, and in 2.3% (2/86) of cases a branch of the internal pudendal. Chantalat et al.⁽²³⁾ also described the origin of the uterine artery via a common trunk with the umbilical artery in the majority of the cases in cadaveric, surgical, and radiologic groups. In the cadaveric group, 50 out of 60 (83.3%) uterine arteries emerged via a common trunk with the umbilical, six (10%) as a separate branch of the internal iliac, and four (6.7%) as a branch of the internal pudendal artery. In the surgical group, a common trunk of the umbilical and uterine arteries was noted in 82 cases (82%), 16 uterine arteries presented as independent branches of the internal iliac, and two of the superior gluteal arteries. In the angiography group, a common trunk of the uterine and umbilical arteries was described in 42 out of 58 cases (76.5%). The uterine artery was a branch of the internal iliac, superior gluteal, and obturator arteries in eight (11.8%), six (8.8%), and two (2.9%) cases, respectively. Finally, Holub et al.⁽²²⁾ described the uterine artery as a branch of the umbilical and internal pudendal arteries in 76.5% and 23.5% of cases, respectively.

The uterine artery is an artery found in females that anatomically corresponds to the artery to the ductus deferens in males. Traditionally, it originates from internal iliac artery. However, following our results, the uterine artery does not always follow

Table 1. Origin of the uterine artery in the literature

A/A	Author	Year	Study type	Patient	Sides studied	Origin of uterine artery			Superior gluteal
						Internal iliac	Bif (IGA)	Trif (IGA, SGA)	
						Separate			
1	Yunxiu et al. ⁽¹⁴⁾	2019	CT Angiography	112	224	22.8% (51)	-	12.9% (29)	-
2	Kozlov et al. ⁽¹⁵⁾	2018	Cadaveric	19	38	60%	-	-	1.6% CT
3	Arfi et al. ⁽¹⁸⁾	2017	CT Angiography	43	86	25.6% (22)	-	-	9.3% (8)
4	Albulescu et al. ⁽¹⁹⁾	2014	CT Angiography	110	200	37%	10%	29%	-
5	Chantalat et al. ⁽²³⁾	2013	Cadaveric	30	60	10% (6)	-	-	-
			Surgical	50	100	16% (16)	-	-	2% (2)
			CT Angiography	34	58	11.8% (8)	-	-	8.8% (6)
6	Obimbo et al. ⁽¹⁶⁾	2010	Cadaveric	53	106	89.7% (95)	-	-	-
7	Naguib et al. ⁽²⁰⁾	2008	CT Angiography	49	95	93% (89)	1% (1)	-	-
8	Holub et al. ⁽²²⁾	2005	Surgical	100	81	-	-	-	-
9	Gomez-Jorge et al. ⁽²¹⁾	2003	Arteriograms (UFE)	257	38% of 514	43%	-	6%	-
10	Roberts et al. ⁽¹⁷⁾	1966	Cadaveric	167	44	56.2% (27)	-	-	-
11	Lipshutz ⁽¹³⁾	1918	Cadaveric	93	67	89.5% (60)	-	-	-

Table 1. Contined

A/A	Author	Origin of uterine artery						
		Inferior gluteal	Umbilical	Superior vesical	Inferior vesical	Middle rectal	Internal pudendal	Obturator
1	Yunxiu et al. ⁽¹⁴⁾	64.3% (144)	-	-	-	-	-	-
2	Kozlov et al. ⁽¹⁵⁾	1.6%*	27% and 1.8%*	-	3% and 1.4%*	2%	1.6%*	-
3	Arfi et al. ⁽¹⁸⁾	-	62.4% (54)*	-	-	-	2.3% (2)	-
4	Albulescu et al. ⁽¹⁹⁾	24%	-	-	-	-	-	-
5	Chantalat et al. ⁽²³⁾	-	83.3% (50)*	-	-	-	6.7% (4)*	-
		-	82% (82)*	-	-	-	-	-
		-	76.5% (42)*	-	-	-	-	2.9% (2)
6	Obimbo et al. ⁽¹⁶⁾	10.3% (11)	-	-	-	-	-	-
7	Naguib et al. ⁽²⁰⁾	-	-	-	-	-	1% (1)*	5% (4)*
8	Holub et al. ⁽²²⁾	-	76.5% (62)	-	-	-	23.5% (19)	-
9	Gomez-Jorge et al. ⁽²¹⁾	51%	-	-	-	-	-	-
10	Roberts et al. ⁽¹⁷⁾	-	39.6% (19)	-	2.1% (1) [CT]	-	2,1% (1)	-
11	Lipshutz ⁽¹³⁾	-	-	6% (4)*	-	-	3% (2)*	1.5% (1)*

CT: Computed tomography, UFE: Uterine fibroid embolization, Bif: Bifurcation, Trif: Trifurcation, IGA: Inferior gluteal artery, SGA: Superior gluteal artery, * Branches in a common trunk

the typical route and origin. Except for the anterior trunk of the internal iliac, the uterine artery may originate from the inferior gluteal and umbilical artery, either directly or as a common stem. As reported in this review, these variations are quite frequent. There are rare cases in which the uterine artery comes from other arteries such as the superior gluteal, internal

pudendal, obturator or vesical arteries. Moreover, there are some case reports of uncommon anatomic variations, such as the origin of uterine artery being from external iliac artery⁽²⁴⁾ and inferior epigastric artery⁽²⁵⁾. The absence of uterine arteries has also been observed^(26,27) with large ovarian vessels being present taking up the uterus blood supply.

The uterine artery and its high vascular ligation is a vital step during pelvic and gynecologic surgeries, such as hysterectomy. Often, pelvic pathologies such as fibroids, endometriosis, adhesions from previous pelvic surgeries, or ovarian remnants can distort the anatomic relations and create technical challenges during laparoscopic hysterectomies. Retroperitoneal dissection, in order to ligate the uterine artery at its vascular origin, can circumvent these obstacles, resulting in a safer procedure⁽⁶⁾.

Uterine artery anatomy and flow play an important role during pregnancy. The uterine artery becomes more tortuous, large, and with increased flow in pregnant women⁽²⁸⁾. Ultrasound is a useful tool in the evaluation of the uterine artery during the first and second trimesters of pregnancy, most commonly used as uterine Doppler ultrasound, for the prediction of the later development of pre-eclampsia, intrauterine fetal growth restriction, placental abruption, and stillbirth⁽²⁾.

Furthermore, the clinical importance of understanding the uterine artery variations lies in the fact that during specific clinical processes, such as in uterine artery embolization, the plethora of anatomic variations of the uterine artery make the associated procedures quite challenging. As first described by O'Leary et al.⁽²⁹⁾, one method of controlling postpartum hemorrhage is by bilateral ligation of the uterine arteries. Other methods include bimanual compression, which can be internal bimanual uterine compression or external bimanual compression or a medical approach, which includes oxytocin, tranexamic acid, blood transfusions or oral misoprostol. Occlusion of uterine vessels can be achieved via transcatheter arterial embolization, which is nowadays considered as the first-line therapy to control post-partum hemorrhage due to its characteristics of fast pace, excellent effect, wide indication, minimal invasiveness, and uterine preservation⁽³⁰⁾. Embolization makes it possible to avoid hysterectomy, while theoretically preserving the possibility of another pregnancy⁽⁵⁾. Cheng et al.⁽³¹⁾ described how menstruation and fertility could be preserved successfully for future pregnancy after embolization.

Uterine artery embolization illustrates an important role in uterine fibroid and leiomyoma management. Embolization of the uterine arteries reduces the blood supply to the uterus, reduces the size of fibroids, resulting in decreased pain and dysmenorrhea with a high level of clinical improvement without the need of hysterectomy and better outcomes than myomectomy⁽³²⁾. Uterine artery embolization has the advantage of managing the complications of myomectomy and other uterine procedures in a safe and effective way⁽³³⁾. It may also deal with other causes of bleeding such as uterine artery pseudoaneurysm⁽³⁴⁾, an underestimated clinical occurrence with many reports⁽³⁵⁻³⁷⁾, and 3.3% prevalence according to Dossou et al.⁽⁵⁾.

The importance of uterine artery embolization as well as the major role of the uterine artery in pregnancy and pelvic surgery marks the necessity for deep understanding of this vessel and its anatomic variations. Many studies described the origin of

uterine arteries in different ways. Some of them used the Adachi classification on the iliac artery⁽³⁸⁾, and some simply reported the vessel from which the artery originated. The characterization of this peculiar and significant artery requires specific classification because of the plethora of anatomic variations of uterine artery, and the different descriptions reported in the literature. In this review, the main origins of the uterine artery were the internal iliac artery, umbilical artery, and inferior gluteal artery. When the uterine artery comes from the internal iliac artery, it can branch separately or as bifurcation or trifurcation with other arteries, mainly the inferior and superior gluteal arteries. The internal pudendal artery is also reported to be the origin of the uterine artery in a notable percentage of cases. All these variations raise the challenge for a recognizable and accepted classification.

Uterine arteries play an important role in clinical practice. Internal iliac artery is described as their most common vascular origin. However, this review highlights that the main vessels of origin for uterine arteries are the internal iliac, umbilical, and inferior gluteal artery. Nevertheless, classification and further research for this peculiar anatomic structure is fundamental in the future.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.L., N.T., I.T., Concept: P.S., K.V., D.F., Design: Data Collection or Processing: K.L., N.T., I.T., G.T., P.S., K.V., D.F., Analysis or Interpretation: K.L., N.T., I.T., G.T., P.S., K.V., D.F., Literature Search: K.L., N.T., P.S., K.V., D.F., Writing: K.L., N.T., I.T., G.T., P.S., K.V., D.F.

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References

1. Arulkumaran S, Karoshi M, Keith LG, Lalonde AB, B-Lynch C, eds. A Comprehensive Textbook of Postpartum Hemorrhage An Essential Clinical Reference for Effective Management. In: Sapiens Publishing Ltd. 2nd ed. London: Paula and David Bloomer 2012:177-84.
2. Gómez O, Figueras F, Martínez JM, del Río M, Palacio M, Eixarch E, et al. Sequential changes in uterine artery blood flow pattern between the first and second trimesters of gestation in relation to pregnancy outcome. *Ultrasound Obstet Gynecol* 2006;28:802-8.
3. Katz MD, Sugay SB, Walker DK, Palmer SL, Marx MV. Beyond Hemostasis: Spectrum of Gynecologic and Obstetric Indications for Transcatheter Embolization. *Radiographics* 2012;32:1713-31.
4. Wan AY, Shin JH, Yoon HK, Ko GY, Park S, Seong NJ, et al. Post-operative hemorrhage after myomectomy: safety and efficacy of transcatheter uterine artery embolization. *Korean J Radiol* 2014;15:356-63.
5. Dossou M, Debost-Legrand A, Déchelotte P, Lémery D, Vendittelli F. Severe secondary postpartum hemorrhage: A historical cohort. *Birth* 2015;42:149-55.

6. Peters A, Stuparich MA, Mansuria SM, Lee TT. Anatomic vascular considerations in uterine artery ligation at its origin during laparoscopic hysterectomies. *Am J Obstet Gynecol* 2016;215:393.
7. Selçuk İ, Yassa M, Tatar İ, Huri E. Anatomic structure of the internal iliac artery and its educative dissection for peripartum and pelvic hemorrhage. *Turk J Obstet Gynecol* 2018;15:126-9.
8. Selçuk İ, Ersak B, Tatar İ, Güngör T, Huri E. Basic clinical retroperitoneal anatomy for pelvic surgeons. *Turk J Obstet Gynecol* 2018;15:259-69.
9. Ouyang Z, Liu P, Yu Y, Chen C, Song X, Liang B, et al. Role of ovarian artery-to-uterine artery anastomoses in uterine artery embolization: Initial anatomic and radiologic studies. *Surg Radiol Anat* 2012;34:737-41.
10. Chaudhry R, Chaudhry K. Anatomy, abdomen and pelvis, uterine arteries. *StatPearls* 2019.
11. Barral J-P, Croibier A, Barral J-P, Croibier A. Uterine vessels. *Visc Vasc Manip* 2011:240-44.
12. Jackson LA, Ramirez DMO, Carrick KS, Pedersen R, Spirtos A, Corton MM. Gross and Histologic Anatomy of the Pelvic Ureter: Clinical Applications to Pelvic Surgery. *Obstet Gynecol* 2019;133:896-904.
13. Lipshutz B. A Composite Study of the Hypogastric Artery and its Branches. *Ann Surg* 1918;67:584-608.
14. Yunxiu H, Kefei W, Guorong W, et al. Value of the CT Angiography in Displaying the Anatomical Variations of the Origin of Uterus Artery. *Acta Acad Med Sin.* 2019;41:216-9.
15. Kozlov SV, Dvoretzskii DD, Alekseenko LA, Omelchenko A, Kartamysheva VD. Anatomical Variants of Uterine Arteries. *Ukr J Med Biol Sport* 2018;3:32-7.
16. Obimbo MM, Ogeng JA, Saidi H. Variant anatomy of the uterine artery in a Kenyan population. *Int J Gynecol Obstet* 2010;111:49-52.
17. Roberts WH, Krishningner GL. Comparative study of human internal iliac artery based on Adachi classification. *Anat Rec* 1967;158:191-6.
18. Arfi A, Arfi-Rouche J, Barrau V, Nyangoh Timoh K, Touboul C. Three-dimensional computed tomography angiography reconstruction of the origin of the uterine artery and its clinical significance. *Surg Radiol Anat* 2018;40:85-90.
19. Albulescu D, Constantin C, Constantin C. Uterine artery emerging variants - angiographic aspects. *Curr Heal Sci J* 2014;40:214-6.
20. Naguib NNN, Nour-Eldin N-EA, Hammerstingl RM, et al. Three-dimensional Reconstructed Contrast-enhanced MR Angiography for Internal Iliac Artery Branch Visualization before Uterine Artery Embolization. *J Vasc Interv Radiol* 2008;19:1569-75.
21. Gomez-Jorge J, Keyoung A, Levy EB, Spies JB. Uterine artery anatomy relevant to uterine leiomyomata embolization. *Cardiovasc Intervent Radiol* 2003;26:522-7.
22. Holub Z, Lukac J, Kliment L, Urbanek S. Variability of the origin of the uterine artery : Laparoscopic surgical observation. *J Obs Gynaecol Res* 2005;31:158-163.
23. Chantalat E, Merigot O, Chaynes P, Lauwers F, Delchier MC, Rimailho J. Radiological anatomical study of the origin of the uterine artery. *Surg Radiol Anat* 2014;36:1093-99.
24. Saraiya PV., Chang TC, Pelage JP, Spies JB. Uterine artery replacement by the round ligament artery: An anatomic variant discovered during uterine artery embolization for leiomyomata. *J Vasc Interv Radiol* 2002;13:939-41.
25. Uflacker A, Sopata CE, Haskal ZJ. Inferior Epigastric Uterine Artery. *J Vasc Interv Radiol* 2017;28:23.
26. Wagner E, Soneji K, Semaan A, Yu S. Bilateral agenesis of the uterine arteries. *Res Rep Gynaecol Obs* 2017;1:20-2.
27. Maclaran KA, Edmonds DK, Tait P. Absence of uterine arteries discovered at fibroid embolisation. *Br J Radiol* 2009;82:228-30.
28. Osol G, Mandala M. Maternal uterine vascular remodeling during pregnancy. *Physiology (Bethesda)* 2009;24:58-71.
29. O'Leary JL, O'Leary JA. Uterine artery ligation in the control of intractable postpartum hemorrhage. *Am J Obstet Gynecol* 1966;94:920-4.
30. Chen C, Lee SM, Kim JW, Shin JH. Recent Update of Embolization of Postpartum Hemorrhage. *Korean J Radiol* 2018;19:585-96.
31. Cheng HH, Tsang LLC, Hsu TY, et al. Transcatheter arterial embolization as first-line rescue in intractable primary postpartum hemorrhage: Assessment, outcome, and subsequent fertility. *J Formos Med Assoc* 2017;116:380-7.
32. Segars JH, Parrott EC, Nagel JD, et al. Proceedings from the Third National Institutes of Health International Congress on Advances in Uterine Leiomyoma Research: comprehensive review, conference summary and future recommendations. *Hum Reprod Update* 2014;20:309-33.
33. Wan AY-H, Shin JH, Yoon H-K, et al. Post-operative hemorrhage after myomectomy: safety and efficacy of transcatheter uterine artery embolization. *Korean J Radiol* 2014;15:356-63.
34. Yi SW. Extravasating uterine pseudoaneurysm: A rare cause of postpartum haemorrhage. *J Obstet Gynaecol (Lahore)* 2017;37:416-20.
35. Karmous N, Ayachi A, Derouich S, Mkaouar L, Mourali M. Rupture of uterine artery pseudoaneurysm: role of ultrasonography in postpartum hemorrhage management. *Pan Afr Med J* 2016;25:136.
36. Chummun K, Kroon N, Flannelly G, Brophy D. Severe postcoital bleeding from a uterine artery pseudoaneurysm 4 months after cesarean delivery. *Obstet Gynecol* 2015;126:638-41.
37. Cornette J, Van Der Wilk E, Janssen NM, et al. Uterine artery pseudoaneurysm requiring embolization during pregnancy. *Obstet Gynecol.* 2014;123:453-6.
38. Adachi B, Hasebe K. Das Arteriensystem Der Japaner. Kyoto; 1928. <https://lib.ugent.be/en/catalog/rug01:001663571>. Accessed July 1, 2019.



Leriche's syndrome and twin pregnancy

Leriche sendromu ve ikiz gebelik

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Abstract

Leriche's syndrome is characterized by chronic obstruction of the abdominal aorta and iliac arteries. A patient with Leriche's syndrome presented with twin pregnancy and severe preeclampsia at 32 weeks' gestation. Cesarean delivery was performed and the patient was admitted to the intensive care unit. Magnetic resonance angiography showed total occlusion of the distal abdominal aorta, common, and external iliac arteries. There were extensive collateral vessels between the lumbar arteries and iliolumbar arteries. The patient was discharged in an improved clinical condition. Leriche's syndrome and pregnancy demonstrating complete aortic, common, and external iliac artery occlusion is very rare in the literature. Despite complete occlusion, viability of the fetus can be achieved with collateral vessels.

Keywords: Aortoiliac occlusion, Leriche's syndrome, pregnancy

Öz

Leriche sendromu abdominal aorta ve iliak arterlerin kronik obstrüksiyonu ile karakterizedir. Olgu 32. gebelik haftasında ikiz gebelik ve ağır preeklampsi tanısı ile refere edildi. Sezaryen doğum gerçekleştirildi ve hasta yoğun bakıma alındı. Manyetik rezonans anjiyografi ile distal abdominal aorta, common iliak ve eksternal iliak arterlerde total oklüzyon görüldü. Lumbar ve iliolumbar arterler arasında yoğun kollateral damarlar mevcuttu. Kliniği düzelen hasta taburcu edildi. Aortik, common ve eksternal iliak arterlerde komplet oklüzyon ile giden Leriche sendromu ve gebelik olgusu literatürde oldukça azdır. Komplet oklüzyona rağmen kollateral damarların gelişimi ile fetüsün viabilitesi elde edilebilir.

Anahtar Kelimeler: Aortoiliak oklüzyon, Leriche sendromu, gebelik

Introduction

Leriche syndrome, also known as aortoiliac occlusive disease, is characterized by chronic obstruction of the abdominal aorta and iliac arteries⁽¹⁾. The disease was first described by Robert Graham in 1814⁽²⁾. Leriche syndrome was named after a French surgeon, Rene Leriche, who first operated on the condition⁽¹⁾. We aimed to report a case of Leriche syndrome and twin pregnancy presenting with severe preeclampsia because there are scant data regarding total aortoiliac occlusion and pregnancy in the literature.

Case report

A 53-year-old gravida-1-para-0 at 32 weeks' gestation was referred to hospital for dyspnea and uncontrolled hypertension. Her medical history revealed chronic hypertension, pregestational diabetes mellitus (DM), and aortoiliac occlusive disease. She had a twin pregnancy using egg donation in another

country. She was treated with a regimen of alpha methyl dopa (3x250 mg), acetylsalicylic acid (1x100 mg), and enoxaparin (1x6000 IU). Her antenatal visits were irregular. In his initial physical examination blood pressure was 190/90 mm Hg. A fetal ultrasound examination showed a dichorionic diamniotic twin pregnancy. Hematologic and serum biochemical tests were within normal limits. Urine dipstick analysis revealed 3+ proteinuria. Elevated blood pressure persisted despite antihypertensive drugs such as calcium channel blockers and a diagnosis of superimposed preeclampsia was made. A decision for immediate delivery was made. Cesarean section was performed due to a breech cephalic presentation. A male infant weighing 1.730 g and female infant weighing 1.980 g were delivered. The patient was admitted to the intensive care unit due to a history of aortoiliac occlusive disease and severe hypertension. The postoperative period was uneventful and aortofemoropopliteal magnetic resonance (MR) angiography was performed at postoperative day 5. MR angiography showed total occlusion of the distal abdominal aorta. Common iliac

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and external iliac vessels were bilaterally occluded. Extensive collateral vessels were seen between the lumbar arteries and iliolumbar arteries (Figure 1). The patient was discharged on postoperative day 6. The infants were discharged in good condition. Informed consent was obtained.

Discussion

Leriche syndrome is a rare atherosclerotic occlusive disease characterized by total occlusion in the abdominal aorta and/or both iliac arteries⁽¹⁾. Aortoiliac lesions were categorized by the Trans-atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II), and Leriche syndrome can be categorized as a type D aortoiliac lesion⁽³⁾. It has been reported that Leriche syndrome usually affects men in the third to sixth decades of life⁽⁴⁾. Hyperlipidemia, hypertension, DM, and smoking are described as risk factors in the literature⁽⁵⁾. Oocyte donation, maternal age, chronic hypertension, and pregestational DM were possible risk factors in our case. A previous study of 77 patients with aortoiliac disease showed increased pregnancy loss compared with the control group. In this study, a stenotic lesion of >50% was accepted for peripheral arterial occlusive disease⁽⁶⁾. Sass et al.⁽⁷⁾ reported a case of pregnancy with an aortic prosthesis for Leriche syndrome. In this case, the patient had previously undergone surgery and the abdominal aorta was the only affected aortic area. In comparison with these reports, there was total occlusion of the abdominal aorta, common, and external iliac

arteries in our case. Patients with aortoiliac occlusive disease can be asymptomatic or present with several symptoms such as claudication, severe pain during rest, paresis, and absence of femoral pulses, which are highly dependent on the location of the occlusive lesion and development of collateral vessels⁽⁸⁾. We observed extensive collateral vessels in the distal region of the internal iliac artery. We can argue that these anastomoses between the lumbar arteries and iliolumbar arteries maintained normal uterine perfusion and the asymptomatic presentation of our patient can be explained also by impressive formation of retroperitoneal anastomoses. It is difficult to state an optimal obstetric management and follow-up for asymptomatic cases with aortoiliac occlusive disease because there are only few reports regarding aortoiliac occlusive disease and pregnancy in the literature.

We reported twin pregnancy and Leriche syndrome with complete aortic, common, and external iliac artery occlusion. Despite complete occlusion, viability of a fetus can be achieved with collateral vessels. Multidisciplinary teams must be involved in the care of these women.

Ethics

Informed Consent: It was obtained.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Data Collection or Processing: A.M., K.A. Analysis or Interpretation: C.T.E., Literature Search: A.M., K.A., Writing: C.T.E., H.E.

Conflict of Interest: We declare that we have no conflict of interest.

Financial Disclosure: We do not have a financial relationship with the organization that sponsored the research.

References

1. Leriche R, Morel A. The syndrome of thrombotic obliteration of the aortic bifurcation. *Ann Surg* 1948;127:193-206.
2. Graham R. Case of obstructed aorta. *Med Chir Trans* 1814;287-456-9.
3. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. TASC II Working Group. Inter-society consensus for the management of peripheral arterial disease (TASC II). *J Vasc Surg* 2007;45 Suppl: 5-67.
4. Beckwith R, Huffman ER, Eiseman B, Blount SG Jr. Chronic aortoiliac thrombosis: a review of sixty-five cases. *N Engl J Med* 1958;258:721-6.
5. Frederick M, Newman J, Kohlwes J. Leriche syndrome. *J Gen Intern Med* 2010;25:1102-4.
6. van den Bosch MA, Mali WP, Bloemenkamp DG, Eikelboom BC, Kemmeren JM, Tanis BC, et al. Increased pregnancy loss in young women with aortoiliac disease. *Atherosclerosis* 2002;164:121-7.
7. Sass N, Poli AH, Torloni MR, Gibim PG, Andrade JD, Izukwa NM. Pregnancy and delivery in a patient with aortic prosthesis for Leriche syndrome. *Sao Paulo Med J* 2003;121:34-6.
8. Wooten C, Hayat M, du Plessis M, Cesmebasi A, Koesterer M, Daly KP, et al. Anatomical significance in aortoiliac occlusive disease. *Clin Anat* 2014;27:1264-74.

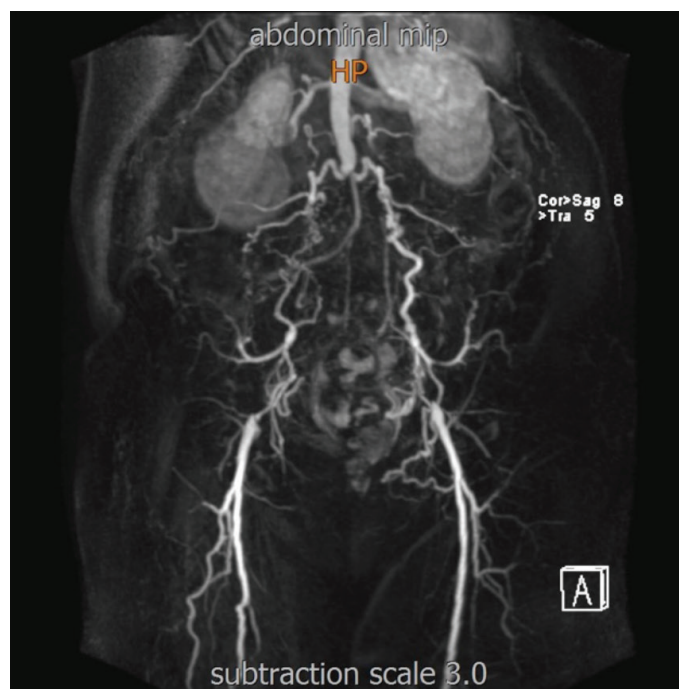


Figure 1. Magnetic resonance angiography showed total occlusion of distal abdominal aorta. Common iliac and external iliac vessels were bilaterally occluded. Extensive collateral vessels were seen between the lumbar arteries and iliolumbar arteries



Ovarian hyperstimulation syndrome presenting with isolated unilateral right-side hydrothorax: A report of two cases and systematic review of the literature

İzole tek taraflı hidrotoraks şeklinde bulgu veren ovaryan hiperstimülasyon sendromu: İki olgunun takdimi ve literatürün sistematik olarak gözden geçirilmesi

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Abstract

Although hydrothorax may accompany abdominal ascites in women with severe ovarian hyperstimulation syndrome (OHSS), there are few cases reported with isolated pleural effusion. Herein, we report two patients with isolated hydrothorax without any significant abdominal fluid following infertility treatment, along with a systematic review of the literature to describe risk factors for this rare entity. Two women with isolated pleural effusion without significant abdominal ascites were reported. The available literature was screened from Ovid-SP and PubMed to review OHSS cases with isolated hydrothorax. Two women aged 28 and 31 years were admitted to hospital with chest pain, tachypnea, and tachycardia after infertility treatment. They had right pleural effusion without abdominal fluid and the symptoms relieved after thoracentesis. Similar to our cases, we identified 24 case reports (n=41 women) in the literature according to eligible criteria. On the day of triggering, estradiol (E₂) level was <4000 pg/mL in 81% of reported cases and hematocrit (HCT) was <45% in 44% of cases at the time of diagnosis. Isolated hydrothorax is an unpredictable event, which may even complicate women with low E₂ levels or HCT concentrations. Physicians should keep in mind the possibility of isolated hydrothorax when respiratory symptoms are significant but abdominal ascites is not evident.

Keywords: Ovarian hyperstimulation syndrome, unilateral hydrothorax, in-vitro fertilization, controlled ovarian stimulation

Öz

Siddetli ovarian hiper-stimülasyon sendromu (OHSS) olan hastalarda abdominal asite hidrotoraksın da eşlik etmesi karşılaşılan bir durumdur, ancak literatürde izole hidrotoraks ile başvuran sadece birkaç olgu bildirilmiştir. Bu yazıda infertilite tedavisini takiben batında belirgin asit olmadan izole hidrotoraksı olan iki hastayı rapor etmeyi ve bu nadir durum için risk faktörlerini tanımlayabilmek için sistematik bir derleme yapmayı amaçladık. Belirgin asiti olmadan izole sağ taraf hidrotoraksı olan iki kadın rapor edildi. İzole hidrotoraks ile başvuran OHSS olgularını gözden geçirmek için Ovid-SP ve PubMed veri tabanlarında literatür taraması yapıldı. İnfertilite tedavisinden hemen sonra yaşları 28 ve 31 olan iki kadın göğüs ağrısı, taşipne ve taşikardi nedeniyle hastaneye/acil-servise başvurdu. Hastaların ultrasonda batında serbest sıvı yokken akciğer grafisinde sağ tarafta izole hidrotoraks saptandı. Her iki hastanın da torasentez sonrası semptomları hafifledi. Literatürde olgularımıza benzer şekilde, derlememizin dahil etme kriterlerine uygun 24 olgu takdiminde 41 kadın saptandı. Risk faktörleri yönünden literatürdeki hasta verileri değerlendirildiğinde rapor edilen tüm olguların %81'inde ovulasyon tetikleme günü kan östradiol seviyesi <4000 pg/mL iken, tanı anında olguların %44'ünde hematokrit <%45 idi. İzole hidrotoraks, düşük östradiol düzeyleri ve hematokrit konsantrasyonları olan kadınları bile komplike edebilecek ön görülemez bir olaydır. Klinisyenler, solunum semptomları varlığında belirgin abdominal asit olmasa bile izole hidrotoraks olasılığını akıllarına getirmelidir.

Anahtar Kelimeler: Ovaryan hiperstimülasyon sendromu, tek taraflı hidrotoraks, in vitro fertilizasyon, kontrollü ovaryan stimülasyon.

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Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication caused by ovarian stimulation procedures during infertility treatment. This syndrome occurs due to the release of cytokines from luteinized follicles, mainly vascular endothelial growth (VEGF), which induces vascular permeability, leakage of the intravascular liquid to third spaces, and a tendency to thromboembolic events⁽¹⁾. The recognized risk factors for severe OHSS include younger age, low body mass index (BMI), high antral follicle count or anti-Müllerian hormone level, elevated peak estradiol concentration, and ≥ 20 oocytes collected during an in-vitro fertilization cycle^(2,3).

The incidence of severe OHSS is around 1% and there has been an increasing interest for novel strategies to decrease the syndrome^(1,4). In women with severe OHSS, although hydrothorax might already accompany significant abdominal ascites in up to 10% of cases^(2,5), a clinical presentation of isolated pleural effusion without any abdominal ascites is a rare entity. In that context, there is lack of data for the explanatory mechanism and risk factors that might predict the appearance of isolated hydrothorax without any significant abdominal ascites. We performed a systematic review to describe risk factors for isolated hydrothorax and described a possible explanation that could indicate a mechanism for this clinic entity.

Case Reports

Case 1

A 28-year-old woman was admitted with a 3-year history of primary infertility and oligo-anovulation. Her BMI was 19.7 kg/m² and her total AFC was 27. A low-dose step-up protocol with a daily dosage of 75 IU (Puregon, MSD, İstanbul, Turkey) for ovulation induction was employed. On the 9th day of ovarian stimulation (OS), folliculometry with ultrasonography revealed one follicle of >17 mm, two follicles between 13-14 mm, and two follicles between 11-13 mm with an estradiol level of 2100 pg/mL. We decided to trigger (Ovitrelle, Merck, İstanbul) and perform intrauterine insemination (IUI) 36 hours later because she had only one leading follicle exceeding 17 mm.

Eight days after triggering, she was admitted to the emergency department with right chest pain, dyspnea, tachypnea, and tachycardia. The heart rate was 110/minute, blood pressure was 105/70 mm Hg, and her body temperature was 37.1°C. In a physical examination, diminished respiration sounds on the right hemi-thorax and a mild abdominal discomfort was noticed. In laboratory tests, the hemoglobin (Hb) was 15.1 g/dL, hematocrit (HCT) was 45.9%, and white blood cell (WBC) count was 16.4×10^3 . The serum electrolytes, liver enzymes, creatinine value, and cardiac panel were within the normal range; there was a slight increase in D-Dimer level up to 2.1 mg/L. Under ultrasonography, there was only minimal fluid accumulation in the recto-uterine pouch. After chest X-ray imaging (Figure 2a), computed tomography was planned, which

revealed a right-sided unilateral pleural effusion with a 55-mm deep accompanying atelectasis (Figure 2b). Under fluoroscopy guidance, a total of 1500 mL serous characterized fluid was drained via transthoracic aspiration with a 22-G needle (Cook, Inc., Bloomington, IN, USA). Immediately after intervention, the symptoms were relieved and the HCT declined to 37% one day after the procedure. The patient was discharged from hospital four days later but she failed to conceive.

Case 2

A 31-year-old woman with a normal BMI (21.4 kg/m²) was referred to our infertility clinic with a 4-year history of primary infertility. The AFC was 23 and the anti-müllerian hormone level was 8.49 ng/mL. Her menstrual periods were regular. A tubal patency assessment with hysterosalpingography (HSG) revealed normal findings. However, her husband had mild oligospermia. Intra-cytoplasmic sperm injection (ICSI) and embryo transfer (ET) cycle was employed (Table 1) with a daily dosage of 150 IU (Puregon, MSD, İstanbul, Turkey). On the 9th day of stimulation, three follicles of ≥ 17 mm, eight follicles between 14-16 mm, and five follicles between 11-13 mm were identified with an estradiol level of 2890 pg/mL. We decided to trigger with a GnRH-analogue (Decapeptyl, Ferring, Kiel, Germany) with dose of 0.2 mg because she had 16 follicles ≥ 11 mm in a GnRH-antagonist cycle. A total of 15 oocytes were collected and 1500 IU hCG (Pregnyl, Merck, Kiel, Germany) was injected one hour after retrieval to support luteal phase⁽⁶⁾. Two days after oocyte collection, she was admitted with severe dyspnea and tachycardia. In the laboratory tests, the Hb was 14.9 g/dL, HCT was 44.0%, and the WBC count was 15.8×10^3 . The serum electrolytes, liver enzymes, creatinine value, and cardiac panel were within the normal range. Bilateral ovaries were observed enlarged with multiple anechoic cysts in ultrasound screening, but there was no peritoneal fluid. Thorax ultrasound was performed immediately after abdominal ultrasonography after tilting the patient on her left/right side and revealed a 50-mm wide horizontal pleural effusion on the right side (Figure 2c). Chest X-ray also confirmed unilateral right-side pleural effusion and a total of 1500 mL serous characterized fluid was drained under fluoroscopy. Immediately after intervention, the symptoms were relieved. HCT declined to 39% one day after the procedure and she was discharged. ET was cancelled and all embryos were vitrified.

Review of the Literature

Two authors (A.T. and S.M.) independently searched the PubMed & Ovid SP databases from January 1st, 1990, to March 1st, 2016. The full electronic search strategy were as described: OHS (Mesh) OR OHSS (tiab) AND isolated pleural effusion (majr) OR unilateral pleural effusion OR pleural effusion OR isolated hydrothorax (tiab) OR unilateral hydrothorax (tiab) OR isolated hydrothorax (tiab).

All reported cases of isolated hydrothorax after infertility treatment were included, but accompanying ascites were taken

Table 1. The available English literature reporting isolated hydrothorax without accompanying abdominal ascites as unusual manifestation of ovarian hyper stimulation syndrome (1990 – 2017)

Reference	Quality score	Age (years)	Treatment	Peak E2 level pg/mL	Retrieved oocyte (n)a	Starting day of symptoms after triggeringd	Admitted service or department	Hydrothorax side	Laboratory findings Hemoglobin / Hematocrit / White blood cell	Total drainage (ml)
Hsieh et al.(23)	6	30	GIFT	NA	NA	6	Emergency	Right	NA / NA / NA	6800
Levin et al.(20)	5	28-43 (5cases)	IUI to IVF	NA	NA	NA	NA	Right	NA / NA / NA	NA
Arikan et al.(19)	6	29	ICSI	1510	NA	14	OB/ GYN	Left dominance	NA/ 49.0% / 35.7x10 ³	4000
		30	IVF	694	22	8	OB/ GYN	NA	NA/ 47.0% / 15.2x10 ³	Conservative
Man et al.(18)	5	27a (24-29)	IUI	NA	NA	9	Pulmonary Disease	Right	NA / NA / NA	2000
		27a (24-29)	IUI	NA	NA	12	Pulmonary Disease	Left	NA / NA / NA	2000
		27a (24-29)	IVF	NA	NA	NA	Pulmonary Disease	Right	NA / NA / NA	1500
		27a (24-29)	IVF	NA	NA	NA	Pulmonary Disease	Right	NA / NA / NA	1200
Friedler et al.(17)	7	29b	ICSI	2536	27	14	OB/ GYN	Right	11.8 mg/dL / 35.0% / 13.9x10 ³	1700
		29b	ICSI	3000	22	7	OB/ GYN	Right	10.9 mg/dL / 32.0% / 9.8x10 ³	Conservative
		33	ICSI	3000	19	7	OB/ GYN	Right	15.4 mg/dL / 46.0% / 20x10 ³	4500
Wood et al.(16)	7	29	IVF	3479	18	9	OB/ GYN	Right	17.4 mg/dL / 51.4 % / NA	800
Jacob et al.(15)	7	31	IVF	3405 ^c	24	14	OB/ GYN	Left	17.0 mg/dL / NA / 28x10 ³	Conservative
Roden et al.(14)	7	34	IVF	3214	NA	7	Pulmonary Disease	Right	NA / 46.0% / 12.7x10 ³	1000
		33	IVF	NA	NA	14	Pulmonary Disease	Right	NA / 48.0% / 13.6x10 ³	Conservative
		29	IVF	NA	NA	8	Pulmonary Disease	Right	NA / 50.0% / 18.6x10 ³	Conservative
Rabinerson et al.(14)	6	35	IVF	7340	13	16	OB/ GYN	Right	NA / NA / NA	6800
Tansuthiwong et al.(13)	7	38	IVF	3322	10	14	OB/ GYN	Right	NA/ 44.0% / NA	3800
Thomas et al.(11)	7	35	IVF	NA	NA	4	Emergency	Right	14.8 mg/dL / NA / 22.2x10 ³	10000
Aldawood and Felemban(12)	7	36	IVF	4358 ^c	25	5	OB/ GYN	Right	NA / 49.0% / 15x10 ³	7200

Table 1. Continued

Reference	Quality score	Age (years)	Treatment	Peak E2 level pg/mL	Retrieved oocyte (n) ^a	Starting day of symptoms after triggering ^d	Admitted service or department	Hydrothorax side	Laboratory findings Hemoglobin / Hematocrit / White blood cell	Total drainage (ml)
Murray and Rombauts ⁽¹⁰⁾	8	33	ICSI	9868	26	7	OB/ GYN	Right dominance	13.8 mg/dL / 40.0% / NA	Right: 7850, Left: 450
Qublan and Barakat ⁽⁹⁾	7	29	IVF	2700	NA	NA	OB/ GYN	Right	NA / 35.0% / 13.9x10 ³	1300
Tang et al. ⁽⁷⁾	6	35	IVF	2052	8	NA	Emergency	Right	13.6 mg/dL / 38.6% / 15.9x10 ³	Conservative
Khairy et al. ⁽²¹⁾	6	41	IVF	NA	9	NA	Emergency	Right	NA / 42.0% / 13x10 ³	6000
Ciepiela et al. ⁽⁸⁾	7	32	ICSI	NA	8	8	OB/ GYN	Right	NA / NA / NA	1600
Beji et al. ⁽⁶⁾	6	26	IVF	NA	NA	4	Emergency	Right dominance	11.7 mg/dL / 49.0% / 21x10 ³	5300 (Total right, left)
Yildizhan et al. ⁽²⁴⁾	6	24	IVF	4000	NA	NA	Emergency	Right	18.0 mg/dL / 52.0% / 29x10 ³	T/S, NA
Gupta et al. ⁽²⁵⁾	7	21	IUI to IVF	1272	10	16	OB/ GYN	Right dominance	NA / 34.0% / 11x10 ³	Conservative
George et al. ⁽²⁶⁾	8	24	IUI to IVF	4000	NA/freeze	4	OB/ GYN	Right	NA / 40.2% / NA	900
Mullin et al. ⁽²⁷⁾	7	25	IVF	3731	44	4	OB/ GYN	Right	NA / 44.0% / NA	2900
	41	41	IVF	2552	18	6	OB/ GYN	Right	NA / 48.4% / NA	4850
Junqueira et al. ⁽²⁸⁾	6	27	IVF	NA	NA	9	Thoracic surgery	Right dominance	NA / 40.5% / 23.2x10 ³	Right: 10700, Left: 6640
	30	30	IVF	NA	NA	12	Thoracic surgery	Right dominance	NA / 53.7% / 20.4x10 ³	Right: 22360, Left: 650
	33	33	IVF	NA	NA	8	Thoracic surgery	Right dominance	NA / 47.0% / 26.3x10 ³	Right: 11580, Left: 8100
Bass et al. ⁽²⁹⁾	6	29	IVF	NA	NA	15	Emergency	Right	16.3 mg/dL / 48.8% / 23.2x10 ³	T/S, NA
Current report	7	28	IUI	2100	NA	8	Emergency	Right	15.1 mg/dL / 45.9% / 16.4x10 ³	1500
	31	31	ICSI	2890	21/freeze	4	OB/ GYN	Right	14.9 mg/dL / 44.0% / 15.8x10 ³	1500

^aMean age; ^bsame patient with 2 cycles; ^cConverted from pmol/L; ^dCalculated according to ET day; NA: not applicable or not available; T/S: Thoracentesis

as exclusion criteria. From the two databases, a total of 132 articles were identified. After screening from the title, abstract and text, 50 were subsequently removed due to being duplicate ($n=34$), not including information on OHSS ($n=45$), and not being written in the English language ($n=3$). Of the remaining 50 articles assessed for eligibility, isolated hydrothorax was not confirmed in 18 reports and data were not obtained in eight studies (Figure 1). After exclusion, the remaining 24 articles⁽⁶⁻²⁹⁾ were scrutinized for the side of the isolated pleural effusion and for the patients' information (Figure 2). Finally, two authors (S.M. and A.T.) extracted the data from full-text articles that presented information about patients' clinical manifestation and cycle characteristic ($n=24$), as depicted in Table 1. Additionally, the quality assessment of the case reports was performed by using the tool suggested by Murat et al.⁽³⁰⁾ in 2018. This tool was established for the evaluation of the methodologic quality of case reports and case series. Four categoric domains, which were selection, ascertainment, causality, and reporting, were assessed through eight specific questions and binary responses were scored⁽³⁰⁾. An aggregate score was preferred for all included studies in order to standardize the assessment (Supplementary file).

Results of the literature review

A total of 41 patients with isolated hydrothorax as a single manifestation of OHSS have been reported in 24 English

articles in the literature including the current report. Of them, one patient was reported twice for two consecutive IVF cycles. The median (minimum-maximum) female age and BMI of these patients were 30 (range: 21-43) years and 24 (19.5-30.4) kg/m^2 , respectively. Thirty-six of them had reported women with primary infertility, and four had been under treatment for secondary infertility. With respect to infertility etiology, polycystic ovary syndrome was the most encountered reason ($n=9$). Other reasons were as follows: five couples had unexplained infertility, two couples had male factor, one had hyperprolactinemia, one had endometriosis, one had tubal factor, and the remaining reports had no data (Table 2). The median (minimum-maximum) duration of infertility was 4 (minimum: 2; maximum: 10) years.

According to the available data, 14 patients were stimulated with a long agonist and five patients with an antagonist protocol in an IVF cycle. In addition, six patients had been treated with superovulation in an IUI cycle in which rescue-IVF was subsequently planned in three of them due to hyperstimulation. Seven patients were treated with ICSI and the remaining 31 with IVF. The duration of ovarian stimulation was between 7 and 13 days. The median total FSH dose consumption during ovarian stimulation was 1550 (minimum: 675, maximum: 2925) IU. All cases in the literature, except patient 2 who we presented, were prescribed recombinant or urinary hCG for triggering final oocyte maturation. The estradiol level before triggering

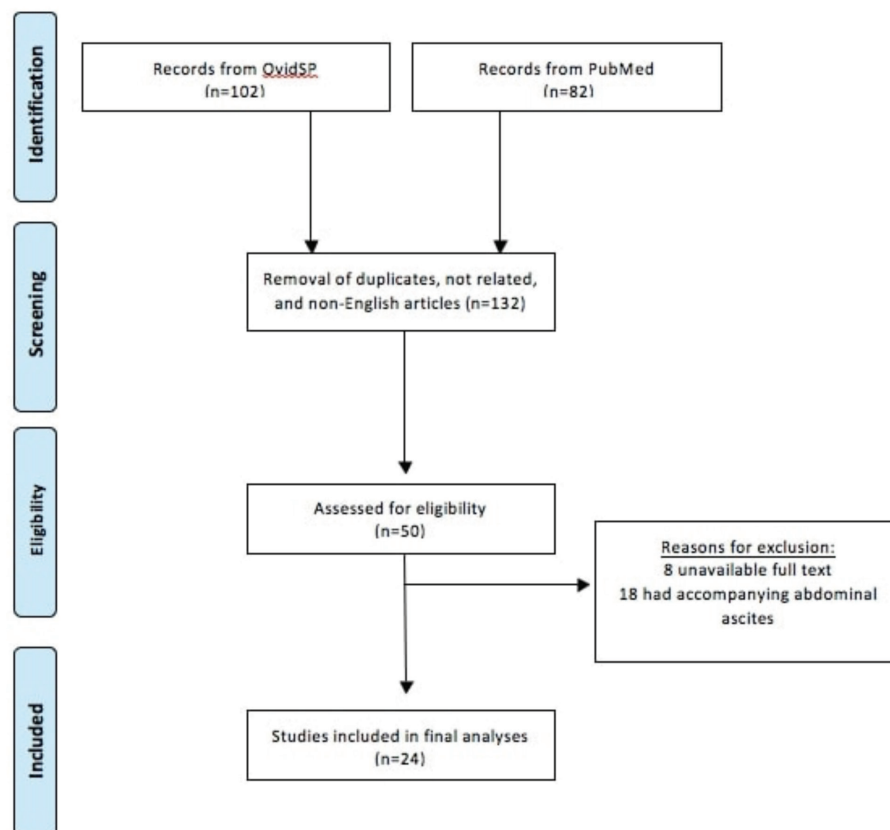


Figure 1. Flow diagram

was between 694 and 9868 pg/mL, as depicted in Table 1. In the available literature, the median number of retrieved oocytes was 19 (minimum: 8, maximum: 44), and the median number of transferred embryos was 3 (minimum: 1, maximum: 4) in the 17 cycles.

The main symptom of patients causing admittance to hospital was dyspnea accompanied by cough, orthopnea, right upper quadrant pain, and mild abdominal pain. More than half of the patients 56.1% (23/41) were examined departments other than Ob/Gyn. The presence of hydrothorax was diagnosed through chest X-ray/CT-scans, and the absence of abdominal fluid was diagnosed using ultrasonography in the cases included in this study. The minimum and maximum starting days of symptoms after ovulation triggering were 4 and 16, respectively. Available data suggest that range of Hb, HCT, and WBC count were 10.9-18.0 mg/dL, 32.0-52.0%, and 9.8×10^3 - 29×10^3 , respectively. At the time of hospital admission, 92.5% (37/40) of patients had isolated right side (n=31) or right side dominant (n=6) hydrothorax. While 82.9% (34/41) of patients needed to thoracentesis either with thorax tube or needle, the remaining 17.1% (7/41) were followed conservatively. Among those patients, 14.7% (5/34) required repetitive thoracentesis. The amount of total drainage fluid once or repetitively was between

800-22,320 mL (Table 1). One patient was complicated with pulmonary emboli, and one patient needed mechanical ventilation. At the time of follow-up, all patients had full-recovery.

In terms of ET outcomes, data were available for 19 patients. Of these, two patients (10.5%) had miscarriage, and 11 patients (57%) had ongoing pregnancy or live birth.

The median quality score of the included studies was 7 (interquartile range: 1), ranging from 6 to 8. Three (12%), 11 (44%), and 11 (44%) studies had eight, seven, and six points, respectively, which indicates moderate to good quality⁽³⁰⁾.

Discussion

In both cases of isolated pleural effusion, dyspnea and tachycardia were the main symptoms without abdominal discomfort at the time of hospital admittance. Additionally, patient 2 appears to be the first case complicated by isolated pleural effusion after triggering the final oocyte maturation with a GnRH agonist, but with 1500 IU hCG for luteal rescue. The discrepancy between the severity of symptoms and visualization of only a small amount of abdominal fluid was noteworthy, suggesting that pleural effusion should be considered in such patients even when there is no apparent risk factor for OHSS. Nevertheless, at the same visit, we were able to visualize pleural effusion using ultrasonography with the same probe and hence drainage of the fluid improved the symptoms dramatically.

Of the included case series in this systematic review, most had good quality scores. Although the definitive etiopathology is not clear, it has been postulated that diaphragmatic lymphatic and multiple macroscopic defects might potentially explain the fluid leakage from the abdominal cavity to the thorax⁽²¹⁾. However, these assumptions fail to clarify cases with no significant ascites in the abdomen, but with enough pleural fluid to induce severe dyspnea and tachypnea. In that context, Kirschner et al.⁽³¹⁾ proposed that porous diaphragm syndromes might be a possible explanation for this entity. The authors constructed the hypothesis based on the presence of diaphragmatic fenestrations creating peritoneopleural

Table 2. Type and etiology of the infertility of the included cases (n=41)

Etiology of infertility, n (%)	Type of infertility, n (%)
Oligo-anovulation (Polycystic ovary syndrome), n=9 (22.5%)	Primer infertility, n=36 (90%)
Unexplained infertility, n=5 (12.5%)	Secondary infertility, n=4 (10%)
Male factor, n=2 (22.2%)	
Hyperprolactinemia, n=1 (2.5%)	
Endometriosis, n=1 (2.5%)	
Tubal factor, n=1 (2.5%)	
Unspecified etiology, n=21 (52.5%)	

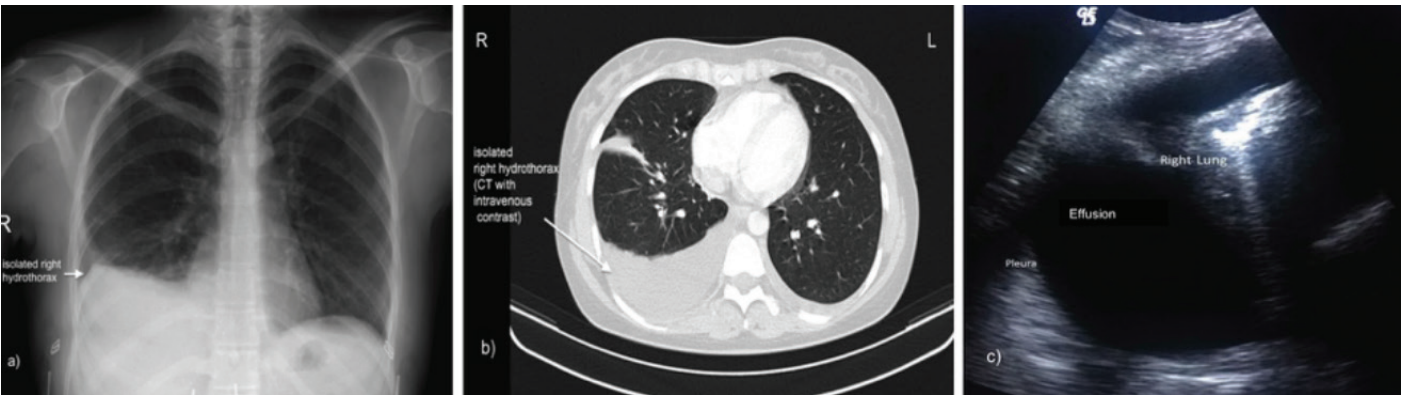


Figure 2. The imaging of isolated pleural effusion under X-ray (a), computed tomography (b), and (c) ultrasonography

communications. In this situation, the reduced hydrostatic pressure in the right upper quadrant of the abdominal cavity and intestinal sweeping would contribute to fluid accumulation in these areas. Afterwards, through the porous fenestrations located in the diaphragm, the piston-like motion of the liver capsule transfers fluid to the pleural cavity even when there is no large amount of abdominal ascites. In that context, four morphologic types of diaphragmatic pores were defined as visualized during video-thoracoscopic repair of defects in a patient with hepatic disorders-related hydrothorax⁽³²⁾: type-1, no macroscopic defect; type-2, blebs lying on diaphragm; type-3, broken defect (fenestrations) of the diaphragm; and type-4, multiple gaps of the diaphragm. The authors reported that the amount of the fluid in the pleura was not correlated with the type of diaphragmatic defect⁽³²⁾. Further invasive procedures were not offered to our patients presented herein because there was possibility of not achieving visualization of diaphragmatic pores, even with video-thoracoscopy.

Unilateral hydrothorax is an unexpected finding of OHSS, presenting with right side dominance. According to the available literature, pleural fluid becomes clinically apparent 5 to 16 days after oocyte retrieval and serum estradiol concentrations may be 684-9800 pg/mL, as reported with various cases (Table 1). After symptoms become significant, isolated pleural effusion might be encountered in these cases in spite of normal HCT and lack of significant abdominal ascites that might be suggestive of only mild OHSS for the physician. Therefore, physicians should consider pleural effusion in patients who mainly admit with symptoms of dyspnea within the first few weeks after triggering when there is no amount of abdominal fluid to account for the severity of symptoms. Most cases in the literature were treated with a single thoracentesis procedure. The success rate in infertility treatment, ongoing pregnancy or live birth does not appear to be negatively affected.

In the differential diagnosis of isolated hydrothorax, pulmonary embolism should be ruled out. Making a prompt diagnosis is important because the treatment and prognosis for these conditions are completely different. For physicians working in reproductive medicine, it is worth emphasizing that performing thoracic ultrasound immediately after abdominal ultrasound with the patient tilted on her sides shortens the diagnosis time. However, when ultrasonography fails to demonstrate an abdominal or pleural effusion in patients with dyspnea and tachypnea, spiral computerized tomography should be performed for a definitive diagnosis.

OHS is a life-threatening complication, as such an individualized approach including an assessment of risk factors, application of appropriate ovarian stimulation protocols, modification of regimens according to patient characteristics, and administration of an alternative to standard-dose hCG or GnRH-agonist for final oocyte maturation may decrease the risk of OHSS in patients receiving infertility treatment⁽³³⁾.

Study Limitations

The main limitation of the present study is not visualizing diaphragmatic pores. However, as we mentioned above, other than video-thoracoscopy, a clinically feasible non-invasive method is not available to demonstrate diaphragmatic pores.

Conclusion

Isolated right-sided hydrothorax is a rare, unexpected, and unpredictable event, which may appear even in the setting of mild OHSS. Physicians should be aware of the possibility, diagnosis, and management of this finding. Although the hypothesis based on diaphragm pores might be a potential explanation for pathogenesis of pleural effusion, further research is required for additional risk factors such as inflammation mediators, vasoactive substances, and serosal surface defects that may accompany a porous diaphragm. Advances in imaging technology may allow physicians to demonstrate these potential pores in further studies.

Ethics

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M., A.T., V.T., G.B., Concept: S.M., A.T., V.T., G.B., Design: S.M., A.T., V.T., G.B., Data Collection or Processing: S.M., A.T., V.T., G.B., Analysis or Interpretation: S.M., A.T., V.T., G.B., Literature Search: S.M., A.T., V.T., G.B., Writing: G.S., A.E.K., E.K., E.Ç. S.M., A.T., V.T., G.B.

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References

1. B. Lunenfeld, [Ovarian stimulation: a new approach based on recent physiological and clinical data. Future perspectives], *Contracept Fertil Sex* 1993;4:1-7.
2. Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv* 1989;44:430-40.
3. Whelan JG 3rd, Vlahos NF. The ovarian hyperstimulation syndrome. *Fertil Steril*. 2000;73:883-96.
4. M. Practice Committee of the American Society for Reproductive. Ovarian hyperstimulation syndrome. *Fertil Steril* 2003;80:1309-14.
5. Abramov Y, Elchalal U, Schenker JG. Pulmonary manifestations of severe ovarian hyperstimulation syndrome: a multicenter study. *Fertil Steril* 1999;71:645-51.
6. Beji O, Brahmi N, Thabet H, Mokline A, Abidi N, Blél Y, et al. Compressive pleural effusion after ovarian hyperstimulation syndrome--a case report and review. *Fertil Steril* 2008;89:1826.
7. Tang HH, Tsai YC, Kang CY, Chung MT, Loo TC, Huang KF. Atypical ovarian hyperstimulation syndrome with isolated pleural effusion but without ascites or hemoconcentration. *Taiwan J Obstet Gynecol* 2007;46:180-2.
8. Ciepiela P, Brelik P, Baczowski T, Kurzawa R. Isolated severe hydrothorax with respiratory distress as a main manifestation

- of ovarian hyperstimulation syndrome preceded by respiratory tract infection caused by *Haemophilus influenzae*. *Ginekol Pol* 2007;78:570-3.
9. Qublan HS, Barakat M. Respiratory distress caused by a unilateral hydrothorax as only manifestation of ovarian hyperstimulation syndrome. *J Obstet Gynaecol* 2006;26:585-6.
 10. Murray A, Rombauts L. Unilateral pleural effusion as the main presentation of "early onset" severe ovarian hyperstimulation syndrome. *Fertil Steril* 2004;81:1127-9.
 11. Thomas F, Kalfon P, Niculescu M. Acute respiratory failure, lactic acidosis, and shock associated with a compressive isolated right pleural effusion following ovarian hyperstimulation syndrome. *Am J Med* 2003;114:165-6.
 12. Aldawood AS, Felemban AA. Isolated unilateral pleural effusion as the only manifestation of the ovarian hyperstimulation syndrome. *Saudi Med J* 2003;24:1397-9.
 13. Tansutthiwong AA, Srisombut C, Rojanasakul A. Unilateral massive pleural effusion as the only principal manifestation of severe ovarian hyperstimulation syndrome. *J Assist Reprod Genet* 2000;17:454-6.
 14. Roden S, Juvin K, Homasson JP, Israel-Biet D. An uncommon etiology of isolated pleural effusion. The ovarian hyperstimulation syndrome. *Chest* 2000;118:256-8.
 15. Jacob S, Byrne P, Harrison RF. Symptomatic cystic swelling at the root of the neck with left sided pleural effusion as a presentation of ovarian hyperstimulation syndrome. *Br J Obstet Gynaecol* 1999;106:986-8.
 16. Wood N, Edozien L, Lieberman B. Symptomatic unilateral pleural effusion as a presentation of ovarian hyperstimulation syndrome. *Hum Reprod* 1998;13:571-2.
 17. Friedler S, Rachstein A, Bukovsky I, Ron-El R, Raziel R. Unilateral hydrothorax as a sole and recurrent manifestation of ovarian hyperstimulation syndrome following in-vitro fertilization. *Hum Reprod* 1998;13:859-61.
 18. Man A, Schwarz Y, Greif J. Pleural effusion as a presenting symptom of ovarian hyperstimulation syndrome. *Eur Respir J* 1997;10:2425-6.
 19. Arikan G, Giuliani A, Gucer F, Auner H, Urdl W. Rare manifestations of the ovarian hyperstimulation syndrome: a report of two cases. *Clin Exp Obstet Gynecol* 1997;24:154-6.
 20. Levin MF, Kaplan BR, Hutton LC. Thoracic manifestations of ovarian hyperstimulation syndrome. *Can Assoc Radiol J* 1995;46:23-6.
 21. Khairy M, El-Toukhy T, Emovon E, Khalaf Y. Hydrothorax as the sole manifestation of ovarian hyperstimulation syndrome: unusual case and literature review. *Reprod Biomed Online* 2007;14:715-7.
 22. Rabinerson D, Shalev J, Royburt M, Ben-Rafael Z, Dekel A. Severe unilateral hydrothorax as the only manifestation of the ovarian hyperstimulation syndrome. *Gynecol Obstet Invest* 2000;49:140-2.
 23. Hsieh MJ, Tsao TC, Cheng PJ. Ovarian hyperstimulation syndrome with minimal ascites and massive pleural effusion: report of a case. *J Formos Med Assoc* 1994;93:882-4.
 24. Yildizhan R, Adali E, Kolusari A, Kurdoglu M, Ozgokce C, Adali F. Ovarian Hyperstimulation Syndrome with pleural effusion: a case report. *Cases J* 2008;1:323.
 25. Gupta S, Sathya B, Padhy N, Kundavi S, Thomas BE, Varma TR. Isolated bilateral pleural effusion as the sole manifestation of late onset ovarian hyperstimulation syndrome. *J Hum Reprod Sci* 2009;2:83-6.
 26. George K, Aleyamma T, Kamath M, Chandy A, Mangalaraj AM, Muthukumar K, Londhe V. Symptomatic unilateral pleural effusion: A rare presentation of ovarian hyperstimulation syndrome. *J Hum Reprod Sci* 2010;3:49-51.
 27. Mullin CM, Fino ME, Reh A, Grifo JA, Licciardi F. Symptomatic isolated pleural effusion as an atypical presentation of ovarian hyperstimulation syndrome. *Case Rep Obstet Gynecol* 2011;2011:967849.
 28. Junqueira JJ, Bammann RH, Terra RM, Castro AC, Ishy A, Fernandez A. "Pleural effusion following ovarian hyperstimulation. *J Bras Pneumol* 2012;38:400-3.
 29. Bass LC, Dillard TA, Forseen CJ, Davis WB. An unusual cause of massive pleural effusion. *Am J Med Sci* 2012;344:505-7.
 30. Murat MH, Sultan S, Haffar S, Bazerbachi F. Methodological quality and synthesis of case series and case reports. *BMJ evidence-based medicine* 2018;23:60-63.
 31. Kirschner PA. Porous diaphragm syndromes. *Chest Surg Clin N Am* 1998;8:449-72.
 32. Huang PM, Chang YL, Yang CY, Lee YC. The morphology of diaphragmatic defects in hepatic hydrothorax: thoracoscopic finding. *J Thorac Cardiovasc Surg* 2005;130:141-5.
 33. Practice Committee of the American Society for Reproductive Medicine. Electronic address and M. Practice Committee of the American Society for Reproductive. Prevention



Bilateral iliac artery aneurysm: A rare cause of postpartum recurrent hemorrhage

Bilateral iliak arter anevrizması: Postpartum rekürren kanamanın nadir bir nedeni

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Abstract

Postpartum hemorrhage (PPH) is a critical health problem that may result in maternal death. In cases of impaired maternal hemodynamics, several surgical therapies such as hypogastric artery ligation or postpartum hysterectomy may be employed to control the bleeding. A 30-year-old multiparous patient who had given birth via spontaneous vaginal delivery had undergone hysterectomy and then hypogastric artery ligation due to postpartum hemorrhage. The patient was referred to our clinic due to uncontrolled bleeding and she experienced recurrent episodes of massive hemorrhage during her follow-up in our clinic. Pelvic angiography performed by interventional radiologists to detect the bleeding focus revealed arteriovenous fistula and aneurysm in the right internal iliac artery and incomplete ligation of the left internal iliac artery. The bleeding was controlled by selective embolization through coiling of the fistula in the right internal iliac artery and branches of the left uterine artery. PPH is still an important cause of maternal mortality and vascular structural anomalies must be borne in mind in cases with delayed onset.

Keywords: Iliac artery aneurysm, postpartum hemorrhage, maternal mortality

Öz

Postpartum kanamalar, maternal ölüme neden olabilecek kritik bir sağlık sorunudur. Bozulmuş maternal hemodinamik durumlarda, kanamayı kontrol etmek için hipogastrik arter ligasyonu veya postpartum histerektomi gibi çeşitli cerrahi tedaviler kullanılabilir. Otuz yaşındaki bir multipar spontan vajinal yolla doğum yapmış olan hasta histerektomi ve postpartum kanama nedeniyle hipogastrik arter ligasyonu geçirmişti. Hasta kontrolsüz kanama nedeniyle kliniğimize sevk edildi ve kliniğimizde takiplerinde tekrarlayan masif kanama atakları geçirdi. Girişimsel radyologlar tarafından kanama odağını saptamak için yapılan pelvik anjiyografide kanama odağını tespit etmek için sağ internal iliak arterde arteriyovenöz fistül ve anevrizma ve sol internal iliak arterin tam ligate olmadığı saptandı. Hastanın sağ iliak arter ve sol uterin arterine selektif embolizasyon yapılarak kanama kontrol altına alındı. Postpartum kanamalar her türlü girişimsel işleme rağmen hala anne ölümlerinin önemli bir nedenidir.

Anahtar Kelimeler: İliak arter anevrizması, doğum sonrası kanama, anne ölümü

Introduction

Postpartum hemorrhage (PPH) is a serious health problem that may result in maternal death by causing an impairment of maternal hemodynamics^(1,2). According to the world health organization, approximately 150.000 women die annually of PPH^(3,4). Postpartum hysterectomy should be the last resort for surgeons due to its morbidity and effects on the patient's fertility. Alternatively, hypogastric artery ligation is a fertility-preserving treatment that is associated with a low success rate

and high levels of complication if not performed by experienced surgeons^(1,5). Pelvic arterial embolization is an alternative treatment for both hysterectomy and hypogastric artery ligation due to its effects on morbidity and patients' fertility. It continues to be a relevant procedure because it is a minimally invasive intervention^(6,7). The current report presents a patient with PPH who underwent embolization, in whom bleeding could not be controlled despite repeated surgical interventions, and in whom a bleeding focus that would explain this clinical condition could not be detected intraoperatively.

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Case report

The 30-year-old, gravidity 3, parity 2 patient had no medical problems during her pregnancy follow-up visits, such as gestational diabetes, preeclampsia, intrauterine growth retardation, and premature delivery. The patient had given birth to a baby weighing 3500 grams via spontaneous vaginal delivery at 39 weeks of gestation and she experienced heavy bleeding at day 6 after birth. The patient did not respond to medical therapies and conventional methods and underwent hysterectomy due to impaired hemodynamics caused by uncontrolled bleeding. The patient was discharged on postoperative day 3 with full recovery. The patient experienced active vaginal bleeding on postoperative day 10 and underwent cuff repair through the vaginal route after exploration. Possible bleeding foci that were inspected during exploration were sutured and then the patient was discharged with full recovery. However, the patient experienced recurrent abundant bleeding on postoperative day 20 for which she was hospitalized and underwent bilateral hypogastric artery ligation. The patient experienced another episode of abundant bleeding on day 7 after the hypogastric artery ligation and she was then referred to our clinic. The patient's hemodynamics was stable on initial examination. Laboratory parameters were normal. Abdominal ultrasonography revealed normal ovaries and no fluid in the abdominal cavity. A vaginal examination revealed no bleeding. The patient experienced heavy bleeding on day 3 after admission to our clinic and she also had impairment in her hemodynamics. Her hemoglobin was 4.8 g/dL and the patient was administered 4 units of erythrocyte suspension. The patient underwent emergency surgery, but no active bleeding focus was detected. A consultation with a radiologist was performed because no bleeding focus was detected intraoperatively, and the patient underwent computed tomography (CT) with contrast enhancement. The CT scans showed findings suggestive of aneurysmal filling in the pelvic area (Figure 1). The patient had ongoing bleeding and she underwent angiography in the interventional radiology clinic. Initial angiography revealed an arteriovenous fistula and aneurysm filling from the right internal iliac artery (Figure 2). The artery fistula was closed with a coil. The right internal iliac artery was totally obstructed by the coil, causing intermittent bleeding after partial intraoperative ligation. On the second day after the intervention, the patient still had bleeding, although the amount had decreased. The patient was re-evaluated by interventional radiologists; the branches of the left uterine artery were angiographically obstructed with microparticles and the partially-ligated left internal iliac artery was totally closed by coils (Figure 3). The patient received 15 units of erythrocyte suspension until the completion of the second procedure. The patient became hemodynamically stable following the procedure and had no recurrent bleeding during the follow-up period; she was discharged with full recovery on postoperative day 15.

Informed consent was obtained from the patient.

Discussion

PPH is defined as 500 mL blood loss after normal delivery and 1000 mL blood loss after cesarean delivery. It is a significant health problem causing an impairment in maternal hemodynamics⁽¹⁾. If there is no response to conservative therapies such as uterine massage and Bakri balloon placement, compression sutures, hypogastric artery ligation, hysterectomy, and pelvic arterial embolization can be employed^(8,9).

The pelvic organs are supplied by the internal iliac artery and ligation of this artery reduces bleeding by decreasing uterine arterial pressure^(10,11). Pelvic arterial embolization was used for the first time in 1979 for obstetric bleeding⁽⁷⁾. Selective arterial embolization offers a success rate ranging from 81% to 96% in different sources⁽¹²⁾. Pelvic arterial embolization is considered a safe procedure because it is a fertility-preserving minimally invasive procedure and, in experienced hands, it is also associated with reduced morbidity⁽¹⁾. However, there are also publications reporting necrosis of the uterus, sciatic nerve, and bladder if not performed under the appropriate conditions^(13,14). Although uterine atony is the most common cause of PPH, vascular pathologies such as arteriovenous malformation (AVM), hemangioma, and vascular tumors must be borne in mind for patients with massive hemorrhage⁽¹⁵⁾. Variations in the pelvic vascular anatomy in particular may cause serious bleeding in the postpartum period. Selective arterial embolization performed after angiography is a safe and effective option in uncontrolled uterine bleeding⁽²⁾. There are reported cases in the literature in which selective pelvic embolization was performed due to PPH of different etiologies. Yu et al.⁽¹⁵⁾ reported the efficacy of selective arterial embolization in a patient with vaginal hemangioma who remained undetected in antenatal follow-up and who experienced massive bleeding after vaginal delivery with episiotomy. The development of collateral vessels between the inferior mesenteric artery (IMA) and uterus is not

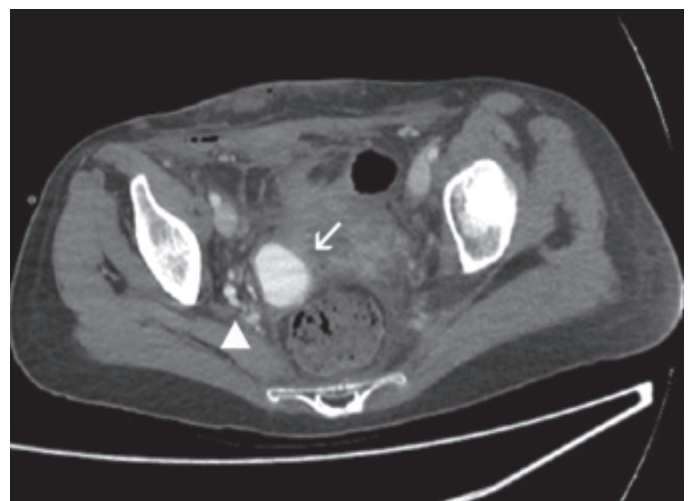


Figure 1. Computed tomography images acquired in the arterial phase show enlarged vascular structured connected to the arteriovenous fistula (arrow head) and aneurysm sac (white arrow)



Figure 2. Right internal iliac artery angiography shows aneurysm sac



Figure 3. Embolization of the artery supplying the aneurysm with detachable coil (white arrow) and embolization of the internal iliac artery with pushable coil (arrow head)

normally observed. However, it has been reported in patients with uterine fibroids and adenomyosis and those with a history of pelvic surgery, uterine artery ligation or embolization⁽¹⁶⁾. Shin et al.⁽²⁾ detected widespread collateral vessels between the IMA and uterine and ovarian arteries during angiography in a patient with massive PPH who had no history of uterine fibroids, pelvic surgery or embolization. They reported that the hemorrhage was controlled by the selective embolization of the distal branches of the IMA. Kim et al.⁽¹⁷⁾ reported a patient with massive PPH due to damage of the collateral network between the superior rectal branch of the IMA and the vaginal artery following vaginal delivery and they controlled the hemorrhage with embolization.

Vascular pathologies must be considered in patients with PPH with a delayed onset. In a series of 33 patients with PPH reported by Crespo et al.⁽¹⁾, six patients experienced hemorrhage due to laceration of the uterine pseudoaneurysm and three patients had laceration of the branches of the vaginal, cervicovaginal and epigastric artery, and they reported the efficacy of selective embolization. In a study by Ganguli et al.⁽¹⁸⁾ reporting on 66 patients with PPH, a 95% success rate was reported for uterine artery embolization and the rate of complication was reported as 4.5%.

The most common causes of pseudoaneurysms occurring in the uterine artery are curettage, vaginal delivery, and genital infections⁽¹⁹⁾. In the study by Kiyokawa et al.⁽²⁰⁾ that included six patients with cesarean scar pregnancy and uterine AVM or pseudoaneurysm, five patients underwent uterine artery embolization. Only one patient who underwent embolization resulted in hysterectomy.

The present case had intermittent hemorrhage but it was severe enough to impair hemodynamics after spontaneous vaginal delivery. A total of three operations were performed for vaginal cuff repair, hysterectomy, and finally hypogastric artery ligation because the bleeding focus could not be detected despite ongoing hemorrhage. The presence of recurrent hemorrhage and failure to detect the bleeding focus despite all plausible surgical interventions led us to consider the patient for vascular pathologies. The failure to control bleeding, after the detection of and intervention to the vascular pathology in the right side, raised the possibility of another pathology in the left side. We therefore emphasize the importance of bearing vascular pathologies in mind in patients with uncontrolled hemorrhage despite optimal surgical interventions. Consistent with the literature, we observed the successful embolization of the aneurysm accompanying the arteriovenous fistula and arterial branches causing bleeding due to incomplete suturing.

In conclusion, emergency intervention in PPH can be life-saving. Selective arterial embolization, which is less invasive and is associated with low complication rates, appears to be an appropriate option for patients unresponsive to conservative therapy.

Ethics

Informed Consent: Informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.P.T.Y., Y.K., S.E., Concept: E.P.T.Y., Y.E.T., Design: E.P.T.Y., Y.E.T., Data Collection or Processing: E.P.T.Y., Y.E.T., Analysis or Interpretation: E.P.T.Y., Y.K., S.E., Literature Search: Y.E.T., Y.K., S.E., Writing: E.P.T.Y., Y.E.T.

Conflict of Interest: The authors declare no conflict of interest.

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References

1. Aguilar-Crespo A, Morales-Roselló J, Sánchez-Ajenjo C, Valle-Tejero A, García-Marcos R, Perales-Marín A. Postpartum hemorrhage with pelvic arterial embolization, study of 33 cases. *J Matern Fetal Neonatal Med*. 2017;16:1-6.
2. Shin SM, Yi KW, Chung HH. Postpartum Uterine Bleeding from the Inferior Mesenteric Artery: Case Report and Review of the Literature. *Gynecol Obstet Invest* 2015;80:60-3.
3. Ojengbade OA, Morhason-Bello IO, Galadanci H, Meyer C, Nsima D, Camlin C, et al. Assessing the role of the non-pneumatic anti-shock garment in reducing mortality from postpartum hemorrhage in Nigeria. *Gynecol Obstet Invest* 2011;71:66-72.
4. Thakur M, Adekola HO, Asaad R, Gonik B. Secondary Postpartum Hemorrhage due to Spontaneous Uterine Artery Rupture after Normal Vaginal Delivery Managed by Selective Arterial Embolization. *AJP Rep* 2016;6:442-4.
5. Negura A. Indications for bilateral ligation of the uterine arteries and the hypogastric arteries in refractory obstetrical hemorrhages. *Rev Fr Gynecol Obstet* 1991;86:495-7.
6. Evans S, McShane P. The efficacy of internal iliac artery ligation in obstetric hemorrhage. *Surg Gynecol Obstet* 1985;160:250-3.
7. Brown BJ, Heaston DK, Poulson AM, Gabert HA, Mineau DE, Miller FJ Jr. Uncontrollable postpartum bleeding: a new approach to hemostasis through angiographic arterial embolization. *Obstet Gynecol* 1979;54:361-5.
8. Holub Z, Pertl J, Voráček J. The surgical technic of ligation of the hypogastric artery. *Cesk Gynecol* 1988;53:763-5.
9. Ferguson JE, Bourgeois FJ, Underwood PB. B-Lynch suture for postpartum hemorrhage. *Obstet Gynecol* 2000;95:1020-2.
10. Torreblanca Neve E, Merchan Escalante G, Walter Tordecillas MA, Acosta Alfaro MA. Ligation of the hypogastric arteries. Analysis of 4000 cases. *Ginecol Obstet Mex* 1993;61:242-6.
11. Fătu C, Frâncu D, Pușor M, Fătu CI. Changes in the arterial pressure after ligation of the hypogastric artery. *Rev Med Chir Soc Med Nat Iasi* 1996;100:149-50.
12. Deux JF, Bazot M, Le Blanche AF, Tassart M, Khalil A, Berkane N, et al. Is selective embolization of uterine arteries a safe alternative to hysterectomy in patients with postpartum hemorrhage? *AJR Am J Roentgenol* 2001;177:145-9.
13. Al-Thunyan A, Al-Meshal O, Al-Hussainan H, Al-Qahtani MH, El-Sayed AA, Al-Qattan MM. Buttock necrosis and paraplegia after bilateral internal iliac artery embolization for postpartum hemorrhage. *Obstet Gynecol* 2012;120:468-70.
14. Palacios-Jaraquemada JM. Buttock necrosis and paraplegia after bilateral internal iliac artery embolization for postpartum hemorrhage. *Obstet Gynecol* 2012;120:1210.
15. Yu BR, Lee GE, Cho DH, Jeong YJ, Lee JH. Genital tract cavernous hemangioma as a rare cause of postpartum hemorrhage. *Obstet Gynecol Sci* 2017;60:473-76.
16. Dixon S, Tapping CR, Chuah PS, Bratby M, Uberoi R, Anthony S. Successful fibroid embolization of pelvic and inferior mesenteric artery collaterals after previous uterine artery embolization. *Acta Radiol* 2012;53:292-5.
17. Kim CH, Jeon GS, Lee SJ, Kang SH. Embolization of the inferior mesenteric artery for post-partum hemorrhage with a vaginal laceration: A case with unusual collateral supply. *J Obstet Gynaecol Res* 2016;42:1878-80.
18. Ganguli S, Stecker MS, Pyne D, Baum RA, Fan CM. Uterine artery embolization in the treatment of postpartum uterine hemorrhage. *J Vasc Interv Radiol* 2011;22:169-76.
19. Chou MM, Hwang JI, Tseng JJ, Huang YF, Ho ES. Cesarean scar pregnancy: quantitative assessment of uterine neovascularization with 3-dimensional color power Doppler imaging and successful treatment with uterine artery embolization. *Am J Obstet Gynecol* 2004;190:866-8.
20. Kiyokawa S, Chiyoda T, Ueno K, Saotome K, Kim SH, Nakada S. Development of pseudoaneurysm in cesarean section scar pregnancy: a case report and literature review. *J Med Ultrason* 2018;45:357-62.



Different pathogenic mechanisms of early-onset preeclampsia, late-onset preeclampsia, and hemolysis, elevated liver enzymes, low platelet syndrome

Erken başlangıçlı preeklampsi, geç başlangıçlı preeklampsi ve hemoliz, yüksek karaciğer enzimleri ve düşük trombosit sayım sendromunun farklı patojenik mekanizmaları

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Keywords: Preeclampsia, cbc, neutrophils, wbc, prediction, hellp syndrome

Anahtar Kelimeler: Preeklampsi, cbc, nötrofiller, wbc, tahmin, hellp sendromu

To the Editor,

We read with interest the article titled “First trimester complete blood cell (CBC) indices in early and late-onset preeclampsia” by Örgül et al.⁽¹⁾ published in the Turkish Journal of Obstetrics and Gynecology in June 2019.

We share the same enthusiasm in the use of a cheap and simple CBC count as an early predictor of poor obstetric outcomes. CBC is the first laboratory investigation performed in every pregnant woman and its value is not limited to diagnosing current medical conditions, it can also be used as a predictor of future events.

In their article, Örgül et al.⁽¹⁾ show that white blood cell (WBCs) and neutrophil counts are significantly elevated in the first trimester of pregnancies with early and late-onset preeclampsia, compared with controls. They also give an excellent clinical tool in finding a specific cut-off value, using receiver operating characteristic curve analysis; specifically, $9.55 \times 10^3/\mu\text{L}$ for WBCs and $6.45 \times 10^3/\mu\text{L}$ for neutrophils.

Unfortunately, in their statistical analysis, when comparing the three groups, they did not perform a post-hoc analysis to compare one group with each other.

In our recent studies, we analyzed CBC indices in pregnancy affected by hemolysis, elevated liver enzymes, low platelet

(HELLP) syndrome vs. controls^(2,3). We found no differences in the first trimester in terms of neutrophil count, and we did not analyze the total WBC count.⁽²⁾ We obtained informed consent from the patients included in our study.

We think that the difference between our study and the study of Örgül et al.⁽¹⁾ is due to the different pathogenesis of HELLP syndrome and preeclampsia. We think that preeclampsia is caused by an early placentation defect, whereas HELLP syndrome is determined by a maternal immunologic “storm” of circulating inflammatory molecules triggered in the third trimester.

Örgül et al.⁽¹⁾ mentioned that early-onset preeclampsia was caused by early placentation defects, and late-onset preeclampsia was more related to maternal characteristics: in this regards, as mentioned earlier, a post-hoc analysis would have been very useful.

Ethics

Informed Consent: We obtained informed consent from the patients included in our study.

Peer-review: Externally peer-reviewed

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References

1. Örgül G, Aydın Haklı D, Özten G, Fadiloğlu E, Tanacan A, Beksac MS. First trimester complete blood cell indices in early and late onset preeclampsia. Turk J Obstet Gynecol 2019;16:112-7.
2. Sisti G, Faraci A, Silva J, Upadhyay R. Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio and Complete Blood Count Components in the First Trimester Do Not Predict HELLP Syndrome. Medicina (Kaunas) 2019;28:55.
3. Sisti G, Faraci A, Silva J, Upadhyay R. Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio, and Routine Complete Blood Count Components in HELLP Syndrome: A Matched Case Control Study. Medicina (Kaunas) 2019;8:55.