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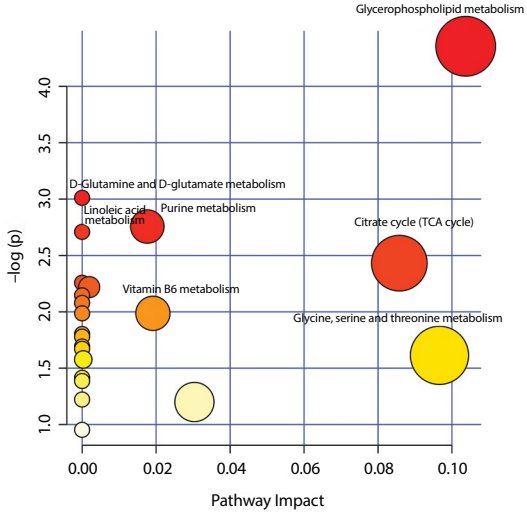

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zawiadamia

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1. Zebranie Zarządu Głównego Polskiego Towarzystwa Ginekologów i Położników odbędzie się w dniu **28 listopada 2019 (czwartek) o godz. 11.00 (I TERMIN) lub o godzinie 11.15 (II TERMIN) i potrwa około 50 minut.**
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   w Restauracji OKWineBar Między Mostami
   ul. Księcia Witolda 1, 50-202 Wrocław
   Planowane Zakończenie Nadzwyczajnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników jest na godz. 14.00

Porządek zebrania ZG PTGiP dnia 28 listopada 2019 r. godzina 11.00 (I TERMIN) oraz godzina 11.15 (II TERMIN):

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2. Stwierdzenie ważności zwołanego zebrania Zarządu Głównego Polskiego Towarzystwa Ginekologów i Położników oraz zdolności uczestników zebrania do podejmowania uchwał jako Zarząd Główny Polskiego Towarzystwa Ginekologów i Położników.
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7. Pytania, wolne wnioski i komunikaty.
8. Zamknięcie zebrania.

Prof. dr hab. n.med. Mariusz Zimmer
Prezes Zarządu Głównego Polskiego Towarzystwa Ginekologów i Położników

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2. Wybór Przewodniczącego Nadzwyczajnego Walnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników.

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4. Wybór protokolanta Nadzwyczajnego Walnego Zgromadzenia Polskiego Towarzystwa Ginekologów i Położników.
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Obesity as a risk factor of in-hospital outcomes in patients with endometrial cancer treated with traditional surgical mode

Slawomir M. Januszek, Edyta Barnas, Joanna Skret-Magierlo, Jakub Sokolowski, Pawel Sczerba, Rafal Januszek, Wojciech Domka, Tomasz Lozinski, Marek Kluza, Tomasz Kluz

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ABSTRACT

Objectives: Abdominal obesity is a risk factor for endometrial cancer. The negative impact of individual parameters of obesity on the procedural effects of endometrial cancer surgical treatment has been suggested. The aim of the current study was to estimate the relationship of particular parameters of obesity and in-hospital outcomes in patients treated surgically due to endometrial cancer.

Material and methods: The study included 70 women treated surgically for endometrial cancer. Pre-operatively, mass, body mass index (BMI), waist circumference, waist-hip ratio and selected anatomical indices were measured. The duration of surgery, hospitalisation, and the loss of haemoglobin served as parameters of in-hospital procedure success. Also, procedural-related complications were estimated.

Results: There were 37 (52.8%) obese females in the current study. They were obese patients presenting more advanced clinical stages of endometrial cancer before operation. The duration of operation (94.9 ± 21.6 min. vs. 76.1 ± 13.5 min., $p < 0.0001$), hospitalisation (12.4 ± 3.4 days vs. 10 ± 2.3 days, $p = 0.0009$) and haemoglobin loss (2.5 ± 0.9 g/dL vs. 1.9 ± 0.8 g/dL, $p = 0.004$) were significantly greater in obese patients. Multivariate analysis, among the independent predictors of the duration of operation, has confirmed the correlation between BMI, waist circumference and weight and the duration of hospitalisation. Waist and hip circumference and BMI coupled with external conjugate dimension and intertrochanteric distance have been linked with haemoglobin loss. The strongest correlation for the duration of operation, hospitalisation and haemoglobin loss was noticed for waist circumference ($r = 0.7$, $r = 0.57$ and $r = 0.59$).

Conclusions: Waist circumference and BMI are strong predictors of in-hospital outcomes among patients with endometrial cancer treated via traditional surgical operation.

Key words: abdominal obesity; BMI; cancer of endometrium; operative time; perioperative outcomes

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INTRODUCTION

Endometrial cancer is the most common cancer of the female reproductive organs. It is ranked fourth in terms of the incidence of malignant tumours. In the years 1963–2008, a significant increase in the incidence of malignant neoplasms of endometrial cancer was observed (1,023 and 4,820 cases, respectively, in 1963 and 2008) [1]. There was also an increase in the percentage of malignant tumours in this organ among all malignancies in women from 5.3% to 7.4%. The standardised incidence rate in 1963 and 2008 was 5.9 and 14.4 per 100,000 women, respectively. According to Bidziński, it is likely that the growing trend in the incidence of malignant neoplasms of the uterus will persist [1]. On the other hand, mortality from endometrial malignancies in the last decades has significantly decreased. Standard mortality rates in 1963 and 2003 were 9.1 and 2.2, respectively [1]. The occurrence of endometrial cancer is associated with economic and cultural fluctuations in developed countries that bring about lifestyle changes, which, among others, involve obesity, diabetes, hypertension, metabolic syndrome, multiparity, treatment with exogenous oestrogens unbalanced with gestagens or treatment with tamoxifen [2, 3]. The prolonged life expectancy in women in Poland and other European countries also contributes to an increase in the incidence of endometrial cancer. Most cases occur

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in postmenopausal women (75%) [1]. Obesity in women aged 50–59 is the most important risk factor for the development of endometrial cancer [2]. It was estimated that an increase in body mass by 10 kg in women in this age range results in a threefold higher risk of disease, an increase in mass above 20 kg increases the likelihood of the disease even tenfold [4]. In a meta-analysis of 19 reviews and prospective studies, Renehan et al. [5] also found that each increase in BMI by 5 kg/m^2 significantly increased a woman's risk of developing endometrial cancer (RR 1.59, 95% CI 1.50–1.68). Not only does obesity have impact on cancer-related death, but through its association with co-morbidities (diabetes mellitus and hypertension), it significantly effects all-cause mortality. The Gynaecologic Oncology Group, in a review among 380 patients with endometrial cancer, found that morbid obesity was associated with higher mortality (HR 2.77, 95% CI 1.21–6.36) from causes other than endometrial cancer or disease recurrence [6]. Endometrial cancer is not a homogeneous tumour, both in terms of histological structure or clinical exponents. Classically, according to Bokhman, two types of histopathology are distinguished: Type 1 — more frequent, endometriotic cancer, Type 2 — less frequent, non-endometroid cancer. Non-endometroid carcinomas include serous and clear cell carcinomas. The second type of endometrial tumours has a vague etiology and worse prognostics [7, 8]. Type 1 is etiopathogenetically associated with excessive oestrogen stimulation and obesity [9]. We assume that in about 80% of endometrial cancers, it is reasonable to find these compounds. According to the World Cancer Report, obesity accounts for 40% of all endometrial cancer cases [2]. Obesity is an important social and health problem. It is estimated that the issue of overweight and obesity affects 50–65% of the European population. In summary, there are discrepancies in the assessment of the influence of obesity regarding the course of surgically treating endometrial cancer due to the use of various parameters of obesity in the literature.

The aim of the current study was to assess the relationship between obesity parameters and procedural indices with emphasis on the duration of operation, hospitalisation and haemoglobin loss after the operation.

MATERIAL AND METHODS

70 patients treated at the Clinical Department of Gynaecology of the Provincial Specialist Hospital due to endometrial cancer in the period from February to August 2011. The study included patients qualified for transabdominal hysterectomy with diagnosed endometrial cancer based on histopathological examination of uterine scrapings. The research project was an observational prospective study of standard treatment. Before the operation, a clinical interview was performed. This included age, births, education in years, prior abdominal surgeries and comorbidities. In the preoperative study, the following parameters were determined: height, weight, waist and hip circumference in cm with a measuring tape, pelvic bone dimensions using a pelvis meter. Also, body mass index (BMI) and waist-hip ratio (WHR) were calculated in the study group. According to the World Health Organization, overweight and individual degrees of obesity are determined by defined ranges of BMI. Overweight was defined as BMI in the range 25–30 kg/m^2. Class I obesity is within the range 30–35 kg/m^2, while class II obesity is within the range of 35–40 and class III obesity values are > 40 kg/m^2. Patients were qualified for the hysterectomy procedure according to the current Polish guidelines [10]. In the current study obesity was defined as the BMI ≥ 30 kg/m^2. The surgery treatment included: removal of the uterus with adnexal tracts, systemic pelvic and para-aortic lymphadenectomy(FIGO I–G1/G2 and myometrium invasion > 50%, G3) removal of the greater omentum (Excision of the greater omentum in serous endometrial carcinoma and sarcocarcinoma) [11]. In the perioperative period, selected parameters were monitored and they were recognised as indicators of in-hospital outcome of operational treatment: the duration of surgery (minutes), the loss of haemoglobin — the difference in the serum concentration before surgery and on the second day after surgery (g/dL), the occurrence of procedural-related complications, and the duration of hospitalisation (days).

The study has been approved by the local University of Rzeszów ethics committee. The protocol complied with the Declaration of Helsinki, and all participants provided written informed consent before enrolment.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Categorical variables are introduced as numbers and percentages. Normality was assessed with the Shapiro-Wilk test. The Mann-Whitney U test was used for non-normally distributed continuous variables. Univariate and multivariate regression analysis were used to find significant predictors of in-hospital outcomes. The analysed procedural indices (duration of operation, duration of hospitalization and the extent of hemoglobin loss) were assessed as continuous variables. Also predictors of clinical outcomes were assessed as continuous variables or dychotomic when appropriate. Multifactorial analysis was performed by backward elimination. Also, Spearman's correlations were calculated for possible relationships between selected factors and pre-specified indicators of in-hospital outcomes. The p-value < 0.05 was considered statistically significant. All analyses were performed with JMP®, Version 13.1.0 (SAS Institute INC., Cary, NC, USA).

RESULTS

General characteristics

The patients' epidemiology and tumour characteristics are presented in Table 1. In the studied group, 81.43% presented a BMI > 25 kg/m^2 (Fig. 1). While 37 patients presented with BMI > 30 kg/m^2 (52.8%). Most often, the patients were diagnosed and operated in stage I of the cancer according to FIGO — Table 2. In the preliminary analysis of the results of surgical treatment, we observed that the average duration of abdominal hysterectomy due to endometrial cancer was 86 ± 20.4 minutes, the average duration of hospitalisation was 11.3 ± 3.1 days, the average loss of haemoglobin measured as a difference measured in g/dL before surgery and on the second day after surgery was 2.2 ± 0.9 (Tab. 3).

The duration of the operation

The duration of the operation was significantly longer in patients with BMI value ≥ 30 kg/m^2 compared patients with BMI value < 30 kg/m^2 (94.9 ± 21.6 vs. 76.1 ± 13.5 days, $p < 0.0001$). The duration of operation time correlated positively with body mass, BMI value, hip circumference, WHR, intertrochanteric distance, interspinous distance, intercristal distance, external conjugate and prior abdominal surgery, while a negative correlation was found with

Table 1. Patients epidemiology and tumour characteristics

Variable			Overal group N = 70 (%)
Place of residence	Rural region		26 (37.2)
	Urban region		24 (34.3)
	Town > 50,000 residents		20 (28.5)
Menstrual status	Before menopause		61 (87.1)
	After menopause		9 (12.9)
Parity	Nulliparous		14 (20.1)
	Uniparous		16 (22.8)
	Multiparous		40 (57.1)
Histopathological type	Endometrioid	G1	36 (51.4)
		G2	24 (34.2)
		G3	5 (7.1)
	Clear-cell		1 (1.4)
	Serous		2 (2.8)
	Adenosquamous		1 (1.4)
	Non-epithelial		1 (1.4)
Staging according to FIGO classification	I		52 (74.3)
	II		11 (14.3)
	III		5 (7.1)
	IV		2 (2.8)
Co-morbidities	Diabetes mellitus		17 (24.8)
	Hypertension		32 (54.8)
	Coronary artery disease		5 (7)
	History of venous occlusive disease		3 (5.2)
	Arrhythmias		6 (8.4)
	Priorcerebral stroke		2 (2.8)
	Chronic heart failure		4 (5.6)
	Bronchial asthma		5 (7)
	Chronic pulmonary obstructive disease		3 (4.2)
	Hyperthyroidism		4 (5.6)
	Hypothyroidism		3 (4.2)
	Cholelithiasis		6 (8.4)
	Diathesisurica		2 (2.8)
	Osteoarthritis		9 (12.6)

the number of births. This is presented in Table 4. Univariate regression analysis confirmed significant relationships between operation duration and body mass, BMI, waist circumference, hip circumference, WHR, intertrochanteric distance, interspinous distance, intercristal distance, prior abdominal surgery, hypertension, diabetes, chronic obstructive pulmonary disease and hypothyroidism. This is presented in Table 5. Multivariate regression analysis among the above-mentioned factors confirmed significant relationships between the duration of the operation and waist circumference ($p < 0.0001$), body mass ($p < 0.0001$), chronic obstructive pulmonary disease ($p = 0.001$), BMI ($p = 0.0002$), intertrochanteric distance ($p = 0.01$) and hypothyroidism ($p = 0.02$).

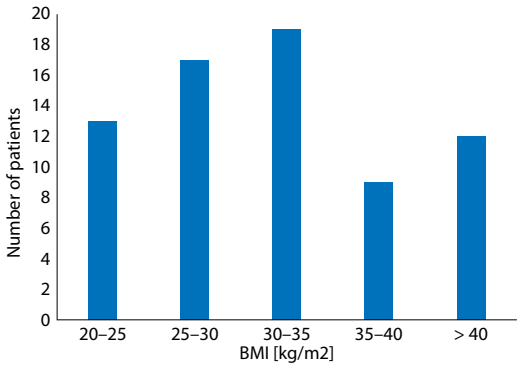

Figure 1. Patient distribution according to body mass index (BMI) value; BMI — body mass index

The duration of hospitalisation

The mean duration of hospitalisation was significantly longer in patients with BMI value ≥ 30 kg/m^2 compared to those with BMI value < 30 kg/m^2 (12.4 ± 3.4 vs. 10 ± 2.3 days, $p = 0.0009$; Tab. 3). The duration of hospitalisation correlated significantly positively with body mass, BMI, waist circumference, hip circumference, WHR, , intertrochanteric distance, interspinous distance, intercristal dimension, external con-

Table 2. Clinical stages according to International Federation of Gynaecology and Obstetrics (FIGO) 2008 — comparison of the number of patients in each stage of cervical cancer depending on the presence of obesity (Chi square test)

Stages of severity	Overall group N = 70	Obese (BMI ≥ 30 kg/m^2) N = 37	Non-obese (BMI < 30 kg/m^2) N = 33	p value
Stage I				
A	34 (51.4)	15 (40.6)	19 (57.4)	0.15
B	16 (22.8)	8 (21.6)	9 (27.2)	0.58
Stage II	11 (14.3)	5 (13.5)	5 (15.4)	0.84
Stage III				
A	5 (7.1)	5 (13.5)	0	–
B	1 (1.4)	1 (2.7)	0	–
C1	1 (1.4)	1 (2.7)	0	–
C2	0	0	0	–
Stage IV				
A	0	0	0	–
B	2 (2.8)	2 (5.4)	0	–

BMI — body mass index

Table 3. Comparison of selected indices in obese and non-obese patients

	Overall group	Obese (BMI ≥ 30 kg/m^2)	Non-obese (BMI < 30 kg/m^2)	p value
Age, years	61.3 ± 10.4	63.3 ± 9	59.1 ± 11.5	0.09
Intertrochromatic dimension, cm	35.3 ± 2.1	36.2 ± 2	34.2 ± 1.8	< 0.0001
Intersubular dimension, cm	26.9 ± 2	27.6 ± 2.1	26.2 ± 1.7	0.0006
Intercristal distance, cm	32.3 ± 3	33.7 ± 2.5	30.7 ± 2.7	< 0.0001
External conjugate, cm	22.2 ± 1.5	22.8 ± 1.4	21.5 ± 1.4	0.0002
Duration of operation, minutes	86 ± 20.4	94.9 ± 21.6	76.1 ± 13.5	< 0.0001
Duration of hospitalisation, days	11.3 ± 3.1	12.4 ± 3.4	10 ± 2.3	0.0009
Loss of haemoglobin, g/dL	2.2 ± 0.9	2.5 ± 0.9	1.9 ± 0.8	0.004

BMI — body mass index

jugate, prior abdominal surgery, clinical stages according to FIGO classification and histopatological grading. This is presented in Table 4. Univariate regression analysis also revealed significant relationships between the duration of hospitalisation and body mass, BMI, waist circumference, hip circumference, WHR, intertrochanteric distance, interspinous distance, external conjugate prior abdominal surgery, hypertension, diabetes, chronic obstructive pulmonary disease, hypothyroidism and heart failure. This is presented in Table 5. Multivariate regression analysis among the above-mentioned factors confirmed that the duration of hospitalisation was significantly associated with waist circumference, hip circumference, prior abdominal surgery hypothyroidism and BMI.

Table 4. Significant correlations between selected indices and haemoglobin loss during operation, duration of operation and hospitalisation

Predictors	Δ Hb before and two days after operation [g/dL]		Duration of operation [min.]		Duration of hospitalisation [days]	
	R coefficient	p value	R coefficient	p value	R coefficient	p value
Weight, kg	0.54	< 0.0001	0.59	< 0.0001	0.48	< 0.0001
Body-mass index, kg/m^2	0.55	< 0.0001	0.57	< 0.0001	0.48	< 0.0001
Waist circumference, cm	0.57	< 0.0001	0.7	< 0.0001	0.59	< 0.0001
Hip circumference, cm	0.46	< 0.0001	0.57	< 0.0001	0.43	0.0002
Waist-hip ratio	0.42	0.0003	0.36	0.002	0.35	0.003
Intertrochromatic dimension, cm	0.33	0.004	0.45	0.001	0.25	0.03
Intersubular dimension, cm	–	–	0.36	0.002	0.31	0.008
Intercristal distance, cm	0.43	0.0002	0.39	0.0007	0.25	0.03
External conjugate, cm	0.48	< 0.0001	0.28	0.02	0.33	0.004
Prior abdominal surgery	0.42	0.0003	0.29	0.014	0.41	0.0005
Number of births	–	–	–0.28	0.017	–	–
Clinical stages, FIGO	0.29	0.01	–	–	0.24	0.04
Histopathology, grading	–	–	–	–	0.25	0.04

Δ Hb — the difference in the serum concentration before surgery and on the second day after surgery

Table 5. Predictors of blood loss, duration of operation and hospitalisation assessed by univariate regression analysis

Predictors	Δ Hb before and two days after operation [g/dL]		Duration of operation [min.]		Duration of hospitalisation [days]	
	ß (95% CI)	p value	ß (95% CI)	p value	ß (95% CI)	p value
Weight, kg	0.018 (0.008–0.028	0.00006	0.641 (0.439–0.842)	< 0.0001	0.081 (0.048–0.115)	< 0.0001
Body-mass index, kg/m^2	0.043 (0.018–0.068)	0.001	1.554 (1.061–2.048)	< 0.0001	0.205 (0.124–0.286)	0.0009
Waist circumference, cm	0.02 (0.01–0.031)	0.0002	0.759 (0.564–0.955)	< 0.0001	0.102 (0.069–0.135)	< 0.0001
Hip circumference, cm	0.018 (0.005–0.032)	0.005	0.681 (0.408–0.954)	< 0.0001	0.082 (0.037–0.126)	0.0005
Waist-hip ratio	4.123 (1.605–6.64)	0.002	102.39 (45.27–159.5)	0.0006	15.55 (6.784–24.33)	0.0007
Intertrochromatic dimension, cm	–	–	3.929 (1.833–6.024)	0.0004	0.424 (0.086–0.761)	0.014
Intersubular dimension, cm	–	–	–	–	0.397 (0.042–0.753)	0.028
Intercristal distance, cm	0.082 (0.013–0.151)	0.02	2.341 (0.793–3.89)	0.004	–	–
External conjugate, cm	0.273 (0.15–0.396)	< 0.0001	–	–	0.534 (0.059–1.009)	0.028
Prior abdominal surgery	0.361 (0.109–0.614)	0.006	7.016 (1.128–12.904)	0.02	1.461 (0.615–2.306)	0.001
Hypertension	0.246 (0.041–0.451)	0.019	7.857 (3.328–12.38)	0.0009	1.033 (0.322–1.743)	0.005
Diabetes mellitus	–	–	4.955 (0.192–9.718)	0.04	1.059 (0.351–1.767)	0.004
COPD	–	–	8.647 (1.23–16.063)	0.023	1.258 (0.116–2.399)	0.031
Hypothyroidism	–	–	11.86 (5.475–18.257)	0.0004	1.708 (0.716–2.7)	0.001
Heart failure	–	–	–	–	1.684 (0.282–3.086)	0.02

Δ Hb — the difference in the serum concentration before surgery and on the second day after surgery; ß — coefficient beta; CI — confidence interval; COPD — chronic obstructive pulmonary disease

Procedure-related haemoglobin loss

The mean haemoglobin loss during periprocedural time was significantly higher in patients with BMI value ≥ 30 kg/m^2 compared to patients with BMI value < 30 kg/m^2 (2.5 ± 0.9 vs. 1.9 ± 0.8 g/dL, p = 0.004; Tab. 3). The degree of haemoglobin loss at the periprocedural time correlated significantly and positively with body mass, BMI, waist circumference, hip circumference, WHR, intertrochanteric distance, intercristal distance, external conjugate, prior abdominal surgery and clinical stages according to FIGO classification. This is presented in Table 4. Univariate regression analysis revealed significant relationships between periprocedural degree of haemoglobin loss and body mass, BMI, waist circumference, hip circumference, WHR, intercristal distance, external conjugate, prior abdominal surgery and hypertension. This is presented in Table 5. Multivariate regression analysis among the above-mentioned factors confirmed a significant relationship between the degree of haemoglobin loss during periprocedural time and external conjugate (p = 0.0002) and prior abdominal surgery (p = 0.001).

Anatomical parameters

Anatomical indices were significantly higher in patients with BMI value ≥ 30 kg/m^2 compared to patients with BMI < 30 kg/m^2, and they included intertrochanteric distance (36.2 ± 2 vs. 34.2 ± 1.8 cm, p < 0.0001), interspinous distance (27.6 ± 2.1 vs. 26.2 ± 1.7 cm, p = 0.0006), intercristal distance (33.7 ± 2.5 vs. 30.7 ± 2.7 cm, p < 0.0001) and external conjugate (22.8 ± 1.4 vs. 21.5 ± 1.4 cm, p = 0.0002). This is presented in Table 3.

Procedure-related complications

There were 5 perioperative complications: infection of the postoperative wound with impaired healing in 2 patients, intraoperative bladder injury in one patient, gastrointestinal obstruction in 1 case and intraoperative bleeding with the need to ligate the internal iliac artery in another patient. On average, patients with procedure-related complications showed higher obesity parameters: BMI (38 vs. 31.7 kg/m^2, p = 0.88) and waist circumference (119.6 vs. 103.7 cm, p = 0.54), compared to patients who had no procedure-related complications, however, those differences did not reach statistical significance.

DISCUSSION

The main findings of the current study are that patients with the mean BMI value > 30 kg/m^2 demonstrated longer duration of hospitalisation and operation, as well as greater haemoglobin loss during the periprocedural period. The second observation worth mentioning is that patients with obesity were diagnosed with endometrial cancer at more advanced stages according to the FIGO classification before the surgical treatment in comparison to non-obese patients. Thirdly, among predictors of the longer duration of operation we confirmed greater BMI, greater waist circumference and body mass. While among predictors of the duration of hospitalisation there were greater waist and hip circumference and greater BMI value. The external conjugate and intertrochanteric dimensions were found to be predictors of greater haemoglobin loss related to the procedure. The strongest correlation for the duration of operation, hospitalisation and haemoglobin loss was noticed for waist circumference. Moreover, patients with procedure-related complications were characterised by greater BMI value and waist circumference. The percentage distribution of obesity parameters showed that a significant percentage of patients with normal BMI presented abdominal obesity in the study group according to IDF criteria. Similar results were obtained in a study on the prevalence of metabolic syndrome in the population of the Tarnawa Dolna commune (70% of patients with abdominal obesity) [12]. The results of the study in the population of women treated for endometrial cancer show a higher incidence of this type of obesity than in the general population (> 90%). Most often, the patients' operations were diagnosed at stage I according to the FIGO classification. The average duration of surgery in the study group operated on for endometrial cancer is comparable to the results obtained at other centres [13, 14]. This study, however, used other parameters of blood loss (blood volume), and the average length of stay in hospital was much shorter. The effect of visceral obesity on the results of surgical treatment has not been studied. However, the influence of obesity measured by the BMI parameter on the duration of surgery and blood loss was demonstrated, which was also confirmed in the presented study for both the BMI parameter and waist circumference. In the current study, the greater waist circumference value is related to longer duration of the operation and hospitalisation, and among other natural explanations of that relationship are greater extent, incision and more complicated surgical procedure in obese compared to non-obese patients. This also definitely reflects directly greater haemoglobin loss during the procedure and remains in close relation to the more advanced disease in this particular group of patients. Interesting data were presented by Kerimoglu et al. [15]. Their study evaluated the effect of the percentage of body fat on surgical outcomes in women with endometrial cancer. In the group patients with an elevated % of body fat, a longer mean operation time was noted than in those with a % of body fat less than 32% (138.2 ± 27.1 vs. 119.3 ± 34.8 min, p = 0.043). Nonetheless, there were no differences between patient groups regarding rates of intraoperative complications, mean duration of hospital stay and postoperative

complications [15]. However, in a cohort of 192 patients with differing BMI, Jan et al. showed that surgical operative time and mean length of hospitalisation in days were not significantly different among the 4 groups. In the obese group, there was significantly higher perioperative blood loss ($p = 0.01$), more wound abscess ($p = 0.05$) and more reinterventions for complications ($p = 0.03$) [16]. The incidence of postoperative complications in developed countries is estimated at 3–22% after surgical operations [11, 13, 15, 17]. Postoperative complications can be divided into early and late. The most common early complications in surgical gynaecology are: bleeding, bladder damage, ureters, intestinal perforation. The most frequent postoperative complications include infections, wound healing disorders, thromboembolic complications, cardiovascular complications, respiratory failure, gastrointestinal obstruction, renal failure, urination disorders, urogenital fistulas, intestinal fistulas. Co-morbidity of obesity and endometrial cancer may be associated with the increased rate of procedure-related complications. It has long been assumed that obesity can hinder the course of surgery. Some studies have shown that obesity is an obstacle to cancer staging in endometrial cancer [13], breast cancer [18] and gastric cancer [19]. A recent study identified EC patients with a body mass index (BMI) of ≥ 40 kg/m^2 at increased risk of developing surgical complications compared to their non-obese counterparts [20]. However, among 514 women with endometrial cancer, Bouwman et al. noted that obese and morbidly obese women experienced significantly more surgical complications than non-obese women ($p = 0.010$) [21]. This was confirmed in the present study.

In a prospective study conducted on a group of 233 patients operated on due to endometrial cancer, it was shown that the BMI value significantly and positively correlates with the duration of the surgery and the loss of haemoglobin during the periprocedural period. However, no relationships were found between obesity, the duration of hospitalisation and the occurrence of postoperative complications [14]. However, a study conducted in Turkey suggests that obesity in this group of patients does not significantly affect the number of acquired lymph nodes, the duration of hospitalisation or the number of procedure-related complications. However, this study was conducted on a relatively small group of 48 patients [22]. There are discrepancies in the literature regarding the influence of obesity on the occurrence of post-operative complications such as: infections, wound healing disorders, pulmonary embolism, and venous thromboembolism. Some authors mention visceral obesity as potentially crucial in understanding the real impact of obesity on the incidence of postoperative complications [22, 23]. On the basis of the conducted study, it could not be unequivocally determined whether there is a relationship between obesity and the occurrence of perioperative complications. The comparative group of patients with complications (5 patients), despite clear differences in parameters, did not demonstrate statistical significance. In the current study, there is no consensus in the assessment of the relationship between obesity and specific postoperative complications. Probably, chronic inflammation and metabolic disturbances observed in abdominal obesity contribute to postoperative complications such as: infections, wound healing disorders, circulatory and respiratory disorders [14]. In our study the highest predictive value for analysed parameters in- hospital outcomes among patients with endometrial cancer were BMI value and waist circumference.

CONCLUSIONS

Obesity predisposes to the later diagnosis of endometrial cancer at a more advanced stage, which may contribute to worse treatment results both in terms of the effectiveness of the procedure and mortality in the follow-up period. Waist circumference seems to be the most sensitive marker, indicating the possibility of extending the duration of the procedure and hospitalization, which is also associated with increased blood loss associated with the procedure. Therefore, waist circumference could serve as a tool for operational risk stratification. Further research will help determine a more accurate relationship between obesity and the occurrence of specific perioperative complications.

Limitations

This study can be referred to as a developmental study because it was conducted on a small group of patients. Also due to the relatively small group of patients studied, in the analysis we conducted, we focused on assessing trends and the relationship of individual indicators with treatment results. However, we have not attempted to isolate cut-off points for individual data above which the risk of losing large amounts of hemoglobin increases significantly.

REFERENCES

1. Zwierko M, Bidziński M. Nowotwory trzonu macicy. Centrum Medyczne Kształcenia Podyplomowego, Warszawa 2011: 5–27.
2. Stewart B. World Cancer Report © International Agency for Research on Cancer. 2008; 5: 428–430.
3. Abacjew-Chmyłk AO, Chmyłko Ł, Wydra DG, et al. Multiple multiparity is a negative prognostic factor for endometrial cancer in Poland. Ginekol Pol. 2016; 87(3): 178–182, doi: 10.17772/gp/60071, indexed in Pubmed: 27306125.
4. Bręborowicz GH. Położnictwo i Ginekologia. Wydawnictwo Lekarskie PZWL Warszawa 2007: 833–842.
5. Renehan AG, Tyson M, Egger M, et al. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008; 371(9612): 569–578, doi: 10.1016/S0140-6736(08)60269-X, indexed in Pubmed: 18280327.
6. von Gruenigen VE, Tian C, Frasure H, et al. Treatment effects, disease recurrence, and survival in obese women with early endometrial carcinoma : a Gynecologic Oncology Group study. Cancer. 2006; 107(12): 2786–2791, doi: 10.1002/cncr.22351, indexed in Pubmed: 17096437.

7. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983; 15(1): 10–17, doi: 10.1016/0090-8258(83)90111-7, indexed in Pubmed: 6822361.
8. Roguz S, Krowiranda KW, Michalski T, et al. [Histological grading of endometrial carcinoma. Clinical and patomorphological analysis]. Ginekol Pol. 2015; 86(5): 340–345, indexed in Pubmed: 26117970.
9. Möllerström G, Carlström K, Lagrelius A, et al. Is there an altered steroid profile in patients with endometrial carcinoma? Cancer. 1993; 72(1): 173–181, doi: 10.1002/1097-0142(19930701)72:1<173::aid-cncr2820720132>3.0.co;2-x, indexed in Pubmed: 8508403.
10. Sznurkowski J, Knapp P, Bodnar L, et al. Recommendations of the Polish Gynecological Oncology Society for the diagnosis andtreatment of endometrial cancer. Current Gynecologic Oncology. 2017; 15(1): 34–44, doi: 10.15557/cgo.2017.0003.
11. Krzakowski M, Herman K, Jassem J. Zalecenia postępowania diagnostyczno–terapeutycznego w nowotworach złośliwych, cz. 2. Via Medica, Gdańsk 2019: 243–247.
12. Żyła ZG, Kinalska I. Ocena częstości występowania zespołu metabolicznego w populacji dorosłych mieszkańców gminy Tarnawa Dolna. Przegląd Kardiodiabetologiczny. 2010; 5(2): 93–97.
13. Orekoya O, Samson ME, Trivedi T, et al. The Impact of Obesity on Surgical Outcome in Endometrial Cancer Patients: A Systematic Review. J Gynecol Surg. 2016; 32(3): 149–157, doi: 10.1089/gyn.2015.0114, indexed in Pubmed: 27274182.
14. Santoso JT, Barton G, Riedley-Malone S, et al. Obesity and perioperative outcomes in endometrial cancer surgery. Arch Gynecol Obstet. 2012; 285(4): 1139–1144, doi: 10.1007/s00404-011-2116-2, indexed in Pubmed: 22020677.
15. Kerimoglu OS, Pekin A, Yilmaz SA, et al. Effect of the percentage of body fat on surgical, clinical and pathological outcomes in women with endometrial cancer. J Obstet Gynaecol Res. 2015; 41(3): 449–455, doi: 10.1111/jog.12554, indexed in Pubmed: 25330937.
16. Jan S, Duquesne M, Marret H, et al. [Influence of Body Mass Index on management and prognosis of women with endometrial cancer]. Gynecol Obstet Fertil. 2014; 42(11): 766–771, doi: 10.1016/j.gyobfe.2014.07.034, indexed in Pubmed: 25153444.
17. Eisenhauer EL, Wypych KA, Mehrara BJ, et al. Comparing surgical outcomes in obese women undergoing laparotomy, laparoscopy, or laparotomy with panniculectomy for the staging of uterine malignancy. Ann Surg Oncol. 2007; 14(8): 2384–2391, doi: 10.1245/s10434-007-9440-6, indexed in Pubmed: 17522943.
18. Derossis AM, Fey JV, Cody HS, et al. Obesity influences outcome of sentinel lymph node biopsy in early-stage breast cancer. J Am Coll Surg. 2003; 197(6): 896–901, doi: 10.1016/j.jamcollsurg.2003.08.005, indexed in Pubmed: 14644276.
19. Li Sk, Zhou Yb, Zhou Cf, et al. [Influence of obesity on short-term surgical outcome in patients with gastric cancer]. Zhonghua Wei Chang Wai Ke Za Zhi. 2010; 13(2): 133–136, indexed in Pubmed: 20186625.
20. Gunderson CC, Java J, Moore KN, et al. The impact of obesity on surgical staging, complications, and survival with uterine cancer: a Gynecologic Oncology Group LAP2 ancillary data study. Gynecol Oncol. 2014; 133(1): 23–27, doi: 10.1016/j.ygyno.2014.01.041, indexed in Pubmed: 24680587.
21. Bouwman F, Smits A, Lopes A, et al. The impact of BMI on surgical complications and outcomes in endometrial cancer surgery--an institutional study and systematic review of the literature. Gynecol Oncol. 2015; 139(2): 369–376, doi: 10.1016/j.ygyno.2015.09.020, indexed in Pubmed: 26407479.
22. Erkanli S, Kayaselçuk F, Bagis T, et al. Impact of morbid obesity in surgical management of endometrial cancer: surgical morbidity, clinical and pathological aspects. Eur J Gynaecol Oncol. 2006; 27(4): 401–404, indexed in Pubmed: 17009636.
23. Doyle SL, Lysaght J, Reynolds JV. Obesity and post-operative complications in patients undergoing non-bariatric surgery. Obes Rev. 2010; 11(12): 875–886, doi: 10.1111/j.1467-789X.2009.00700.x, indexed in Pubmed: 20025695.

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The parameters to estimate postoperative severe complications classified through Clavien-Dindo after upper abdominal surgery in patients with primary and recurrent ovarian cancer

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ABSTRACT

Objectives: The more surgical effort and performing extensive upper abdominal surgery (UAS) are often required to accomplish the highest rates of optimally cytoreduction in patients with ovarian cancer. Nonetheless, the rate of complications increases with extensive surgery. We have studied the upper abdominal surgery complications by Clavien-Dindo Classification (CDC) and analyzed parameters affecting post-operative severe complications classified through Clavien-Dindo.

Material and methods: A retrospective cohort of patients diagnosed with epithelial ovarian cancer from January 1st 2009 to April 30th 2016 was evaluated. Patients who underwent at least one UAS procedure with or without optimal cytoreduction for epithelial ovarian cancer (stage IIIC–IV or recurrent) were included. Postoperative complications were recorded according to the Clavien-Dindo Classification.

Results: In total, 58 patients were included. There were 120 UAS procedures performed on the 58 patients. Diaphragm peritonectomy was the most performed surgery (50%, 29/58), and then the other UAS procedures were liver surgery (39.7%, 23/58), cholecystectomy (24.1%, 14/58), splenic surgery (24.1%, 14/58), full-thickness diaphragm resection (22.4%, 13/58), pancreatic surgery (19%, 11/58), resection of tumor from porta hepatis (17.2%, 10/58), celiac lymph node excision (8.6%, 5/58), partial gastrectomy (1.7%, 1/58), respectively. Thirteen patients (22.4%) had post-operative grade 3–5 complications according to CDC within 30 days after surgery.

Conclusions: This current study demonstrated that the addition of extensive upper abdominal surgery procedures were not associated with increased postoperative severe complications in patients with recurrent or advanced ovarian cancer. These procedures are safe and feasible for patients in need and also can be performed with acceptable mortality and morbidity.

Key words: upper abdominal surgery; Clavien-Dindo; ovarian cancer; postoperative complication

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INTRODUCTION

Ovarian cancer is the second most common malignancy of reproductive tract in women and epithelial ovarian cancer (EOC) is the most fatal gynecologic cancer; the number of new cases of ovarian cancer is 11.9 per 100,000 whereas the number of deaths is 7.5 per 100,000 [1]. Despite the devastating survival statistics compared to other gynecologic cancers, there has been a decline in the mortality rate of EOC. Death rates have been falling on average 2.2% each year between 2004–2013 and the 5-year survival rate has an upward trend, increasing from 33.7% in 1977 to 46.2% in 2008, although the majority of ovarian cancer patients have metastasis to upper abdominal organs at diagnosis and found to have stage III or stage IV disease [1]. Progress in life expectancy of ovarian cancer patients can mainly be attributed to advances in cytoreductive surgery and implementation of platin based chemotherapy [2, 3].

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Major developments in medical treatment of EOC are: introduction and combination of paclitaxel to platin based chemotherapy in 1996 [4]; the emergence of intraperitoneal chemotherapy in 2006 [5]; and targeted chemotherapies and poly [adenosine diphosphate (ADP)] ribose polymerase (PARP) inhibitors recently [6]. The evolution of surgical treatment of EOC was towards more radical, extensive procedures that consisted of radical oopherectomy, pelvic peritonectomy, bowel resections and anastomosis, and then upper abdominal procedures were added to armory of gynecologic oncologists [7]. The concept of optimal cytoreduction was changing and brought to an end at maximal cytoreduction in order to improve overall survival, despite the new medical treatment modalities [8]. There has been afierce debate over extensive maximal debulking surgery versus neoadjuvant treatment. However, many studies have shown that primary cytoreductive surgery (CRS) aiming no residual disease was the most important modifiable prognostic factor affecting survival [9–11]. A dedicated gynecologic oncology team performing extensive upper abdominal surgery (UAS) such as diaphragm stripping and/or resection, liver resection, cholecystectomy, splenectomy, distal pancreatectomy, resection of tumor from porta hepatis, celiac lymph node excision and partial gastrectomy is required to accomplish the highest rates of optimally cytoreduction or complete resection [11–13]. The comprehension of CRS and the biology of a tumor was established by many reports on the impact of CRS and upper abdominal surgery on oncological outcomes. However, systematic evaluation of complications of upper abdominal surgery is seldom studied. Additionally, standardization is required for classification of surgical complications. There is no consensus among gynecologic oncologist on how to report surgical complications.

Clavien-Dindo Classification (CDC), mainly used by general surgeons, has been proposed to rank a complication in an objective, reliable and reproducible manner [14]. The point of CDC is mainly on the therapeutic consequences of a complication. Therefore, we have studied the upper abdominal surgery complications by CDC and analyzed parameters affecting post-operative severe complications classified through Clavien-Dindo.

MATERIAL AND METHODS

We obtained Institutional Review Board approval (number: 04/03/15:55) and then identified all patients with International Federation of Gynecology and Obstetrics (FIGO) stage IIIC and IV epithelial ovarian cancer or recurrent epithelial ovarian cancer who underwent extensive upper abdominal surgery at the Suleyman Demirel University Hospital from January 1, 2009 thru April 30, 2016. The medical records of all patients were retrospectively reviewed for the following data: age, body mass index (BMI) American Society of Anesthesiologist (ASA) score, Eastern Cooperative Oncology Group (ECOG) performance status, FIGO stage, pre-operative albumin, serum cancer antigen (CA125), hemoglobin levels, ascites, upper abdominal surgery procedures, estimated blood loss, intraoperative blood transfusion, duration of surgery, residual disease after surgery, length of hospital stay, and finally post-operative complications within 30 days and pathologic data.

We included patients who had undergone at least one upper abdominal surgery procedure with or without optimal cytoreduction for epithelial ovarian cancer (FIGO stages IIIC–IV or recurrent). We excluded patients who had received neoadjuvant chemotherapy or histologically confirmed non-epithelial ovarian cancers, low malignant potential tumors from the study.

These patients were classified according to residual disease (RD); RD 0: no residual disease, RD 1–10: residual disease 1–10 mm, and RD > 10: gross residual disease is more than 10 mm. Extensive surgical procedures were performed on the upper abdomen included diaphragm peritonectomy, full-thickness diaphragm resection, liver surgery (partial liver resection or segmental hepatectomy or liver capsule metastasectomy), cholecystectomy, splenic surgery (splenectomy or resection of tumor on the surface of spleen without splenectomy), pancreatic surgery (distal pancreatectomy or resection of tumor on the pancreatic capsule), partial gastric resection, celiac lymph node excision, and resection of tumor from porta hepatis.

We recorded post-operative complications with the Clavien-Dindo Classification. We accepted post-operative complications or death associated with surgery if occuring within 30 days after surgery. Complications were evaluated in five categories depending on their severity in the CDC (1: no treatment or simple medical treatment, and 5: death) (Tab. 1). We subdivided patients into two groups; grade 1–2 complications as mild and grade 3–5 as a severe group. We focused on those grades with serious clinical outcomes. If patients had more than one complication, we noted the highest grade complication in the analysis. Adjuvant chemotherapy was routinely administered within 6 weeks of the operation.

Mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov Simirnov Test. Mann-Whitney U Test and Independent Sample T Test were used in the analysis of quantitative data. The Chi-square test was used to analyze qualitative data, and the Fisher test was used when the chi-square test conditions were not met. SPSS 22.0 program was used in the analyzes.

Table 1. Clavien-Dindo Classification of surgical complications

Grade	Definition
Grade I	Any deviation from the normal course without the need for pharmacological treatment or surgical, endoscopic and radiologic interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included
Grade III	Requiring surgical, endoscopic or radiological intervention
IIIA	Intervention not under general anesthesia
IIIB	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)* requiring IC/ICU management
IVA	Single organ dysfunction (including dialysis)
IVB	Multiorgan dysfunction
Grade V	Death of a patient

*Brain hemorrhage, ischemic stroke, subarachnoid bleeding, but excluding transient ischemic attacks; CNS — central nervous system; IC — intermediate care; ICU — intensive care unit

RESULTS

Fifty-eight patients with EOC who underwent upper abdominal surgery at our institution were included in this study between January 1, 2009 and April 30, 2016. All patients underwent cytoreductive surgery by exploratory laparotomy. The demographic characteristics and surgical outcomes were abstracted in Table 2.

The mean age was 62.2, mean BMI was 27.6 kg/m^2, mean ascites volume was 919.3 ml, mean preoperative serum hemoglobin level was 12.4 g/dL, mean preoperative serum albumin level was 3.6 g/dL, mean preoperative serum CA125 was 799 u/mL, mean estimated blood loss was 387.1 mL, mean operative time was 319.1 minutes and mean post-operative hospital stay was 13.4 days. An intra-operative blood transfusion was required in 33 patients (56.8%).

The most common ECOG performance status score was 0 (36.2%) and 65.5% of patients had an ASA class of 2. The majority of patients had serous histology (87.9%) and grade 3 tumors (67.3%).

Thirty-two out of 58 patients (55.2%) had primary disease and 26 patients (44.8%) had recurrent disease. According to the results of cytoreductive surgery, no gross residual disease after surgery was in 58.6% (n: 34/58, RD 0), gross residual disease ≤ 10 mm in 12.1% (n: 7/58, RD 1–10mm), and gross residual disease > 10 mm in 29.3% (n: 17/58, RD > 10 mm). In patients who underwent debulking surgery was primary cytoreduction in 55.2% (32/58), secondary cytoreduction in 31.1% (18/58), tertiary cytoreduction in 13.7% (8/58) of cases respectively. Twenty-seven out of 32 patients (84.3%) who had primary disease was stage IIIC and 5 patients (15.7%) were stage VI according to FIGO classification.

There were 120 UAS procedures performed on the 58 patients, and multiple procedures were performed in many of patients. Diaphragm peritonectomy was the most

Table 2. Patient and clinical characteristics

	Min–max	Mean + SD/N–%
Age [years]	42.0–92.0	62.2 + 10.6
BMI [kg/m^2]	18.0–34.4	27.6 + 4.1
Preoperative serum hemoglobin [g/dL]	9.2–15.4	12.4 + 1.4
Preoperative serum albumin [g/dL]	1.6–4.8	3.6 + 0.6
Preoperative serum CA125 [u/mL]	11.0–5005.0	799.0 + 1140.1
Ascites volume [mL]	0–7000.0	919.3 + 1490.1
Operative time [min]	140.0–570.0	319.1 + 116.9
Length of hospitalization [days]	5.0–42.0	13.4 + 8.2
Estimated blood loss [mL]	100–1700.0	387.1 + 332.7
Intra-operative units of blood transfused		33 56.8
ECOG performance status		
0		21 36.2
1		16 27.6
2		12 20.7
3		9 15.5
ASA score		
1		3 5.2
2		38 65.5
3		17 29.3
Cytoreduction		
Primary		32 55.2
Secondary		18 31.1
Tertiary		8 13.7
FIGO stage		
IIIC		27 46.6
IV		5 8.6
Recurrent disease		26 44.8
Residual disease		
RD 0		34 58.6
RD 1–10 mm		7 12.1
RD > 10 mm		17 29.3
Histology		
Serous		51 87.9
Endometrioid		3 5.2
Mucinous		1.7
Carcinosarcoma		3.5
Transitional		1 1.7
Tumor grade		
1		7 12.1
2		12 20.6
3		39 67.3

PO — postoperative; BMI — body mass index; ASA — American Society of Anesthesiologist; ECOG — Eastern Cooperative Oncology Group; FIGO — International Federation of Gynecology and Obstetrics; CA125 — cancer antigen; RD — residual disease; SD — standard deviation

performed surgery (50%, 29/58), and then the other UAS procedures were liver surgery (39.7%, 23/58), (right posterior bisegmentectomy (segment 6–7); 1/58, intraparenchymal tumor resections; 10/58, liver capsule metastasectomy; 12/58), cholecystectomy (24.1%, 14/58), splenic surgery (24.1%, 14/58) (splenectomy; 12/58, resection of the tumor on the surface of spleen; 2/58), full-thickness diaphragm resection (22.4%, 13/58), pancreatic surgery (19%, 11/58) (distal pancreatectomy; 4/58, resection of tumor on the pancreatic capsule; 7/58), resection of tumor from porta hepatis (17.2%, 10/58), celiac lymph node excision (8.6%, 5/58), partial gastrectomy (1.7%, 1/58), respectively. Other surgical procedures implemented to patients were hysterectomy, unilateral/bilateral salpingo-oophorectomy, pelvic lymph node dissection, para-aortic lymph node dissection, omentectomy, peritonectomy, small bowel resection, large bowel resection, ileostomy, appendectomy, cardiophrenic lymph node dissection, VATS (video assisted thoracoscopic surgery), IP (intraperitoneal) catheter, and HIPEC (hyperthermic intraperitoneal chemotherapy) (Tab. 3).

Thirteen patients (22.4%) had post-operative grade 3–5 complications according to Clavien-Dindo Classification within 30 days after surgery (Tab. 4). Ten patients (17.2%) were reported as grade 3 complication, 1 patient (1.7%) was reported as grade 4 complication, and 2 patients (3.5%) were reported as grade 5 complication. Grade 3 complications were treated surgical, endoscopic or radiological intervention. Grade 4 complication was a life-threatening complication and treated at intensive care unit.

Two patients had grade 5 complications (mortalities) within 30 days of surgery (3,5%). The first patient died due to short bowel syndrome in the 28th post-operative day; she was a 49-year-old patient with an ECOG performance status of 2 who underwent multiple surgical procedures (right diaphragm peritonectomy, splenectomy, distal pancreatectomy, total colon resection, resection of the small intestine segment after the 70th cm of the treitz ligament, jejunostomy, HIPEC and RD 0 tertiary cytoreduction) for recurrent serous ovarian carcinoma. The second patient died due to acute cardiopulmonary failure in the 5th post-operative day; she was an 87-year-old patient with an ECOG performance status of 3 and cardiac failure who underwent multiple surgical procedures (total abdominal hysterectomy with bilateral salpingo-oophorectomy, splenectomy, cholecystectomy, distal pancreatectomy, porta hepatis disease resection, total colectomy, ileostomy and optimal cytoreduction) for a stage 4 serous ovarian carcinoma. The patient's status was stable until the third postoperative day. On the third postoperative day, respiratory arrest occurred suddenly. She was taken to the intensive care unit and was connected to a ventilator

Table 3. Data of surgical procedures implemented to patients

	Patients (N: 58) n %
Upper abdominal surgery procedures (n: 120)	
Diaphragm peritonectomy	29/58 50
Full-thickness diaphragm resection	13/58 22.4
Splenic surgery	14/58 24.1
Pancreatic surgery	11/58 19.0
Cholecystectomy	14/58 24.1
Partial gastrectomy	1/58 1.7
Liver Surgery	23/58 39.7
Celiac lymph node resection	5/58 8.6
Porta Hepatis disease resection	10/58 17.2
Other procedures	
Hysterectomy	34/58 58.6
Unilateral/bilateral salpingo-oophorectomy	34/58 58.6
Pelvic lymph node dissection	25/58 43.1
Para-aortic lymph node dissection	24/58 41.3
Omentectomy	39/58 67.2
Peritonectomy	29/58 50
Small bowel resection	13/58 22.4
Large bowel resection	21/58 36.2
Ileostomy	2/58 3.4
Colostomy	4/58 6.9
Anastomosis	19/58 32.7
Appendectomy	14/58 24.1
Cardiophrenic lymph node dissection	1/58 1.7
VATS	7/58 12.1
IP catheter	5/58 8.6
HIPEC	5 /58 8.6

VATS — video assisted thoracoscopic surgery; IP — intraperitoneal; HIPEC — hyperthermic intraperitoneal chemotherapy

Table 4. Post-operative grade 3–5 complications according to Clavien-Dindo Classification

Type of complication	UAS Patients (N: 58%)
Anastomotic insufficiency	2/58 3.5
Short bowel syndrome*	1/58 1.7
Pleural effusion	1/58 1.7
Pneumothorax	1/58 1.7
Pulmonary embolism	1/58 1.7
Postop bleeding	1/58 1.7
Acute cardiopulmonary failure*	1/58 1.7
Intra-abdominal abscess	1/58 1.7
Wound infection	2/58 3.5
Evisceration	2/58 3.5
Total complication	**13/58 22.4**

*Postoperative 30-day mortality (3.5%); UAS — Upper Abdominal Surgery

device and monitored. Her status worsened and she died on postoperative day 5.

We analyzed the parameters for prediction of the major postoperative complication and mortality after extensive upper abdominal surgery. There was no statistical difference in age, BMI, ascites, preoperative serum hemoglobin level, preoperative serum albumin level, preoperative serum CA125 level, estimated blood loss, operative time, ECOG performance status score, ASA score, FIGO stage and residual disease between complicated and uncomplicated patients ($p > 0.05$). Only the length of the post-operative hospital stay was statistically significant between complicated and uncomplicated patients ($p < 0.05$) (Tab. 5).

No statistical difference was found between the length of the post-operative hospital stay and the types of upper abdominal surgical procedures ($p > 0.05$) (Tab. 6).

There was also no statistical difference in upper abdominal surgery procedures between complicated and uncomplicated patients ($p > 0.05$). Only diaphragm peritonectomy was statistically significant ($p < 0.05$) (Tab. 7).

DISCUSSION

The amount of residual tumors after surgery in patients with advanced stage cancer is closely related to disease free and overall survival [11]. Ovarian cancer tends to spread to the upper abdominal anatomic sites and organs and therefore upper abdominal surgery has a key role to achieve the optimally cytoreduction rate [15]. There are very few studies to indicate the complication rate in patients with extensive upper abdominal surgeries. Kuhn et al. [12] reported that the rate of perioperative serious complication increased in patients with advanced ovarian cancer who underwent UAS compared to standard surgery for tumor debulking. Chi et al. demonstrated that the rate of postoperative major complications in patients underwent extensive UAS was 22% and the rate of postoperative mortality was 1.4%. This postoperative mortality and morbidity rate was acceptable [16]. In a population-based systematic review an average postoperative mortality after primary cytoreductive surgery for advanced stage epithelial ovarian cancer was reported as 2.5–3.7% [17]. In our study, the mortality rate was 3.4% and was similar to the literature.

Table 5. Comparison of parameters between patients with or without severe complications

		PO severe complication no		PO severe complication yes		p
		Mean + SD/n–%	Min–Max	Mean + SD/n–%	Min–Max	
Age [years]		61.5 ± 10.2	42–92	64.5 ± 12.1	45–87	0.355
BMI [kg/m^2]		27.7 ± 3.8	18–34	27.2 ± 5.0	21–34	0.532
Preoperative serum hemoglobin [g/dL]		12.4 ± 1.3	10–15	12.4 ± 1.8	9–15	0.881
Preoperative serum albumin [g/dL]		3.7 ± 0.7	1.6–4.8	3.5 ± 0.5	2.6–4.2	0.170
Preoperative serum CA125 [u/mL]		827.8 ± 1190.5	11–5005	699.2 ± 981.4	22–3372	0.758
Ascites volume [mL]		815 ± 1454	0–7000	1281 ± 1617	0–4000	0.665
Operative time [min.]		313.8 ± 118.4	140–570	337.7 ± 114.4	180–560	0.520
Length of hospitalization [days]		11.0 ± 4.4	5–24	21.9 ± 12.0	5–42	0.001
Estimated blood loss [mL]		353.3 ± 282.7	100–1300	503.8 ± 462.1	100–1700	0.285
ECOG performance status	0	16 35.6%		5 38.5%		0.393
	1	14 31.1%		2 15.4%		
	2	9 20.0%		3 23.1%		
	3	6 13.3%		3 23.1%		
ASA score	1	3 6.7%		0 0.0%		0.896
	2	29 64.4%		9 69.2%		
	3	13 28.9%		4 30.8%		
FIGO Stage	3C	22 48.9%		5 38.5%		0.600
	4	2 4.4%		3 23.1%		
	Recurrent	21 46.7%		5 38.5%		
Residual Disease	No Visible	27 60.0%		7 53.8%		0.210
	1–10 mm	3 6.7%		4 30.8%		
	> 10 mm	15 33.3%		2 15.4%		

T-test, Mann-Whitney U test, Chi-square test; PO — postoperative; BMI — body mass index; ASA — American Society of Anesthesiologist; ECOG — Eastern Cooperative Oncology Group; FIGO — International Federation of Gynecology and Obstetrics; CA125 — cancer antigen; SD — standard deviation

Table 6. Comparison of length of hospital stay and types of surgical procedures

	Length of Hospital Stay			
	Min–Max	Median	Mean + SD	p
Diaphragm Resection No Yes	5.0–42.0 8.0–33.0	11 12	13.2 + 8.4 14.2 + 7.5	0.437
Diaphragm Peritonectomy No Yes	5.0–24.0 5.0–42.0	10 12	10.8 + 4.2 16.1 + 10.2	0.057
Splenic Surgery No Yes	5.0–42.0 5.0–33.0	11 11	13.4 + 8.2 13.7 + 8.2	0.827
Cholecystectomy No Yes	5.0–42.0 5.0–24.0	11 11	13.8 + 8.9 12.2 + 5.1	0.956
Gastric Surgery No Yes	5.0–42.0 28.0–28.0	11 28	13.2 + 8.0 28.0 + –	0.142
Pancreatic Surgery No Yes	5.0–42.0 5.0–33.0	10 13	12.8 + 8.0 16.1 + 8.9	0.164
Liver Surgery No Yes	5.0–38.0 5.0–42.0	11 12	12.7 + 7.0 14.7 + 9.8	0.566
Porta Hepatis Disease Resection No Yes	5.0–42.0 5.0–24.0	11 12	13.6 + 8.6 12.8 + 5.6	0.965
Celiac Lymph Node Resection No Yes	5.0–42.0 8.0–18.0	11 11	13.5 + 8.5 12.4 + 4.4	0.813

Mann-Whitney U test

Table 7. Comparison of postoperative severe complications and types of surgical procedures

	PO severe complication no	PO severe complication yes	p
	n %	n %	
Diaphragm Resection	11 24.4	2 15.4	0.490
Diaphragm Peritonectomy	19 42.2	10 76.9	**0.028**
Splenic Surgery	9 20.0	5 38.5	0.171
Cholecystectomy	12 26.7	2 15.4	0.402
Gastric Surgery	0 0.0	1 7.7	0.224
Pancreatic Surgery	7 15.6	4 30.8	0.118
Liver Surgery	16 35.6	7 53.8	0.235
Porta Hepatis Disease Resection	6 13.3	4 30.8	0.143
Celiac Lymph Node Resection	4 8.9	1 7.7	1.000

PO — postoperative, x^2 chi-square test (Fisher exact test)

In a previous study, the rate of postoperative major complications (grade 3–5) was reported as 19.8% in patients underwent UAS [18]. In our study, the rate of severe postoperative complications (grade 3–5) was 22.4% and was compatible with this study.

In recent studies, liver surgery, splenectomy, pancreatic surgery, cholecystectomy, celiac lymphadenectomy and resection tumor from porta hepatis were reported as strong predictive factors for postoperative severe complications during cytoreductive surgery for advanced ovarian cancer [19–22]. We did not find any correlation between these procedures and postoperative severe complications. In our study, only diaphragm peritonectomy was associated with postoperative severe complications.

In the literature, the incidence of postoperative pleural effusion after diaphragmatic surgery as part of ovarian cancer debulking surgery ranged from 10% to 59% [23–26]. There is no consensus about use of a chest tube when the pleural space is opened during diaphragm surgery. Some authors do not recommend prophylactic use of a chest tube during diaphragm resection [18, 24–28], on the contrary, some authors routinely recommend chest tube placement [29–32]. Eisenhauer et al. [24] reported that the postoperative pleural effusion developed in 60% of the patients who underwent diaphragm surgery for advanced mullerian cancer and 15% of these patients required a postoperative chest tube placement or thoracentesis. In another study, the rate of postoperative pleural effusion following diaphragmatic peritonectomy with ovarian carcinoma was 30%, and 12.5% of these patients were treated with thoracentesis or chest tube placement to manage symptomatic pleural effusions [25]. In these two studies, routine use of chest tubes were not recommended when the pleural space is opened. In contrast, Chereau and colleagues did not place a chest tube in patients whose pleural cavity was opened during diaphragm surgery with stage III/IV ovarian cancer (38%) and the rate of postoperative chest tube placement was 27%. Therefore, at the end of this study period, they decided to consist-

ently place a chest tube [31]. Einenkel et al. [32] reported a high rate of postoperative chest tube placement (18%) and recommended use of chest tubes during diaphragm resection. In our study, we routinely placed a chest tube during diaphragm resection (22.4%) and necessity postoperative chest tube was 0% after diaphragm resection. We placed postoperative chest tubes because of symptomatic pleural effusion and pneumothorax in only 2 patients (3.4%) whose were not performed diaphragm surgery.

Langstraat et al. [33] showed that low albumin level, emergent surgery, advanced age and stage IV disease were associated with poor surgical outcomes in multivariate analysis. Besides, they observed that increased surgical complexity did not increase the risk of postoperative major complications. In light of this information, extensive surgery should not be avoided in patients who require complex surgeries.

There are some scoring systems to predict postoperative complications. However, these scoring systems are neglected if you can completely remove the tumor in patients with advanced stage cancer [34]. Because, the maximal cytoreductive surgery is the most important prognostic factor for overall survival in patients with advanced ovarian cancer.

Ataseven et al. [35] reported that preoperative serum albumin level was a predictive factor for severe postoperative complications (grade 3–5). However, in another study preoperative serum, albumin levels were not associated with severe postoperative complications [16]. We didn't observed any significant relationship between serum albumin levels in patients with and without postoperative severe complications.

Chi et al. analyzed predictive factors for the risk of severe postoperative complications in patients underwent UAS. Parameters such as BMI, age, ASA score, FIGO stage, and preoperative CA-125 levels were found unrelated. However, ascites volume, estimated blood loss and operative time were reported as predictive factors [16]. In a recent study, BMI was reported as an independent risk factor for severe postoperative complications and mortality in patients underwent primary surgical debulking for ovarian cancer [35]. However, we did not find a correlation between these predictive factors and severe postoperative complications in our study.

Benedetti Panici et al. [18] showed that the types of surgical procedures (diaphragmatic, pancreatic, gastric resection and splenectomy) were significantly related to a longer postoperative stay. In our study, there was no correlation between the types of upper abdominal surgery procedures and the length of hospital stay. However, it was longer in patients with severe complication, this result may be due to longer treatment process.

In conclusion, this current study demonstrated that the addition of extensive upper abdominal surgery procedures were not associated with increased postoperative severe complications in patients with recurrent or advanced ovarian cancer. These procedures are safe and feasible for patients in need and also can be performed with acceptable mortality and morbidity.

REFERENCES

1. National Cancer Institute, Surveillance, Epidemiology, and End Results Program. https://seer.cancer.gov/statfacts/html/ovary.html (01.01.2017).
2. Griffiths CT. Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. Natl Cancer Inst Monogr. 1975; 42: 101–104, indexed in Pubmed: 1234624.
3. Hoskins WJ. Epithelial ovarian carcinoma: principles of primary surgery. Gynecol Oncol. 1994; 55(3 Pt 2): S91–S96, doi: 10.1006/gyno.1994.1346, indexed in Pubmed: 7835815.
4. McGuire WP, Hoskins WJ, Brady MF, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med. 1996; 334(1): 1–6, doi: 10.1056/NEJM199601043340101, indexed in Pubmed: 7494563.
5. Armstrong DK, Bundy B, Wenzel L, et al. Gynecologic Oncology Group. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med. 2006; 354(1): 34–43, doi: 10.1056/NEJMoa052985, indexed in Pubmed: 16394300.
6. Wiggans AJ, Cass GKS, Bryant A, et al. Poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of ovarian cancer. Cochrane Database Syst Rev. 2015(5): CD007929, doi: 10.1002/14651858.CD007929.pub3, indexed in Pubmed: 25991068.
7. Eisenkop SM, Friedman RL, Wang HJ. Complete cytoreductive surgery is feasible and maximizes survival in patients with advanced epithelial ovarian cancer: a prospective study. Gynecol Oncol. 1998; 69(2): 103–108, doi: 10.1006/gyno.1998.4955, indexed in Pubmed: 9600815.
8. Chi DS, Eisenhauer EL, Lang J, et al. What is the optimal goal of primary cytoreductive surgery for bulky stage IIIC epithelial ovarian carcinoma (EOC)? Gynecol Oncol. 2006; 103(2): 559–564, doi: 10.1016/j.ygyno.2006.03.051, indexed in Pubmed: 16714056.
9. Winter WE, Maxwell GL, Tian C, et al. Gynecologic Oncology Group Study. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol. 2007; 25(24): 3621–3627, doi: 10.1200/JCO.2006.10.2517, indexed in Pubmed: 17704411.
10. Fanfani F, Fagotti A, Ercoli A, et al. Is There a Role for Tertiary (TCR) and Quaternary (QCR) Cytoreduction in Recurrent Ovarian Cancer? Anticancer Res. 2015; 35(12): 6951–6955, indexed in Pubmed: 26637921.
11. Bristow RE, Tomacruz RS, Armstrong DK, et al. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol. 2002; 20(5): 1248–1259, doi: 10.1200/JCO.2002.20.5.1248, indexed in Pubmed: 11870167.
12. Kuhn W, Florack G, Roder J, et al. The influence of upper abdominal surgery on perioperative morbidity and mortality in patients with advanced ovarian cancer FIGO III and IV. Int J Gynecol Cancer. 1998; 8: 56–63.
13. Eisenkop SM, Spirtos NM, Friedman RL, et al. Relative influences of tumor volume before surgery and the cytoreductive outcome on survival for patients with advanced ovarian cancer: a prospective study. Gynecol Oncol. 2003; 90(2): 390–396, doi: 10.1016/s0090-8258(03)00278-6, indexed in Pubmed: 12893206.
14. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004; 240(2): 205–213, doi: 10.1097/01.sla.0000133083.54934.ae, indexed in Pubmed: 15273542.
15. Eisenhauer EL, Abu-Rustum NR, Sonoda Y, et al. The addition of extensive upper abdominal surgery to achieve optimal cytoreduction improves survival in patients with stages IIIC-IV epithelial ovarian cancer. Gynecol Oncol. 2006; 103(3): 1083–1090, doi: 10.1016/j.ygyno.2006.06.028, indexed in Pubmed: 16890277.
16. Chi DS, Zivanovic O, Levinson KL, et al. The incidence of major complications after the performance of extensive upper abdominal surgical procedures during primary cytoreduction of advanced ovarian, tubal, and peritoneal carcinomas. Gynecol Oncol. 2010; 119(1): 38–42, doi: 10.1016/j.ygyno.2010.05.031, indexed in Pubmed: 20609464.

17. Gerestein CG, Damhuis RAM, Burger CW, et al. Postoperative mortality after primary cytoreductive surgery for advanced stage epithelial ovarian cancer: a systematic review. Gynecol Oncol. 2009; 114(3): 523–527, doi: 10.1016/j.ygyno.2009.03.011, indexed in Pubmed: 19344936.
18. Benedetti Panici P, Di Donato V, Fischetti M, et al. Predictors of postoperative morbidity after cytoreduction for advanced ovarian cancer: Analysis and management of complications in upper abdominal surgery. Gynecol Oncol. 2015; 137(3): 406–411, doi: 10.1016/j.ygyno.2015.03.043, indexed in Pubmed: 25824857.
19. Merideth MA, Cliby WA, Keeney GL, et al. Hepatic resection for metachronous metastases from ovarian carcinoma. Gynecol Oncol. 2003; 89(1): 16–21, doi: 10.1016/s0090-8258(03)00004-0, indexed in Pubmed: 12694649.
20. Chen LM, Leuchter RS, Lagasse LD, et al. Splenectomy and surgical cytoreduction for ovarian cancer. Gynecol Oncol. 2000; 77(3): 362–368, doi: 10.1006/gyno.2000.5800, indexed in Pubmed: 10831343.
21. Kehoe SM, Eisenhauer EL, Abu-Rustum NR, et al. Incidence and management of pancreatic leaks after splenectomy with distal pancreatectomy performed during primary cytoreductive surgery for advanced ovarian, peritoneal and fallopian tube cancer. Gynecol Oncol. 2009; 112(3): 496–500, doi: 10.1016/j.ygyno.2008.10.011, indexed in Pubmed: 19091388.
22. Magtibay PM, Adams PB, Silverman MB, et al. Splenectomy as part of cytoreductive surgery in ovarian cancer. Gynecol Oncol. 2006; 102(2): 369–374, doi: 10.1016/j.ygyno.2006.03.028, indexed in Pubmed: 16631919.
23. Gouy S, Chereau E, Custodio AS, et al. Surgical procedures and morbidities of diaphragmatic surgery in patients undergoing initial or interval debulking surgery for advanced-stage ovarian cancer. J Am Coll Surg. 2010; 210(4): 509–514, doi: 10.1016/j.jamcollsurg.2010.01.011, indexed in Pubmed: 20347745.
24. Eisenhauer EL, D'Angelica MI, Abu-Rustum NR, et al. Incidence and management of pleural effusions after diaphragm peritonectomy or resection for advanced mullerian cancer. Gynecol Oncol. 2006; 103(3): 871–877, doi: 10.1016/j.ygyno.2006.05.023, indexed in Pubmed: 16815536.
25. Dowdy SC, Loewen RT, Aletti G, et al. Assessment of outcomes and morbidity following diaphragmatic peritonectomy for women with ovarian carcinoma. Gynecol Oncol. 2008; 109(2): 303–307, doi: 10.1016/j.ygyno.2008.02.012, indexed in Pubmed: 18384866.
26. Silver DF. Full-thickness diaphragmatic resection with simple and secure closure to accomplish complete cytoreductive surgery for patients with ovarian cancer. Gynecol Oncol. 2004; 95(2): 384–387, doi: 10.1016/j.ygyno.2004.07.046, indexed in Pubmed: 15491761.
27. Devolder K, Amant F, Neven P, et al. Role of diaphragmatic surgery in 69 patients with ovarian carcinoma. Int J Gynecol Cancer. 2008; 18(2): 363–368, doi: 10.1111/j.1525-1438.2007.01006.x, indexed in Pubmed: 18334014.
28. Cliby W, Dowdy S, Feitoza SS, et al. Diaphragm resection for ovarian cancer: technique and short-term complications. Gynecol Oncol. 2004; 94(3): 655–660, doi: 10.1016/j.ygyno.2004.04.032, indexed in Pubmed: 15350355.
29. Montz FJ, Schlaerth JB, Berek JS. Resection of diaphragmatic peritoneum and muscle: role in cytoreductive surgery for ovarian cancer. Gynecol Oncol. 1989; 35(3): 338–340, doi: 10.1016/0090-8258(89)90074-7, indexed in Pubmed: 2599468.
30. Kapnick SJ, Griffiths CT, Finkler NJ. Occult pleural involvement in stage III ovarian carcinoma: role of diaphragm resection. Gynecol Oncol. 1990; 39(2): 135–138, doi: 10.1016/0090-8258(90)90420-p, indexed in Pubmed: 2227587.
31. Chéreau E, Ballester M, Selle F, et al. Pulmonary morbidity of diaphragmatic surgery for stage III/IV ovarian cancer. BJOG. 2009; 116(8): 1062–1068, doi: 10.1111/j.1471-0528.2009.02214.x, indexed in Pubmed: 19459863.
32. Einenkel J, Ott R, Handzel R, et al. Characteristics and management of diaphragm involvement in patients with primary advanced-stage ovarian, fallopian tube, or peritoneal cancer. Int J Gynecol Cancer. 2009; 19(7): 1288–1297, doi: 10.1111/IGC.0b013e3181a3a833, indexed in Pubmed: 19823067.
33. Langstraat C, Aletti GD, Cliby WA. Morbidity, mortality and overall survival in elderly women undergoing primary surgical debulking for ovarian cancer: a delicate balance requiring individualization. Gynecol Oncol. 2011; 123(2): 187–191, doi: 10.1016/j.ygyno.2011.06.031, indexed in Pubmed: 21794902.
34. Chéreau E, Ballester M, Selle F. Comparison of peritoneal carcinomatosis scoring methods in predicting resectability and prognosis in advanced ovarian cancer. Am J Obstet Gynecol. 2010; 202: 178.e1–178.e10.
35. Ataseven B, du Bois A, Reinthaller A, et al. Pre-operative serum albumin is associated with post-operative complication rate and overall survival in patients with epithelial ovarian cancer undergoing cytoreductive surgery. Gynecol Oncol. 2015; 138(3): 560–565, doi: 10.1016/j.ygyno.2015.07.005, indexed in Pubmed: 26163893.

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The value of PET/CT in determining lymph node metastasis of endometrial cancer

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ABSTRACT

Objectives: In our study, the role of fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT) in determining lymph node metastasis of endometrial cancer was evaluated.

Material and methods: The present retrospectively registered study included 80 patients with endometrial cancer who underwent PET/CT in preoperative period. The patients underwent total hysterectomy with bilateral salpingo-oophorectomy and lymph node dissection. Lymph node status was evaluated in histopathologic examination and PET/CT imaging and, the results were compared.

Results: There were 80 patients (mean age 62 years) in the present study. Lymph node metastasis was detected in five patients, whereas 75 patients had no lymph node metastasis. A total of 1,471 lymph nodes were examined histopathologically, revealing benign findings in 1,433 (pelvic = 1018, paraaortic = 415) and malignant findings in 38 (pelvic = 28, paraaortic = 10) lymph nodes. The accuracy, sensitivity and specificity of PET/CT in determining lymph node metastasis was 95%, 80% and 96% in patient-based evaluation, and 97.4%, 78.9% and 98.6% in lymph node-based evaluation, respectively. The detection sensitivity of PET/CT was 0%, 81.4% and 100% in metastatic lymph nodes with a short diameter of ≤ 4 mm, 5–9 mm and ≥ 10 mm, respectively. PET/CT could detect 73.3% of metastatic lymph nodes that had < 10 mm short diameter.

Conclusions: PET/CT is useful method in detecting lymph node metastasis especially that are disregarded by CT or MR in endometrial cancer. Although PET/CT doesn't fully replace the surgical staging, its utilization in preoperative period may guide surgical procedure.

Key words: FDG PET/CT; endometrial cancer; lymph node metastasis

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INTRODUCTION

The most common gynecological neoplasm in industrialized countries is endometrial cancer [1]. The International Federation of Gynecology and Obstetrics (FIGO) recommends total hysterectomy with bilateral salpingo-oophorectomy and lymph node dissection for surgical staging of endometrial cancer [2, 3]. Prognosis in such patients is affected by numerous factors, such as tumor histology, grade, lymph node metastasis, depth of myometrial invasion, cervical invasion, lymphovascular invasion and patient's age [4, 5]. Pelvic lymph nodes are a common site of involvement in endometrial cancer, and patients with lymph node metastasis have considerably lower survival than patients without nodal metastasis [6, 7]. Randomized studies suggest that a routine systematic pelvic lymphadenectomy may contribute to surgical staging without any effect on survival in early-stage endometrial cancer [8, 9]. In this regard, the major role of lymphadenectomy is to decide on adjuvant therapy by contributing to the staging and the prediction of prognosis in endometrial cancer [10]. Lymph node dissection may cause significant morbidity, and metastasis may not be found in patients undergoing dissection [10]. Thus, the accurate prediction of lymph node status in preoperative period is important if futile lymph node dissection is to be avoided [11]. Magnetic resonance (MR) and computed tomography (CT) may fail to determine lymph node metastasis accurately [12, 13]. Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT) is a commonly used method for the diagnosis and staging of numerous malignancies, and in evaluating response to therapy, providing both functional and anatomical information. It is suggested that functional changes that are detectable on a PET/CT precede morphological changes that can be detected using conventional CT and MR [14].

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Objectives

The authors of the present study assess the role of F-18 FDG PET/CT in determining lymph node metastasis of endometrial cancer.

MATERIAL AND METHODS

Patients

This retrospective study included 80 patients with a histopathological diagnosis of endometrial cancer who underwent preoperative F-18 FDG PET/CT in our department between February 2010 and March 2014.

Surgery

All patients underwent total hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy (external iliac, superficial and deep common iliac lymph nodes). There were positive lymph nodes in the pelvic and/or paraaortic areas on PET/CT scans in 7 patients. Furthermore, there were 43 patients with suspected for paraaortic lymph node metastasis during surgery. Therefore, these fifty patients underwent an additional paraaortic lymphadenectomies (precaval and paracaval, superficial and deep interaortocaval and paraaortic lymph nodes up to the renal vessels). All lymph nodes were evaluated by intraoperative inspection and palpation. Palpable, enlarged or fixed lymph nodes were regarded suspicious for malignancy. Furthermore, frozen section of uterine resection was evaluated in terms of tumor size, grade, histology and myometrial and cervical invasion status. Patients with myometrial invasion < 50% and without cervical invasion endometrioid cancer (grade 1 or tumor diameter < 2 cm grade 2) were considered to be at low risk for paraaortic lymph node metastasis, whereas others high risk. Paraaortic lymphadenectomy was performed in high-risk patients and those with suspicious lymph nodes during the operation.

There was a patient who was suspected to have omental metastasis during the operation, so she underwent omental resection. Although no metastasis was detected in frozen sections of the omentum, microscopic metastasis was found in the final histopathological evaluation. Since the distant metastasis of the patient with stage 4B could not be detected during the operation or preoperative PET/CT, lymphadenectomy was performed.

Histopathologic Evaluation of Lymph Nodes

Uterus, bilateral adnexal tissues, lymph nodes and any other resected tissues were analysed by experienced pathologists.

Lymph nodes were fixed with 10% formalin and then embedded in paraffin. Paraffin blocks were sliced and processed. Tissue sections were firstly stained with hematoxylin and eosin (H&E). If metastasis was detected in this procedure, no further histological examination was performed. If the serial H&E sections were negative, immunohistochemical (IHC) methods were performed. PAN-cytokeratin was used for IHC evaluation. Histopathologically, the lymph nodes were defined as benign and malignant.

F-18 FDG PET/CT

PHILIPS GEMINI TF 16 Slice PET/CT scan was used for imaging. Patients with a blood glucose level below 200 mg/dL following an at least 6 hours of fasting received an intravenous 8–11 mCi FDG injection. The urinary bladder was evacuated approximately 60 minutes after the injection, and the body area from the vertex of the skull to the upper femur was scanned. CT images were captured initially (140 kV, 100 mAs, 5 mm slice) followed by PET scans. PET scanning was performed at 9–10 bed positions depending on the height of the patient, each bed position lasting 90 seconds.

The blood pool activity was taken into consideration while evaluating the FDG uptake by the lymph nodes on PET/CT. Regardless of the lesion size, a lymph node showing a FDG uptake equal to or below the blood pool activity was considered negative for malignancy, and a FDG uptake above this threshold was considered positive for malignancy.

Data and Statistical Analyses

The histopathological examination results were considered as the reference standard in the evaluation of lymph node metastasis. A histopathological examination of the lymph nodes identified the lymph nodes as benign or malignant. The result of PET/CT was considered a true negative (TN) if it showed negative findings, and the result was considered false positive (FP) if the PET/CT showed positive findings in a histopathologically benign lymph node. The result of PET/CT was considered a true positive (TP) if it showed positive findings, and the result was considered a false negative (FN) if it showed negative findings in a histopathologically malignant lymph node. The accuracy, sensitivity and specificity of PET/CT in determining lymph node metastasis was calculated using a standard formula on a patient and lymph node basis.

$$\text{Sensitivity} = TP/(TP + FN), \quad \text{Specificity} = TN/(TN + FP),$$
$$\text{Accuracy} = (TP + TN)/(TP + FP + TN + FN)$$

RESULTS

Patients and Histopathologic Findings

The study included 80 patients who had endometrial cancer with a mean age of 62 ± 5.4 years (range 37–80). All patients underwent a pelvic lymphadenectomy, while 50 patients underwent an additional paraaortic lymphadenectomy. Lymph node metastasis was detected in five patients, whereas 75 patients had no lymph node metastasis.

Of the patients with lymph node metastasis, one had endometroid and 4 had nonendometroid (mixed = 2, serous = 1, undifferentiated = 1) histology. The tumor grade was 2 in 1 patient and 3 in 4 patients. According to FIGO 2009 staging, 2 of the patients were in stage 3C1, 2 of them were in stage 3C2, 1 of them was in stage 4B.

In patients without lymph node metastasis the histologic types were endometrioid carcinoma (n = 47), adenocarcinoma with squamous differentiation (n = 10), squamous carcinoma (n = 8), undifferentiated carcinoma (n = 6), serous carcinoma (n = 2), clear cell carcinoma (n = 1) and mucinous carcinoma (n = 1). 22 cases were grade 1, 32 cases were grade 2 and 21 cases were grade 3. Of the 75 patients, 40 were stage 1A, 12 were stage 1B, 10 were stage 2, 5 were stage 3A and 8 were stage 3B.

When evaluated on an lymph node basis, of the total 1,471 lymph nodes, 1,046 were found in the pelvic area and 425 in the paraaortic area in a histopathological examination. Of these lymph nodes, 1,433 (pelvic lymph node = 1018, paraaortic lymph node = 415) were benign and 38 (pelvic lymph node = 28, paraaortic lymph node = 10) were malignant (Tab. 1).

Lymph Node Findings on PET/CT

Lymph node metastasis was detected in five out of 80 patients, whereas 75 patients had no lymph node metastasis. When evaluated on a patient basis, PET/CT was negative in 72 (TN) and positive (FP) in three of the 75 patients with a benign lymph node. PET/CT was positive in four (TP) and negative (FN) in one out of the five patients with at least one malignant lymph node on a histopathological examination. The results are presented in Table 2. When evaluated on a patient basis, the accuracy, sensitivity and specificity of PET/CT in detecting lymph node metastasis was 95%, 80% and, 96% respectively.

Table 1. Histopathologically evaluated lymph nodes

	Benign	Malign	Total
Pelvic lymph node	1018	28	1046
Paraaortic lymph node	415	10	425
Total	1433	38	1471

Results are shown as number

Table 2. Correlation of PET/CT results with pathologic findings on the basis of patient

	PET/CT positive	PET/CT negative	Total
Pathology positive	4	1	5
Pathology negative	3	72	75
Total	7	73	80

Results are shown as number

Table 3. Correlation of PET/CT results with pathologic findings on the basis of lymph node

		PET/CT positive	PET/CT negative	Total
Pelvic lymph node	pathology positive	22	6	28
	pathology negative	11	1007	1018
Paraaortic lymph node	pathology positive	8	2	10
	pathology negative	9	406	415
Total		50	1421	1471

Results are shown as number

When evaluated on an lymph node basis, PET/CT was positive in 20 (FP) and negative in 1,413 (TN) out of the 1,433 lymph nodes that qualified histopathologically as benign. PET/CT was positive in 30 (TP) and negative in eight (FN) of the 38 lymph nodes that qualified as malignant upon a histopathological examination. The results are presented in Table 3. When evaluated on an lymph node basis, the accuracy, sensitivity and specificity of PET/CT in detecting lymph node metastasis was 97.4%, 78.9% and 98.6%, respectively. Sample lymph node findings of PET/CT that were evaluated based on histopathological results are presented in Figure 1.

When 38 metastatic lymph nodes are grouped according to their short diameters; 3 of them were ≤ 4 mm, 27 were 5–9 mm, and 8 were ≥ 10 mm. PET/CT could not detect any metastatic lymph nodes with a short diameter of ≤ 4 mm, while it was true positive in 22 of those with a short diameter of 5–9 mm and in all of those with ≥ 10 mm. Thus, PET/CT was able to detect 22 of 30 metastatic lymph nodes with a short diameter of less than 10 mm (73.3%). The detection sensitivity of PET/CT was 0% (0/3), 81.4% (22/27) and 100% in metastatic lymph nodes with a short diameter of ≤ 4 mm, 5–9 mm and ≥ 10 mm, respectively.

DISCUSSION

Detecting the presence of lymph node metastasis in endometrial cancer is important in treatment decisions, as well as in predicting prognosis. However, only 20–25% of high-risk patients show nodal metastasis and up to 80% of patients undergo a futile systematic lymphadenectomy [15]. Furthermore, it has been demonstrated that a routine systematic lymphadenectomy offers no survival benefit in early-stage endometrial cancer [8, 9]. Lymph node dissection is currently considered the optimum method in determining the status of lymph nodes in endometrial cancer [11], although there are ongoing efforts to identify a preoperative, predictive non-invasive method. Determining the status of lymph nodes preoperatively avoids futile lymph node dissection, thereby decreasing morbidity and reducing costs. CT and MR are commonly used methods in

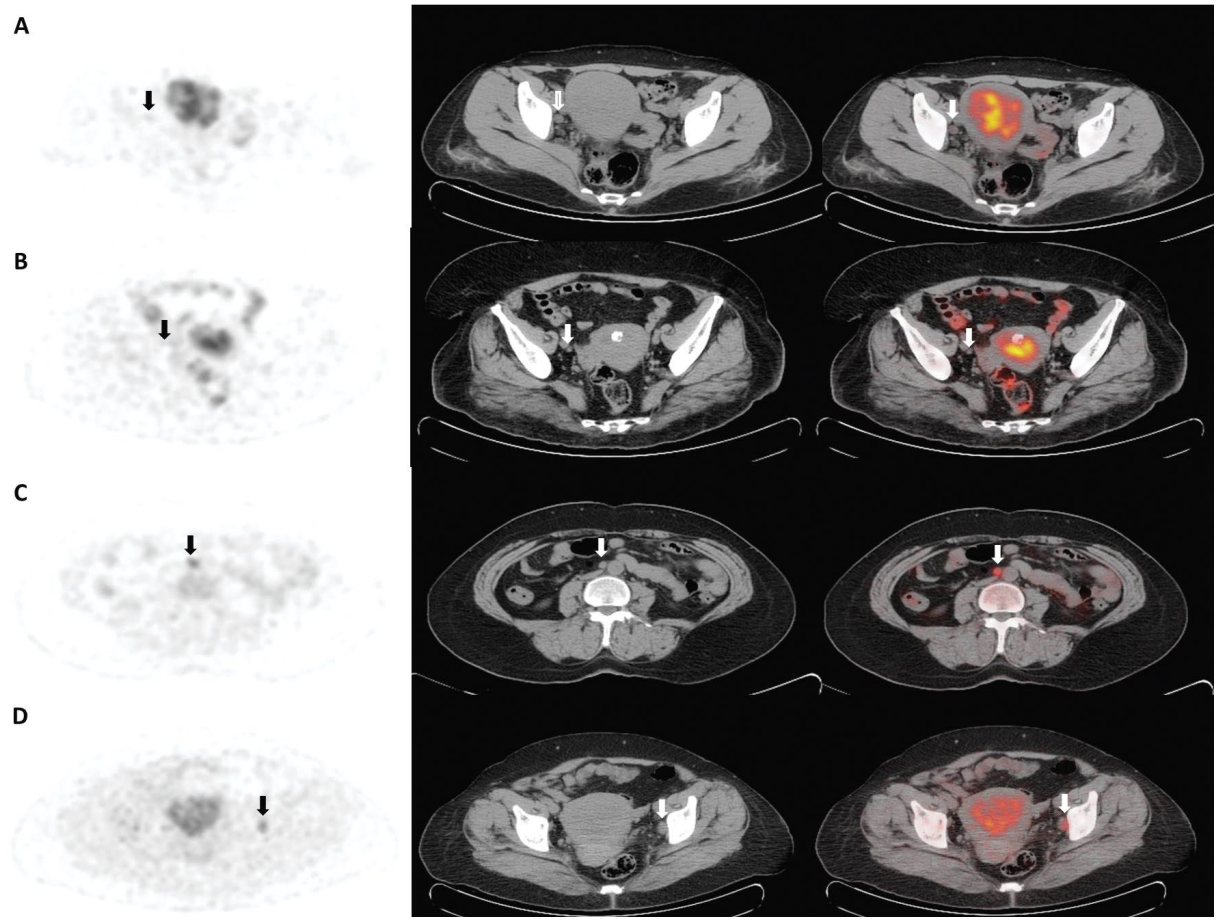

Figure 1. (A–D): Axial PET, CT, and fusion images of the lymph nodes evaluated as true negative (A, arrow), false negative (B, arrow), true positive (C, arrow), and false positive (D, arrow) on PET/CT are presented.; **A.** No lymph nodes with increased FDG uptake were observed in the pelvic or paraaortic regions on the PET/CT of a 44-year-old patient. No metastasis was determined upon a histopathological examination of the patient's 52 lymph nodes (44 pelvic and 8 paraaortic). The PET/CT result was a true negative for all 52 lymph nodes; **B.** No increased FDG uptake was observed in the lymph nodes seen in the pelvic and paraaortic regions in the PET/CT of a 74-year-old patient. A total of 28 lymph nodes were dissected from the pelvic (n: 21) and paraaortic (n: 7) regions in the case, in which metastasis was suspected intraoperatively. Metastasis was determined in the five lymph nodes in the right external and internal iliac regions, while other lymph nodes were determined to be benign. The PET/CT was false negative in those five lymph nodes and true negative in the other 23 lymph nodes; **C.** An increased FDG uptake was noted in one lymph node in the paraaortic region and in three lymph nodes in the pelvic region in a PET/CT of a 49-year-old patient. Metastasis was determined positive in the four lymph nodes histopathologically, and hence PET/CT findings were evaluated to be true positive; **D.** An increased FDG uptake was noted in three lymph nodes in the paraaortic region and in three lymph nodes in the pelvic region on a PET/CT. No metastasis was determined to be positive histopathologically in the total number of 30 lymph nodes (22 pelvic, 8 paraaortic) in this case. The PET/CT findings were considered false positive in six lymph nodes and true negative in the remaining 24 lymph nodes

the preoperative evaluation of patients with endometrial cancer. However, these conventional methods are predicated on the size of the lymph nodes in the evaluation and a short diameter of ≥ 10 mm is the most accepted criterion for the identification of suspected lymph nodes. On this point, it is inevitable that < 10 mm short diameter metastatic lymph nodes are underestimated by CT or MR. However, FDG PET/CT provides functional data while also identifying morphological changes, suggesting that it may be superior to anatomical visualization alone in the evaluation of the status of lymph nodes [14]. In the present study, there were 30 metastatic lymph nodes with < 10 mm short diameter. PET/CT was able to detect 22 of them (73.3%) and so had a great value in the detection of subcentimeter metastatic lymph nodes which have been disregarded by CT and MR. CT and MR are asserted to have a sensitivity of 29–66% and a specificity of 73–99% in various studies [12, 16, 17]. A study involving 287 patients with endometrial cancer found that PET/CT was more sensitive than MR in detecting lymph node metastasis in a patient-based evaluation (70% vs. 34%), although specificity rates were similar (95.4% vs. 95%). The sensitivity and specificity of PET/CT were found to be 79.4% and 96.7% in an lymph node-based evaluation, with MR showing rates of 51.6% and 97.6%, respectively [18]. In a meta-analysis of 13 patient-based studies involving a total of 861 patients with endometrial cancer, the

pooled specificity of FDG PET/CT in detecting lymph node metastasis was 94% (93–96%) and the pooled sensitivity was 72% (63–80%) [14]. In another meta analysis of seven studies (243 patients with endometrial cancer), the pooled specificity and sensitivity of PET or PET/CT in detecting lymph node metastasis was 94.7% (90.4–97.4%) and 63% (48.7–75.7%), respectively [19]. In a study by Crivellaro et al. [15] involving 76 high-risk patients (serous/clear cell carcinoma, grade 2 with deep myometrial invasion, grade 3) with clinical stage 1 endometrial cancer, the accuracy, sensitivity and specificity of PET/CT in detecting lymph node metastasis were 94.7%, 78.6% and 98.4% in a patient-based evaluation, and 95.8%, 67.6% and 98.2% in an lymph node-based evaluation, respectively. In a study involving 40 patients with stage 1A–C endometrial cancer, the accuracy, sensitivity and specificity of PET/CT in detecting lymph node metastasis were found to be 97.8%, 53.3% and 99.6% in a lymph node-based evaluation, respectively. When the lymph nodes were grouped according to their diameters, sensitivity was 16.7%, 66.7% and 93.3% in lesions measuring ≤ 4 mm, 5–9 mm and ≥ 10 mm in diameter, respectively [20]. In a study by Kitajima et al. [21] involving patients with endometrial cancer and cervical cancer and using a similar lymph node classification, sensitivity was 12.5%, 66.7% and 100%, respectively. Another study using FDG-PET detected no lymph node metastasis smaller than 6 mm [22]. The accuracy, sensitivity and specificity of PET/CT in detecting lymph node metastasis in the present study were found to be 95%, 80% and 96% in a patient-based evaluation, and 97.4%, 78.9% and 98.6% in an lymph node-based evaluation. Also, the detection sensitivity of PET/CT was 0%, 81.4% and 100% in metastatic lymph nodes with a short diameter of ≤ 4 mm, 5–9 mm and ≥ 10 mm, respectively. PET/CT could not detect any three metastatic nodes 4 mm or smaller. This is probably because of the currently used PET/CT techniques cannot detect micrometastatic diseases due to their low spatial resolution. The specificity of PET/CT in the present study was similar to literatüre results and, the sensitivity was higher than some of those reported in literature. The authors consider that lymph nodes evaluated in studies with variable sizes and the inability to recognize small-size lymph node metastasiss may result in different sensitivity rates being reported in such studies. For example, in our study, the rate of metastatic lymph nodes with a short diameter of ≤ 4 mm was 3/38 whereas in the study by Kitajima it was 16/45. Furthermore, the selection of low- or high-risk patients in some studies may have also affected the results.

CONCLUCIONS

The present study is limited by its retrospective design and the relatively small number of patients with lymph node metastasis. That said, PET/CT showed high sensitivity, specificity and accuracy in the detection of lymph node metastasis, and had a great value in the detection of subcentimeter ones. Therefore, PET/CT can direct the surgical procedure even though it does not completely replace the surgical staging.

REFERENCES

1. Signorelli M, Guerra L, Buda A, et al. Role of the integrated FDG PET/CT in the surgical management of patients with high risk clinical early stage endometrial cancer: detection of pelvic nodal metastases. Gynecol Oncol. 2009; 115(2): 231–235, doi: 10.1016/j.ygyno.2009.07.020, indexed in Pubmed: 19695685.
2. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009; 105(2): 103–104, doi: 10.1016/j.ijgo.2009.02.012, indexed in Pubmed: 19367689.
3. Creasman W. Revised FIGO staging for carcinoma of the endometrium. Int J Gynaecol Obstet. 2009; 105(2): 109, doi: 10.1016/j.ijgo.2009.02.010, indexed in Pubmed: 19345353.
4. Uharcek P, Mlyncek M, Lajtman E, et al. Prognostic factors in endometrial carcinoma. J Obstet Gynaecol Res. 2008; 34(5): 776–783, indexed in Pubmed: 18958927.
5. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. Ann Oncol. 2016; 27(1): 16–41, doi: 10.1093/annonc/mdv484, indexed in Pubmed: 26634381.
6. Manetta A, Delgado G, Petrilli E, et al. The significance of paraaortic node status in carcinoma of the cervix and endometrium. Gynecol Oncol. 1986; 23(3): 284–290, doi: 10.1016/0090-8258(86)90128-9, indexed in Pubmed: 3957115.
7. Gal D, Recio FO, Zamurovic D, et al. Lymphvascular space involvement--a prognostic indicator in endometrial adenocarcinoma. Gynecol Oncol. 1991; 42(2): 142–145, doi: 10.1016/0090-8258(91)90334-2, indexed in Pubmed: 1894173.
8. Kitchener H, Swart AMC, Qian Q, et al. ASTEC study group. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. Lancet. 2009; 373(9658): 125–136, doi: 10.1016/S0140-6736(08)61766-3, indexed in Pubmed: 19070889.
9. Benedetti Panici P, Basile S, Maneschi F, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. J Natl Cancer Inst. 2008; 100(23): 1707–1716, doi: 10.1093/jnci/djn397, indexed in Pubmed: 19033573.
10. May K, Bryant A, Dickinson HO, et al. Lymphadenectomy for the management of endometrial cancer. Cochrane Database Syst Rev. 2010(1): CD007585, doi: 10.1002/14651858.CD007585.pub2, indexed in Pubmed: 20091639.
11. Park JY, Lee JJ, Choi HJ, et al. The Value of Preoperative Positron Emission Tomography/Computed Tomography in Node-Negative Endometrial Cancer on Magnetic Resonance Imaging. Ann Surg Oncol. 2017; 24(8): 2303–2310, doi: 10.1245/s10434-017-5901-8, indexed in Pubmed: 28550488.
12. Connor JP, Andrews JI, Anderson B, et al. Computed tomography in endometrial carcinoma. Obstet Gynecol. 2000; 95(5): 692–696, doi: 10.1016/s0029-7844(99)00626-2, indexed in Pubmed: 10775731.
13. Ryoo UiN, Choi CH, Yoon JiY, et al. MR imaging in endometrial carcinoma as a diagnostic tool for the prediction of myometrial invasion and lymph node metastasis. Cancer Res Treat. 2007; 39(4): 165–170, doi: 10.4143/crt.2007.39.4.165, indexed in Pubmed: 19746183.
14. Bollineni VR, Ytre-Hauge S, Bollineni-Balabay O, et al. High Diagnostic Value of 18F-FDG PET/CT in Endometrial Cancer: Systematic Review and Meta-Analysis of the Literature. J Nucl Med. 2016; 57(6): 879–885, doi: 10.2967/jnumed.115.170597, indexed in Pubmed: 26823564.
15. Crivellaro C, Signorelli M, Guerra L, et al. Tailoring systematic lymphadenectomy in high-risk clinical early stage endometrial cancer: the role of 18F-FDG PET/CT. Gynecol Oncol. 2013; 130(2): 306–311, doi: 10.1016/j.ygyno.2013.05.011, indexed in Pubmed: 23707673.
16. Rockall AG, Sohaib SA, Harisinghani MG, et al. Diagnostic performance of nanoparticle-enhanced magnetic resonance imaging in the diagnosis of lymph node metastases in patients with endometrial and cervical cancer. J Clin Oncol. 2005; 23(12): 2813–2821, doi: 10.1200/JCO.2005.07.166, indexed in Pubmed: 15837995.
17. Rockall AG, Meroni R, Sohaib SA, et al. Evaluation of endometrial carcinoma on magnetic resonance imaging. Int J Gynecol Cancer. 2007; 17(1): 188–196, doi: 10.1111/j.1525-1438.2007.00805.x, indexed in Pubmed: 17291252.

18. Kim HJ, Cho A, Yun M, et al. Comparison of FDG PET/CT and MRI in lymph node staging of endometrial cancer. Ann Nucl Med. 2016; 30(2): 104–113, doi: 10.1007/s12149-015-1037-8, indexed in Pubmed: 26546334.
19. Chang MC, Chen JH, Liang JA, et al. 18F-FDG PET or PET/CT for detection of metastatic lymph nodes in patients with endometrial cancer: A systematic review and meta-analysis. Eur J Radiol. 2012; 81(11): 3511–3517, doi: 10.1016/j.ejrad.2012.01.024.
20. Kitajima K, Murakami K, Yamasaki E, et al. Accuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. AJR Am J Roentgenol. 2008; 190(6): 1652–1658, doi: 10.2214/AJR.07.3372, indexed in Pubmed: 18492920.
21. Kitajima K, Murakami K, Yamasaki E, et al. Accuracy of integrated FDG-PET/contrast-enhanced CT in detecting pelvic and paraaortic lymph node metastasis in patients with uterine cancer. Eur Radiol. 2009; 19(6): 1529–1536, doi: 10.1007/s00330-008-1271-8, indexed in Pubmed: 19184037.
22. Suzuki R, Miyagi E, Takahashi N, et al. Validity of positron emission tomography using fluoro-2-deoxyglucose for the preoperative evaluation of endometrial cancer. Int J Gynecol Cancer. 2007; 17(4): 890–896, doi: 10.1111/j.1525-1438.2007.00859.x, indexed in Pubmed: 17343574.

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The role of nesfatin and selected molecular factors in various types of endometrial cancer

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ABSTRACT

Objectives: Endometrial cancers (ECs) are the most common gynaecological cancers in well developed countries. Diabetes and metabolic syndrome are among the biggest risk factors. Nesfatin-1, the adipokine derivative of NUCB2 (nucleobindin derivative 2) is linked to the clinical course of EC. Molecular factors, including mutations in MLH1 and MHS2 genes, c-MET and ARID1A are also related to prognosis in endometrial cancer.

Material and methods: Using sections of paraffin-embedded preparations and immunohistochemistry, the expression of NESF1, MLH1, MSH2,c-MET and ARID1A were examined.

Results: In this study on protein expression, EC tissues manifested (although insignificantly) an elevated expression of NESF-1 in type II EC. In type I EC, NESF-1 expression was significantly higher in G1 in comparison to G2 and G3 together. A significantly lower expression of MLH1 was demonstrated in type I EC.

Conclusions: The most pronounced expression involved c-MET in all EC I and EC II tissues (in over 80% of cases). A tendency was detected for a high expression of NESF-1 in patients with type II EC, who also exhibited a high expression of MSH2.

Key words: endometrial cancer; NESF-1; MLH1; MSH2; c-MET; ARID1A

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INTRODUCTION

Endometrial cancer (EC) is the most commonly occurring gynaecological malignancy in economically developed countries. In Europe, the incidence rate amounts to 13.6 per 100.000 women. In many European countries an increased incidence of this tumor has been noted since 2005 [1–3]. In over 90% of EC cases, the cancer develops after the age of 50, with a median age of 63; in 10–15% it is diagnosed before the age of 45 [2, 4].

Bokhman's hypothesis led to the identification of two types of EC differing in etiology, biology and clinical course [5].

Type I EC (*endometrioid adenocarcinoma*) involves 80% of all EC cases. In most cases it is sporadic and linked to unbalanced estrogen stimulation and metabolic syndrome. This EC type manifests slow clinical course and positive prognosis. This type of cancer contains mutations in the *PTEN, KRAS, CTNNB1, P1K3CA* genes and in mismatch repair *(MMR)* genes, typical for Lynch syndrome [2, 6–8].

Type II EC (*non-endometrioid adenocarcinoma*) histologically encompasses serous, clear cell, poorly differentiated cancers of aggressive biology and an unfavorable clinical course. They contain mutations in *TP53*, *HER2-neu* and *BRCA* [2, 9, 10–12].

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Type I EC with a high histological grade (G3) manifests a similarly poor prognosis to uterine serous carcinoma (USC), belonging to type II [6, 3, 13, 14], some well-differentiated, early diagnosed endometrioid EC gives recurrent disease, which suggests that the traditional Bokman's division may not be up to date.

Recent results have shown that more EC types exist. The heterogeneity of ECs has been proven by genomic analysis of endometrioid and serous cancers. The Cancer *Genome Atlas* (TCGA) developed in 2013, indicates there are 4 types of ECs [15]:

— *unique nucleotides spectrum* ultra-mutated tumors *(POLE)* — associated with favourable outcome,
— highly-mutated with microsatellite instability, mostly with MLH1 promoter methylation,
— a group of low frequency of mutations with low gene copy numbers,
— a group with high gene copy numbers and a low frequency of mutations, mainly serous cancers, connected to a poor prognosis.

It is suggested that the Genomic Atlas classification (TGGA) should be linked to the histological classification of ECs, particularly ECs of poor differentiation [16].

The acknowledged and recognized factors which affect the clinical course and type of oncological treatment of EC include the histological type, grading (G) and clinical stage of disease. However, these traditional prognostic factors do not allow for tailoring of treatment to individual patients which could spare many side effects for over-treated women and reduce the number of recurrences in under-treated patient groups.

Molecular studies also indicate the relationship between EC clinical course and several molecular variables, such as: condition of hormonal receptors (ER, PR), ARID1A and c-MET linked to poor prognosis, metastases and mutations in MMR genes detected in Lynch syndrome [17–21]. Recent published studies indicated a relationship between Nesfatin-1 (NESF-1) with its precursor, NUCB2-nucleobindin 2, and the clinical course of EC [22, 23]. NESF-1 is associated with obesity and diabetes type II (main risk factors for EC) and participates in the regulation of hunger and fat storage, it is associated with insulin resistance and glucose homeostasis.

The aim of this study was to estimate the expression of NESF-1, MLH1 and MLH2 belonging to MMR, c-MET and ARID1A in two types of endometrial cancer and determine relationships between the mentioned factors.

MATERIAL AND METHODS

This was a multi-centre, retrospective study. The investigated material involved archival, histological preparations, obtained from uterine endometrial cancer samples taken from patients primarily treated surgically between 2007–2014.

All 146 patients included in this retrospective study were monitored for the entire five to eight-year period from diagnosis of EC through the duration of their treatment and subsequent observation. In order to fulfil the selection criteria for the study, patients must have been diagnosed at one of the centres participating in the study. Only patients where information on FIGO grading and histology were complete, who underwent treatment for EC at the centre where they were originally diagnosed and then monitored for the stated study period by practitioners at that specific centre were included in this study.

Out of a total of 146 patients, 38 (26%) patients were diagnosed at stage IA according to FIGO, 36 patients (24.7%) at stage IB, 37 patients (25.4%) at stage II, 22 (15%) at stage III and 13 (8.9%) at stage IV.

In the studied group, 115 patients were diagnosed with type I EC (78.8%), in 31 type II EC (21.2%) was detected, this included 18 serous types (12.3%), 11 clear cell types and 2 mucinous types (1.4%).

In 38 patients (25%), endometrial cancer manifested a high histological maturity (G1), in 59 patients (38.8%) it displayed an intermediate histological differentiation (G2) while in 55 patients (36.2%) undifferentiated tumors were identified (G3) (Tab. 1).

Table 1. Clinicopathologic characteristics of studied patients with endometrial carcinoma

Clinical Staging acc. to FIGO	**Number of patients**
Endometrioid adenocarcinoma (n = 115)	
IA	33
IB	32
II	27
III	14
IV	9
Serous adenocarcinoma G3 (n = 18)	
IA	4
IB	3
II	7
III	4
Clear cell *adenocarcinoma* G3 (n = 11)	
IA	1
IB	1
II	2
III	4
IV	3
Mucinous adenocarcinoma (n = 2)	
II	1
IV	1
Grading	**Number of patients**
Endometrioid adenocarcinoma (n = 115)	
G1	36
G2	56
G3	23

The mean age of the entire patient group studied was 65.3; in women with endometrioid cancer the mean age was 64.7 (34–83) and in women with non-endometrioid cancer it was 67.6 (40–83) ($p > 0.05$).

The tissue material was fixed in 10% buffered formalin, pH 7.4 and placed in a processor. The neoplastic tissue was embedded in paraffin at 60°C using standard histopathological techniques. The paraffin blocks were sliced in a microtome to 4–5 um thick sections, placed on adhesive glass slides and left for one hour at a temperature of 60°C. In the study, the immunohistochemical method — DAKO Envision™ Flex+ (Dako system, Dako, Santa Clara, USA) was applied. Antigens were detected in the paraffin sections using the Target Retrieval Solution, high pH, DAKO in the PT-link (Dako, Santa Clara, USA) at a temperature of 97°C, for 20 min.

Nesfatin-1 was estimated using Nesfatin-1/Nucleobinding-2 Antibody (Novus Biologicals, Littleton, USA, NBP1-87383). In order to detect antigens present in the tissue material antibodies were used against ARID1A (Novus Biological, Littleton, USA, NBP1-88932), Met (Santa Cruz Biotechnology, Santa Cruz, USA, clone C-12, MLH1 (Leica NCL-L, Buffalo Grove, USA. Clone E-305), MSH2 (Invitrogen, Carlsbad, USA , clone FE11).

The intensity of NESF-1, ARID1A, c-MET, MLH1, MSH2 staining was estimated using a 4-degree scale:
no reaction
+ 1 to 50 immunopositive cells (cell nuclei or the cytoplasm)
++ 50 to 75 immunopositive cells
+++ 75 to 100 immunopositive cells
per 10 visual fields.

A positive reaction was accepted in the case of preparations manifesting ++ or +++ staining.

Statistical calculations were made using the Mann-Whitney, Kruskall-Wallis and Spearman's tests (STATISTICA StatSoft Inc, USA). Statistical significance was concluded when p was less than 0.05.

RESULTS

A high expression of NESF-1, MLH1, MSH2, c-MET and ARID1A was detected, respectively, in 53.1%, 57.7%, 47.3%, 88.7% and 48.2% patients with endometrial cancer.

The immunohistochemical reaction with antibodies against NESF-1 and c-MET was seen in the cytoplasm; MLH1, MSH2 and ARID1A manifested nuclear localization.

Histopathological type

In respect to histopathological diagnosis the patients formed two groups: endometrioid type (n = 115) and non-endometrioid type (n = 31), which included patients with serous, clear cell and mucinous cancers.

Positive expression of nesfatin was determined in 50.99% of patients with type EC I and in 61.3% of patients with type II EC. No significant difference was disclosed between the groups ($p = 0.410$). MLH1 expression was statistically lower in type EC I in comparison to type II EC (51.9% vs 77.4%, $p = 0.013$). No significant difference was disclosed in the expression of MSH2, c-MET and ARIDIA between the two subgroups ($p < 0.05$) (Fig.1).

Clinical stage according to FIGO

Patients with endometrial cancer were subdivided depending on clinical stage into early stage (IA) and later stage of the disease (IB–IV). In the entire group of patients with endometrial cancer, no relationship was detected between intensity of NESF-1 expression and the stage of clinical ad-

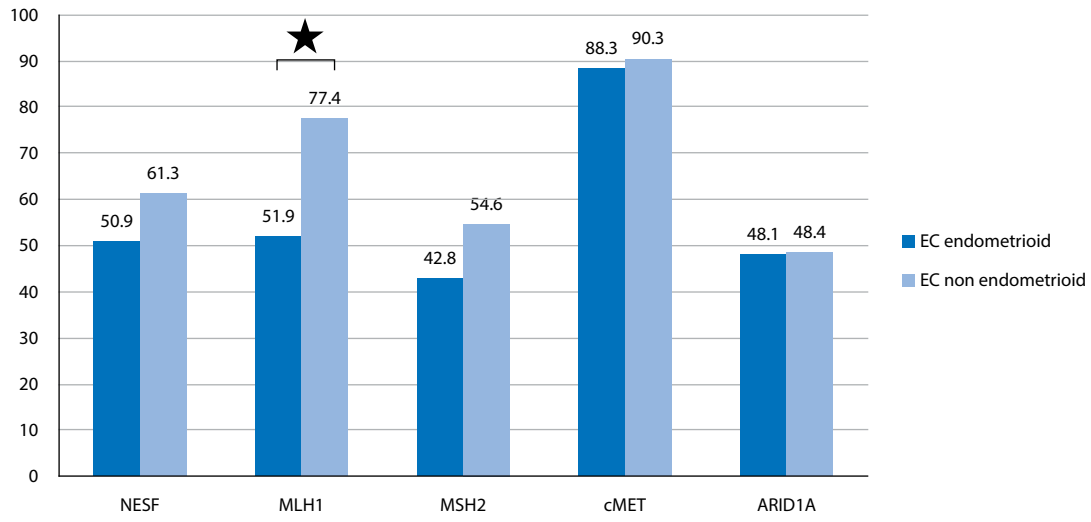

Figure 1. Percentage of endometrial cancer cases manifesting a high expression of a given protein in endometrioid and non-endometrioid cancer cells; ★ $p < 0.05$

vancement of the disease (p = 0.382). Also no such relationship was detected for MLH1, MSH2, c-MET and ARIDIA.

Grading

Among all the analyzed patients no relation was detected between the level of NESF-1 expression and histological grade of cancer differentiation (p = 0.3145). Furthermore, no such relationship was detected for MLH1, MSH2, c-MET and ARID1A (p > 0.05) (Fig. 2, 3). The group of patients with type I EC with grade 1 manifested a statistically higher expression of nesfatin than the patients with histologically less mature cancer (G1 68.97% vs G2 + G3 45.45%, p = 0.0487). The relationship between the level of studied protein expression and histological grading of non-endometrioid cancers was not analyzed since the latter by definition are poorly differentiated cancers (G3).

Correlation between evaluated proteins

Among all the studied patients with ECs, a high expression of NESF-1 correlated with a high expression of the MLH1 protein (p = 0.039). No relationship was detected between the level of NESF-1 expression and MSH2, c-MET and ARID1A in the entire population of patients with endometrial cancer (p = 0.43, p = 0.24, p = 0.4158).

DISCUSSION

NESF-1, the amino acid derivative of NUCB2 carries prognostic significance in type I EC; its immunoreactiv-

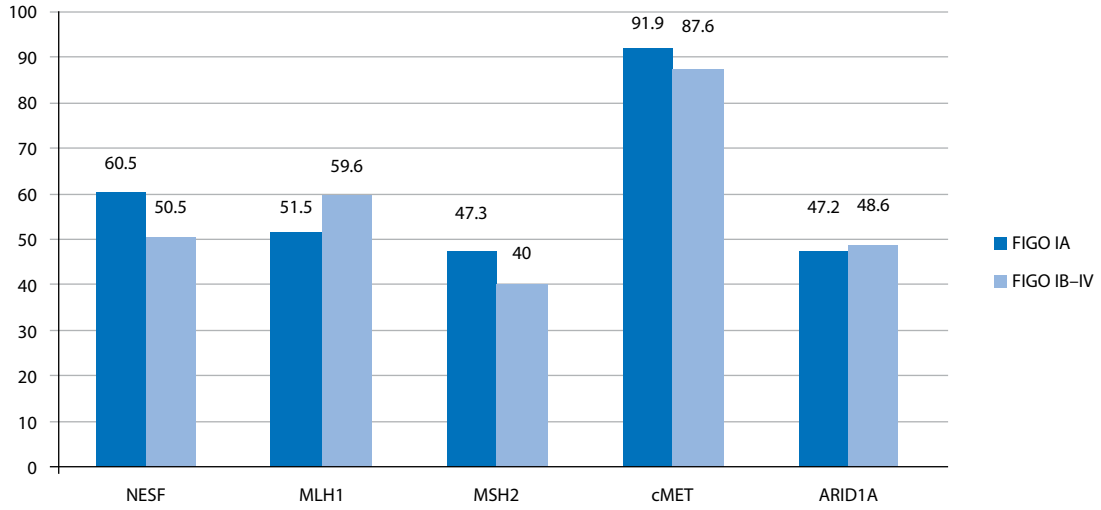

Figure 2. Percentage of endometrial cancer cases manifesting a high expression of a given protein in relation to the clinical stage

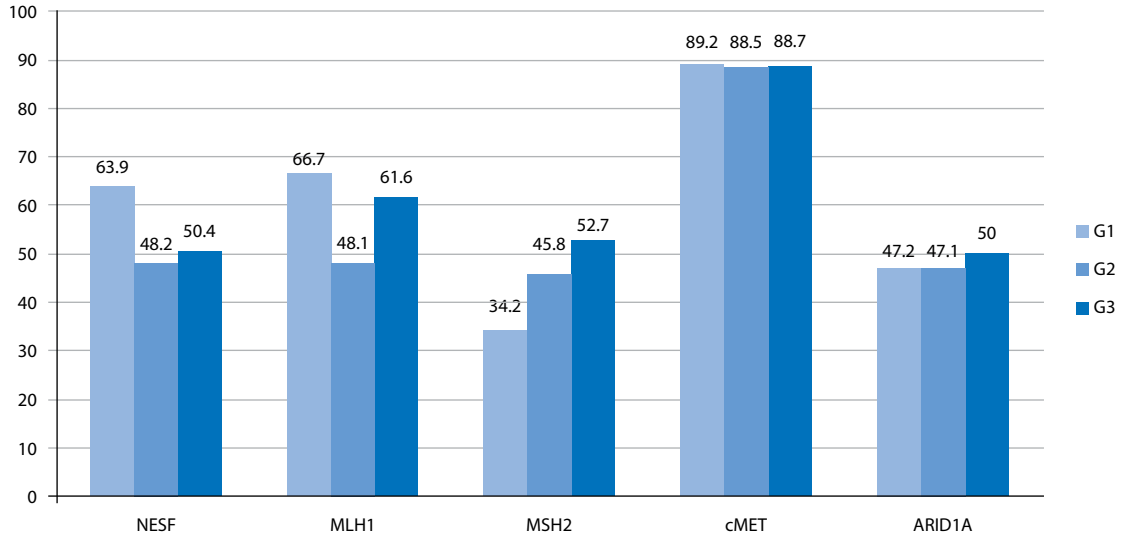

Figure 3. Percentage of endometrial cancer cases in the entire population of patients manifesting high expression of a given protein in relation to histopathological grading

ity correlated with an augmented risk of relapse and poor clinical course [23]. In the presented study, two groups of patients were analysed — women with endometrial cancer endometrioid type (EC I) and non-endometrioid (EC II). The expression of NESF-1 was more pronounced in type II EC (61.3%) with a poorer prognosis compared to type I EC (NESF-1 expression in 50.9% cells). However, no statistically significant values were determined ($p = 0.410$). The evaluation of expression in various grades of histological grading determined that patients with type I EC at G1 manifested a higher expression of NESF-1 than those with type I EC at both G2 and G3 (G1 — 68.97% compared to G2 and G3 — 50.4%, $p = 0.0487$). Similar results were observed in studies of Takagi et al. [23] in type I EC, though no statistical significance was identified (in G1 cancers the proportion of positive reaction was higher than in G2 and G3).

Mutations in MLH1 and MSH2 linked with Lynch syndrome carry a risk of developing type I EC. In the group of patients presented in this study, the expression of MLH1 protein was significantly higher in type II EC. Among all 146 patients, a high expression of NESF-1 correlated with a high expression of MLH1 ($p = 0.039$), which seems to be slightly controversial. In the literature, a high expression of NESF-1 was linked to poorer prognosis, while MLH1, associated with type I EC, was linked to better prognosis [2, 23, 24]. Dividing the patients according to the histological type of the tumor, a tendency was noted for a high expression of NESF-1 in patients with a high expression of MSH2 ($p = 0.0596$). This might indicate that despite the normal function of the MMR protein group, a high expression of NESF-1 is significant for prognosis in the patient group.

c-Met, also called tyrosine-protein kinase Met, is proto-oncogene tyrosine kinase, located on chromosome 7q21-31. Physiologically, it is essential for the disruption of cadherin-based cell–cell contacts and subsequent cell motility which takes place is embryonic development, organogenesis and wound healing [25]. It activates a wide range of different cellular signalling pathways, including those involved in proliferation, motility, migration and invasion. In many human primary tumours, amplification of the c-*MET* gene, with consequent protein overexpression and kinase activation, has been found. In our study, the expression of c-MET was most pronounced among the studied parameters: it was noted in over 80% of all tissues in both type I and type II ECs. No relationship was detected between the expression of c-MET and clinical stage or grading, although in the literature there is a correlation between its expression and metastatic disease and poor prognosis [26, 27].

ARID1A is located on chromosome 1p36.11 and encodes protein ARID1A, an important member of the SWI/SNF complex. This complex is responsible for chromatin remodelling, which regulates gene expression depending on changes in chromatin structures, and participates in replication, transcription and repair processes [28]. Dysfunction in the mechanism leads to carcinogenesis mainly via PI3K/AKT pathway. Mutations in *ARID1A* are found in many cancers and are common in gynaecological cancers such as clear cell and endometrioid ovarian cancer, but also in around 40% of endometrioid EC [29–31]. In our material, expression of ARID1A protein was detected in around 48% of all EC patients.

No differences in the expression of ARID1A protein were revealed in our study between clinical stages and histological grades. No relationship was also detected between ARID1A expression and the studied proteins (NESF-1, MLH1, MSH2, c-MET).

In both subgroups of patients, no other relationships were detected between the levels of expression of NESF, MLH1, MSH2, c-MET and AR1D1A.

CONCLUSIONS

In this study on protein expression, EC tissues manifested (although insignificantly) an elevated expression of NESF-1 in type II EC. In type I EC, NESF-1 expression was significantly higher in G1 in comparison to G2 and G3 combined. A significantly lower expression of MLH1 was demonstrated in type I EC. The most pronounced expression involved c-MET in all EC I and EC II tissues (in over 80% of cases). A tendency was detected for a high expression of NESF-1 in patients with type II EC, who also exhibited a high expression of MSH2.

REFERENCES

1. Globocan 2012 Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr (16.01.2015).
2. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-up. Int J Gynecol Cancer. 2016; 26(1): 2–30, doi: 10.1097/IGC.0000000000000609, indexed in Pubmed: 26645990.
3. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol. 2014; 15(7): e268–e278, doi: 10.1016/S1470-2045(13)70591-6, indexed in Pubmed: 24872110.
4. Schmeler KM, Soliman PT, Sun CC, et al. Endometrial cancer in young, normal-weight women. Gynecol Oncol. 2005; 99(2): 388–392, doi: 10.1016/j.ygyno.2005.06.029, indexed in Pubmed: 16051336.
5. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983; 15(1): 10–17, doi: 10.1016/0090-8258(83)90111-7, indexed in Pubmed: 6822361.
6. Setiawan VW, Yang HP, Pike MC, et al. Australian National Endometrial Cancer Study Group. Type I and II endometrial cancers: have they different risk factors? J Clin Oncol. 2013; 31(20): 2607–2618, doi: 10.1200/JCO.2012.48.2596, indexed in Pubmed: 23733771.
7. Cossio SL, Koehler-Santos P, Pessini SA, et al. Clinical and histomolecular endometrial tumor characterization of patients at-risk for Lynch syndrome in South of Brazil. Fam Cancer. 2010; 9(2): 131–139, doi: 10.1007/s10689-009-9297-x, indexed in Pubmed: 19821155.
8. Huang M, Djordjevic B, Yates MS, et al. Molecular pathogenesis of endometrial cancers in patients with Lynch syndrome. Cancer. 2013; 119(16): 3027–3033, doi: 10.1002/cncr.28152, indexed in Pubmed: 23760948.
9. Attias-Geva Z, Bentov I, Kidron D, et al. p53 Regulates insulin-like growth factor-I receptor gene expression in uterine serous carcinoma and predicts responsiveness to an insulin-like growth factor-I receptor-directed

targeted therapy. Eur J Cancer. 2012; 48(10): 1570–1580, doi: 10.1016/j.ejca.2011.09.014, indexed in Pubmed: 22033326.
10. Togami S, Sasajima Y, Oi T, et al. Clinicopathological and prognostic impact of human epidermal growth factor receptor type 2 (HER2) and hormone receptor expression in uterine papillary serous carcinoma. Cancer Sci. 2012; 103(5): 926–932, doi: 10.1111/j.1349-7006.2012.02240.x, indexed in Pubmed: 22329832.
11. Bishop EA, Lengyel ER, Yamada SD, et al. The expression of hepatocyte growth factor (HGF) and c-Met in uterine serous carcinoma. Gynecol Oncol. 2011; 121(1): 218–223, doi: 10.1016/j.ygyno.2010.11.031, indexed in Pubmed: 21168200.
12. Bruchim I, Amichay K, Kidron D, et al. BRCA1/2 germline mutations in Jewish patients with uterine serous carcinoma. Int J Gynecol Cancer. 2010; 20(7): 1148–1153, doi: 10.1111/IGC.0b013e3181ef622d, indexed in Pubmed: 21206239.
13. Brinton LA, Felix AS, McMeekin DS, et al. Etiologic heterogeneity in endometrial cancer: evidence from a Gynecologic Oncology Group trial. Gynecol Oncol. 2013; 129(2): 277–284, doi: 10.1016/j.ygyno.2013.02.023, indexed in Pubmed: 23485770.
14. Park JiY, Nam JH, Kim YT, et al. Poor prognosis of uterine serous carcinoma compared with grade 3 endometrioid carcinoma in early stage patients. Virchows Arch. 2013; 462(3): 289–296, doi: 10.1007/s00428-013-1382-8, indexed in Pubmed: 23417747.
15. Kandoth C, Schultz N, Cherniack AD, et al. Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. Nature. 2013; 497(7447): 67–73, doi: 10.1038/nature12113, indexed in Pubmed: 23636398.
16. Piulats JM, Guerra E, Gil-Martín M, et al. Molecular approaches for classifying endometrial carcinoma. Gynecol Oncol. 2017; 145(1): 200–207, doi: 10.1016/j.ygyno.2016.12.015, indexed in Pubmed: 28040204.
17. Kreizman-Shefer H, Pricop J, Goldman S, et al. Distribution of estrogen and progesterone receptors isoforms in endometrial cancer. Diagn Pathol. 2014; 9: 77, doi: 10.1186/1746-1596-9-77, indexed in Pubmed: 24684970.
18. Sho T, Hachisuga T, Nguyen TT, et al. Expression of estrogen receptor-α as a prognostic factor in patients with uterine serous carcinoma. Int J Gynecol Cancer. 2014; 24(1): 102–106, doi: 10.1097/IGC.0000000000000029, indexed in Pubmed: 24335662.
19. Takeda T, Banno K, Okawa R, et al. ARID1A gene mutation in ovarian and endometrial cancers (Review). Oncol Rep. 2016; 35(2): 607–613, doi: 10.3892/or.2015.4421, indexed in Pubmed: 26572704.
20. Li M, Xin X, Wu T, et al. HGF and c-Met in pathogenesis of endometrial carcinoma. Front Biosci (Landmark Ed). 2015; 20(8): 635–643, indexed in Pubmed: 25553470.
21. Bischoff J, Ignatov A, Semczuk A, et al. hMLH1 promoter hypermethylation and MSI status in human endometrial carcinomas with and without metastases. Clin Exp Metastasis. 2012; 29(8): 889–900, doi: 10.1007/s10585-012-9478-0, indexed in Pubmed: 22552371.
22. Markowska A, Marszałek A, Szarszewska M, et al. Nesfatin-1 as a potential marker in endometrial and ovarian carcinomas. Post Biol Komórki. 2016; 43(3): 435–439.
23. Takagi K, Miki Y, Tanaka S, et al. Nucleobindin 2 (NUCB2) in human endometrial carcinoma: a potent prognostic factor associated with cell proliferation and migration. Endocr J. 2016; 63(3): 287–299, doi: 10.1507/endocrj.EJ15-0490, indexed in Pubmed: 26842712.
24. Sasada S, Yunokawa M, Takehara Y, et al. Baseline risk of recurrence in stage I-II endometrial carcinoma. J Gynecol Oncol. 2018; 29(1): e9, doi: 10.3802/jgo.2018.29.e9, indexed in Pubmed: 29185267.
25. Corso S, Comoglio PM, Giordano S. Cancer therapy: can the challenge be MET? Trends Mol Med. 2005; 11(6): 284–292, doi: 10.1016/j.molmed.2005.04.005, indexed in Pubmed: 15949770.
26. Zhuang XP, Jin WW, Teng XD, et al. c-Met and RON expression levels in endometrial adenocarcinoma tissue and their relationship with prognosis. Eur J Gynaecol Oncol. 2015; 36(3): 255–259, indexed in Pubmed: 26189249.
27. Li M, Xin X, Wu T, et al. HGF and c-Met in pathogenesis of endometrial carcinoma. Front Biosci (Landmark Ed). 2015; 20(8): 635–643, indexed in Pubmed: 25553470.
28. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. Annu Rev Biochem. 2009; 78: 273–304, doi: 10.1146/annurev.biochem.77.062706.153223, indexed in Pubmed: 19355820.
29. Mao TL, Ardighieri L, Ayhan A, et al. Loss of ARID1A expression correlates with stages of tumor progression in uterine endometrioid carcinoma. Am J Surg Pathol. 2013; 37(9): 1342–1348, doi: 10.1097/PAS.0b013e3182889dc3, indexed in Pubmed: 24076775.
30. Wiegand KC, Shah SP, Al-Agha OM, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010; 363(16): 1532–1543, doi: 10.1056/NEJMoa1008433, indexed in Pubmed: 20942669.
31. Guan B, Mao TL, Panuganti PK, et al. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. Am J Surg Pathol. 2011; 35(5): 625–632, doi: 10.1097/PAS.0b013e318212782a, indexed in Pubmed: 21412130.

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Comparison of the harmonic scalpel with scissors in women who experience obturator nerve injury during lymph node dissection for gynaecological malignancies

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ABSTRACT

Objectives: Lymphadenectomy is crucial for accurate staging in most gynecological malignancies. Serious complications can occur during the surgery. The present study aimed to present the early and late findings associated with obturator nerve injury, which is rarely observed during lymphadenectomy but can result in serious sequela if not noticed.

Material and methods: The files of the patients who underwent lymphadenectomy at our clinic between 2012 and 2018 were examined. Patients with obturator nerve incisions were identified retrospectively.

Results: In total, 287 women patients underwent lymphadenectomy at our clinic between 2012 and 2018. Examination of surgical notes revealed that nine patients underwent obturator nerve incisions using a scissor or a harmonic scalpel (energy-activated ultrasonic scissors). With respect to management of obturator nerve damage, no significant difference was found between the use of a harmonic scalpel and scissors ($p < 1.000$) and the trendelenburg and lithotomy positions ($p < 0.167$). In addition, no significant difference was found between laparoscopy and laparotomy in terms of surgical type ($p < 0.167$). At 6 months post-operatively, sensory-motor examinations and EMG findings of the patients were completely normal.

Conclusions: Surgeries performed for gynaecological malignancies have high mortality and morbidity rates. Moreover, in the event of a complication such as nerve damage during laparoscopy, successful management of the complication before the patient undergoes laparotomy allows the patient to continue benefitting from the advantages of the laparoscopy. The results of our study show that these high-risk surgeries should be performed in advanced and well-equipped medical centres by teams experienced in gynaecological oncology.

Key words: obturator nerve; lymphadenectomy; harmonic scalpel

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INTRODUCTION

Lymphadenectomy (LN) is crucial for accurate staging in most gynecological malignancies [1]. Therefore, surgical management regarding lymphadenectomy worldwide is very heterogeneous. Systemic pelvic and para-aortic lymphadenectomy does not improve overall or progression-free survival in patients with advanced ovarian cancer with both intra-abdominal resection and clinically negative LN [1].

In endometrial cancer, lymphadenectomy is considered to be a staging component that does not improve prognosis but is only used for proper adjustment of adjuvant therapy [2]. However, these radical surgeries can also lead to certain complications. Prolonged surgical duration and the associated vascular and nerve injuries can significantly increase the morbidity risk of patients [3].

Obturator nerve (ON) injury is one of these rare complications. It usually occurs during lymphadenectomy or during excessive retroperitoneal obturator fossa manipulations [4]. In addition, they are also observed in obturator hernia or endometriosis surgeries, bilateral oophorectomy and aorto-femoral bypass surgeries [3]. Furthermore, ON injury reportedly occurs more frequently during radical prostatectomy [5]. During pelvic surgery, obturator nerve damage can be seen in gynecologic oncologic procedures

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with wide pelvic side wall dissection. Nerve injuries may occur as a result of compression, shearing and stretching [6]. When noticed, such injuries can be repaired with early surgery via end-to-end anastomosis. Through proper nerve repair, patients can continue their daily lives without any loss of function.

MATERIAL AND METHODS

Patients who underwent lymphadenectomy at our clinic for ovarian and endometrial cancers between 2012 and 2018 were included in our study. Patient records were retrospectively examined, and it was confirmed that all the patients underwent pre-operative tests for complete blood count, hepatitis markers, coagulation and tumour markers in addition to other biochemical investigations. All patients underwent electrocardiography, posterior–anterior chest X-ray and pre-operative evaluation by the anaesthesia clinic. All patients were admitted to the ward two days pre-operatively and the appropriate surgical procedure for each patient was determined based on the pre-operative examinations. Abdominal and laparoscopic methods were found to be preferred by the patients. Nine patients underwent iatrogenic ON incisions during the surgery and the two nerve ends were completely separated. Four and five patients were operated for endometrial and ovarian cancers respectively. Electromyography (EMG) was performed during the follow-up period, and the records, which were examined by the neurology clinic, revealed that patients underwent two examinations during the follow-up period as short- and long-term examinations. The first examination was performed 1 month post-operatively, whereas the second examination included evaluation at 6 and 12 months post-operatively.

All analyses were performed using the IBM SPSS 20 statistical analysis software. Data were presented as mean, standard deviation, median, minimum, maximum, percentage and number. The Shapiro–Wilk test was used to analyse the normal distribution of continuous variables. The independent samples t-test and the Mann–Whitney U-test were used compare two independent groups with normally and non-normally distributed variables, respectively. The 2×2 comparisons between categorical variables were made using the Pearson's chi-square test if the expected value was > 5; the Yates chi-square test if the expected value was between 3 and 5and the Fisher's exact test if the expected value was < 3. P values < 0.05 were considered to be statistically significant.

RESULTS

A total of 287 patients underwent lymphadenectomy at our clinic between 2012 and 2018 due to malignancy. Surgery reports revealed that nine patients had complete ON incisions during surgery. Of these nine patients, four were operated for endometrial cancer, and five for ovarian cancer. Table 1 shows the comparison of the demographic and clinical characteristics of the patients. Two of the patients who were operated for endometrial cancer were treated through laparoscopic approach, whereas the other two were treated with abdominal approach. The abdominal approach was used in all patients who were operated for ovarian cancer. Four of the patients underwent ON incision using a harmonic scalpel, and five underwent dissection with scissors. Nerve damage was found to be complete, and no resection of nerve endings was performed in any patient. Nine patients underwent end-to-end anastomosis using 6/0 Prolene sutures. Anastomosis could not be performed in one patient as the patient's nerve endings could not be found. This patient was excluded from the study because the repair could not be performed. With respect to the short- and long-term neurological and EMG examinations, no significant difference was found between the use of harmonic scalpels and scissors to make ON incisions. Six patients had a right ON incision, whereas three a left one. We found no statistically significant difference in terms type of surgery, device used and surgical position between the groups ($p > 0.05$) (Tab. 2). The percentage of the injury during lymphadenectomy in our cohort was 3.4%.

Table 1. The demographic and clinical characteristics of the patients

Variables	Pathological Diagnosis		P
	Endometrial Ca (n = 4)	Ovarian Ca (n = 5)	
Age [years]	55.25 ± 3.10	65.60 ± 9.91	0.142
BMI [kg/m^2]	27.62 ± 6.57	25.07 ± 0.86	0.106
Parity	2.95 ± 2.3	3.04 ± 1.1	0.128
Diabetes mellitus	2 (0.18)	1 (0.50)	0143
Hypertension	4 (0.32)	3 (0.43)	0.128
Neurological abnormalities	0 (0)	0 (0)	—
Smoking	1 (0.04)	1 (0.04)	0.317
Menopause age	55 (0.43)	54 (0.43)	0.215
Previous surgery	1 (0.42)	2 (0.61)	0.143
Previous caesarean	1 (0.23)	2 (0.07)	0.106

In the nine patients who underwent ON repair, the sensory–motor examinations conducted 1 month post-operatively were normal for both lower extremities. Nerve conduction studies were normal in the EMG examinations, whereas sparse acute denervation potentials and regeneration motor unit potentials were monitored 1 month post-operatively in ON-innervated muscles via needle EMG examination. In the long-term follow-up of the patients (at 6 months post-operatively for four patients, at 8 months post-operatively for one patient and at 12 months post-operatively for four patients),

Table 2. Comparison of the type of surgery, device used and surgical position between the different types of pathological diagnosis

		Pathological diagnosis				p
		ENDOMETRIAL CA		OVARIAN CA		
		Count	Column N %	Count	Column N %	
Type of surgery	Laparotomy	2	50.00	5	100.00	0.167
	Laparoscopy	2	50.00	0	0.00	
Device used	Harmonic scalpel	2	50.00	2	40.00	1.000
	Scissors	2	50.00	3	60.00	
Surgical position	Trendelenburg	2	50.00	0	0.00	0.167
	Lithotomy	2	50.00	5	100.00	

Table 3. Charcaterictics of the cases

Cases	Age	Pathological diagnosis	Surgery type	Device used	Surgical Position	Surgery duration	Post-operative Sensory–motor examination (month 1)	EMG Findings (Post-operative month 1)	Long-term sensory–motor and EMG findings
1	51	End CA	Laparoscopy	Harmonic scalpel	Trendelenburg	3 h	Normal	*	Normal
2	55	End CA	Laparoscopy	Harmonic scalpel	Trendelenburg	2 h 50 min	Normal	*	Normal
3	58	End CA	Abdominal	Scissors	Lithotomy	2 h 30 min	Normal	*	Normal
4	57	End CA	Abdominal	Scissors	Lithotomy	4 h 20 min	Normal	*	Normal
5	65	Ovarian CA	Abdominal	Harmonic scalpel	Lithotomy	6 h	Normal	*	Normal
6	72	Ovarian CA	Abdominal	Harmonic scalpel	Lithotomy	3 h 50 min	Normal	*	Normal
7	68	Ovarian CA	Abdominal	Scissors	Lithotomy	8 h 30 min	Normal	*	Normal
8	74	Ovarian CA	Abdominal	Scissors	Lithotomy	3 h 30 min	Normal	*	Normal
9	49	Ovarian CA	Abdominal	Scissors	Lithotomy	3 h 20 min	Normal	*	Normal

*Sparse acute denervation potentials and regeneration mup; CA — cancer

the lower extremity sensory–motor examinations and EMG examinations were found to be normal (Tab. 3).

In one patient who could not undergo ON repair, examinations conducted 1 and 6 months post-operatively showed restriction in the flexion and adduction of the right thigh, and sensory deficits in the medial of the thigh. In the EMG examination conducted 1 month post-operatively, intense and acute denervation potentials were observed in the ON-innervated muscles on the right side, whereas EMG examinations conducted 1 and 6 months post-operatively did not reveal any voluntary muscle activity. EMG examinations were consistent with complete ON damage on the right side.

DISCUSSION

Pelvic lymph node evaluation is extremely important in the management of gynaecologic malignancies [4]. However, certain undesirable conditions may arise during dissection. A rare example of these conditions is ON injury. ON originates from lumbar 2–4 spinal nerves and innervates the adductor muscles [7]. After originating from the lumbar plexus, it descends downwards by passing from the posterior surface of the psoas muscle to its medial end, and then exits the pelvis from the obturator canal [8]. It is divided into the anterior and posterior branches. The anterior branch innervates the inner thigh skin [9], and complete, incomplete or heat-related injury of this nerve can lead to numbness and pain in the thigh and can cause weakness in the adduction of the thigh possibly resulting in gait disorders [3].

In the event that an urgent repair becomes necessary during surgery, the nerve usually recovers without problems. Case presentations in the literature report that following urgent repair during surgery, the patients showed no motor deficits or loss of sensation when examined 6 months post-operatively [3]. In our case series, the ON incisions of nine patients were urgently repaired during surgery, and no deficit was identified in neurological examinations and long-term EMG evaluations. However, ON repair could not be performed in one of the patients because the ON ends could not be found. This developed a restriction of adduction and a loss of sensation in the innervated thigh area. The patient's EMG examination was consistent with ON injury. In our study the percentage of the injury during lymphadenectomy was 3.4%. In a similar study, this rate was given as 2.4% [10].

The severity of symptoms is associated with the extent of ON damage. Grafts can be used in nerve incisions where end-to-end anastomosis is not possible. Ghaemmaghami et al. [11] reported the case of a patient in whom a graft was used successfully in ON injury that occurred during radical hysterectomy and bilateral pelvic lymphadenectomy and reported that there was no neurological or functional loss observed 6 months post-operatively. However, although there was full incision along with thermal damage in our case series, no grafts were used, and successful repair was achieved with end-to-end anastomosis without nerve stretching. In one patient, nerve endings could not be found and repair could not be performed.

Most ON injuries occur as a result of pelvic procedures. It has been reported that most patients are kept in the lithotomy position for a long time [12]. In our case series, seven patients were operated while they were in the lithotomy position, whereas two were operated while they were in the Trendelenburg position. However, a review of the literature shows that surgical intervention in the lithotomy position for extended periods of time increases nerve damage risk [13].

To minimise complications during pelvic lymphadenectomy, it is necessary to have knowledge of anatomically important localisations, and to bear in mind that there can be variations. Studies have shown that anterior and posterior separation of the ON is intrapelvic in 23.22% of the cases, inside the thigh in 25% of the cases and in the obturator channel in 51.78% of the cases [14]. This shows that the nerve may show variation as it travels through the obturator canal. In our study, all of the patients had nerve incision in the obturator fossa.

Noticing an ON injury during surgery and its subsequent rapid repair is very important for post-operative recovery. Timely detection of the nerve damage ensures rapid repair and prevents long-term permanent damage. Song et al. [15] have successfully performed laparoscopic repair of nerve damage in a patient with cervical cancer using an electrosurgical instrument. An electrosurgical instrument was used in five of the patients in our patient group and rapid repair was performed during surgery with no pathological findings being observed 1 and 6 months post-operatively. However, Gocmen et al. [3] reported that the total recovery period of incomplete ON injury was approximately one year. It is very important to protect the nerves in laparoscopic interventions requiring precise dissection and to safely provide haemostasis without damaging the surrounding tissues [16]. Owing to the advances in technology, it is now possible to use laparoscopic instruments relying on energy to perform lymphadenectomy and simultaneously ensure haemostasis [17]. The increase in heat generated in the lateral tissues by this energy can cause thermal damage to the surrounding tissue, as a prolonged surgical duration increases the temperature ultimately causing greater damage. Although new laparoscopic instruments utilising ultrasonic energy are considered to be safer and more reliable than monopolar cautery systems, reliable information on their effects is limited. There are studies in the literature showing that the thermal damage to tissues by ultrasonic scissors is safe [18]. Emam and Cuschieri [19] demonstrated that dissection using ultrasonic energy is effective and safe, but also reported that activation periods of over 10 seconds could increase lateralised thermal damage. It was observed in our study that, in cases with nerve damage, incisions performed using ultrasonic energy or with the aid of scissors both resulted in negligible lateral tissue damage. There was no requirement of grafts in both groups, and nerve ends were repaired without stretching. There was no significant difference between short- and long-term neurological and EMG examinations. Although the number of cases in our series was less, it is considered that energy-activated ultrasonic scissors can be used safely, even in complications such as ON incision.

CONCLUSIONS

Noticing complications during surgeries and rapidly and completely managing them is one of the fundamental principles of surgery. In our case series, we discussed the management of nerve damage that may occur during lymphadenectomy, a surgical procedure with a high complication rate. Based on our findings, we conclude that this micro-surgical procedure, which was performed by experienced hands to remedy a situation noticed during surgery, yielded rather satisfactory long-term results.

Statement of ethics

Research involving human participants and/or animals; All procedures performed in these studies that involved human participants were conducted in accordance with the ethical standards of our institutional ethics review committee and adhered to the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by our institutional ethics review board. Informed consent; All patients were notifed about the use of their deidentifed medical data in our retrospective analysis.

REFERENCES

1. Harter P, Sehouli J, Lorusso D, et al. LION: Lymphadenectomy in ovarian neoplasms—A prospective randomized AGO study group led gynecologic cancer intergroup trial. Journal of Clinical Oncology. 2017; 35(15_suppl): 5500–5500, doi: 10.1200/jco.2017.35.15_suppl.5500.
2. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-up. Int J Gynecol Cancer. 2016; 26(1): 2–30, doi: 10.1097/IGC.0000000000000609, indexed in Pubmed: 26645990.
3. Göçmen A, Şanlıkan F. Immediate repair of an incompletely transected obturator nerve during robotic-assisted pelvic lymphadenec-

tomy. J Minim Invasive Gynecol. 2015; 22(2): 302–304, doi: 10.1016/j.jmig.2014.08.783, indexed in Pubmed: 25218992.
4. Creasman WT, Creasman WT. New gynecologic cancer staging. Obstet Gynecol. 1990; 75(2): 287–288, indexed in Pubmed: 2300358.
5. Lindner A, Schulte-Mattler W, Zierz S. [Postpartum obturator nerve syndrome: case report and review of the nerve compression syndrome during pregnancy and delivery]. Zentralbl Gynakol. 1997; 119(3): 93–99, indexed in Pubmed: 9173773.
6. Zhao W, Jiang W, He C, et al. Laparoscopic repair of obturator nerve transection during pelvic lymphadenectomy. Int J Gynaecol Obstet. 2015; 129(3): 273–274, doi: 10.1016/j.ijgo.2015.01.002, indexed in Pubmed: 25745940.
7. Haninec P, Horak L, Kaiser R. Obturator nerve injury in laparoscopic inguinal hernia mesh repair. Hernia. 2013; 17(6): 801–804, doi: 10.1007/s10029-012-0972-8, indexed in Pubmed: 22836919.
8. Kirchmair L, Lirk P, Colvin J, et al. Lumbar plexus and psoas major muscle: not always as expected. Reg Anesth Pain Med. 2008; 33(2): 109–114, doi: 10.1016/j.rapm.2007.07.016, indexed in Pubmed: 18299090.
9. Vasilev SA. Obturator nerve injury: a review of management options. Gynecol Oncol. 1994; 53(2): 152–155, doi: 10.1006/gyno.1994.1108, indexed in Pubmed: 8188073.
10. Zorlu CG, et al. Pelvic and paraaortic lymphadenectomy as a staging and therapeutic procedure. T Klin J Gynecol Obst. 2000; 10: 36–39.
11. Ghaemmaghami F, Behnamfar F, Saberi H. Immediate grafting of transected obturator nerve during radical hysterectomy. Int J Surg. 2009; 7(2): 168–169, doi: 10.1016/j.ijsu.2008.07.007, indexed in Pubmed: 19359226.
12. Bischoff C, Schönle PW. Obturator nerve injuries during intra-abdominal surgery. Clin Neurol Neurosurg. 1991; 93(1): 73–76, doi: 10.1016/0303-8467(91)90014-g, indexed in Pubmed: 1651197.
13. Kitagawa R, Kim D, Reid N, et al. Surgical management of obturator nerve lesions. Neurosurgery. 2009; 65(4 Suppl): A24–A28, doi: 10.1227/01.NEU.0000335652.61676.CC, indexed in Pubmed: 19927074.
14. Kumka M. Critical sites of entrapment of the posterior division of the obturator nerve: anatomical considerations. J Can Chiropr Assoc. 2010; 54(1): 33–42, indexed in Pubmed: 20195424.
15. Song MJ, Lee CW, Yoon JH, et al. Transection of the obturator nerve by an electrosurgical instrument and its immediate repair during laparoscopic pelvic lymphadenectomy: a case report. Eur J Gynaecol Oncol. 2014; 35(2): 167–169, indexed in Pubmed: 24772921.
16. Yıldırım A, Başok EK, Göçer S, et al. Evaluation of thermal spread during the application of laparoscopic bipolar and ultrasonic devices. Türk Üroloji Dergisi - Turkish Journal of Urology. 2009; 35(3): 250–255.
17. Kim FJ, Chammas MF, Gewehr E, et al. Temperature safety profile of laparoscopic devices: Harmonic ACE (ACE), Ligasure V (LV), and plasma trisector (PT). Surg Endosc. 2008; 22(6): 1464–1469, doi: 10.1007/s00464-007-9650-9, indexed in Pubmed: 18027042.
18. Hruby GW, Marruffo FC, Durak E, et al. Evaluation of surgical energy devices for vessel sealing and peripheral energy spread in a porcine model. J Urol. 2007; 178(6): 2689–2693, doi: 10.1016/j.juro.2007.07.121, indexed in Pubmed: 17945280.
19. Emam TA, Cuschieri A. How safe is high-power ultrasonic dissection? Ann Surg. 2003; 237(2): 186–191, doi: 10.1097/01.SLA.0000048454.11276.62, indexed in Pubmed: 12560776.

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GLUT1 expression is a supportive mean in predicting prognosis and survival estimates of endometrial carcinoma

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ABSTRACT

Objectives: This study will investigate the phenotype of Glucose transporter 1 (GLUT1) in endometrial cancer and the association of its expression with tumor's clinicopathological factors.

Material and methods: Standard immunohistochemistry (IHC) staining protocol was utilized to identify the location and expression pattern of GLUT1 in a panel of 71 endometrial carcinomas compared to 30 normal tissues using tissue microarrays.

Results: High scores of GLUT1 staining are more frequent in cancer cases, it was recognized in 64 (90%) endometrial cancers and 12 (40%) control cases. Tissue histotype (cancer versus non-cancerous) was associated with IHC staining of GLUT1 ($p = 0.000$). Significant association between strong GLUT1 staining of malignant epithelial cells and stage of tumor ($p = 0.000$) was observed, advanced disease stages were more prevalent with high GLUT1 staining in malignant epithelial cells. There is also a significant association between high scores of GLUT1 staining and location of expression in transformed epithelium, cytoplasmic and membranous ($p = 0.000$), 100% of cases with cytoplasmic and membranous expression showed high GLUT1 staining scores. **Considerable varied survival models were observed with positive GLUT1 neoplasm regarding diagnosis, grade, stage, differentiation, and recurrence** (p-values 0.000, 0.000, 0.000, 0.002, and 0.000 respectively). Survival estimates are considerably healthier in positive GLUT1 staining cases of endometrial carcinoma, which have low grade, low stage and no recurrence.

Conclusions: GLUT1 expression has been found upregulated in endometrial carcinoma. IHC staining of GLUT1 can be a supportive mean in predicting prognosis and survival estimates of endometrial carcinoma with specific clinical factors.

Key words: GLUT1; immunohistochemistry; endometrial carcinoma

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INTRODUCTION

Endometrial cancer (EC) is the second most frequent malignant neoplasm of the female reproductive system in USA [1]. **In Saudi Arabia, uterine corpus tumors are the most frequent cancer of female genital system [2]**. More than 400 cases of endometrial cancer were registered in 2015, which represented about 6.4 % of all recently confirmed cancer cases of all sites [2]. The mean age was 61 years (22–99). The most common morphological type is endometrioid adenocarcinoma not otherwise specified (NOS) accounts for 63.8 percent, and less commonly adenocarcinoma (NOS) 11.9%, carcinoma (NOS) 3.2%, serous cystadenocarcinoma 3.2%, papillary serous cystadenocarcinoma 3.0% and others [2]. Diagnosis and management of endometrial neoplasms depend greatly on patients' clinicopathological factors [patient age, tumor size and histological type as well as Fédération Internationale de Gynécologie Obstétrique (FIGO) grade as prognostic signs]. Yet, these clinical factors are not adequate to predict disease's outcomes due to endometrial tumors heterogeneity [3]. Regardless of significant improvements in cancer management and the good prognosis of endometrial tumors, about 15% of all endometrial tumors recur, of which up to 90% of recurrent tumors happen within 3 years [4]. The recurrent disease prognosis is poor; the median survival barely surpasses twelve months. At present, the total number of patients with recurrent endometrial tumor arises [5].

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So, it is a pleading demand to find better diagnostic and prognostic markers and chemotherapeutic agents to facilitate clinical tasks for effective diagnosis, prognosis and treatment of endometrial carcinoma. Concentrated experimental works have been constructed to acquire new markers so as to support disease diagnosis and prognosis, improve patients' risk stratification and advance clinical management [6]. The majority of these biomarkers has not been satisfactorily specific or sensitive; this lead to a big interest in distinguishing biomarkers of transformed cells and tumor micro-environment which could have prognostic or predictive values of response to particular medications that could lead to proper therapy.

Glucose transporter 1 (GLUT1) is upregulated in a wide spectrum of human malignancies and its expression is absent in most types of normal epithelial cells. The expression of GLUT1 appears to be a potential marker for malignant transformation [7–8]. GLUT1 has been considered to have a significant role in the development of different neoplasms once overexpressed. Many recent studies associated GLUT1 expression with increased malignant potential, invasiveness, diagnosis, prognosis and survival in different neoplasms, including prostate, breast, colorectal, ovarian, lung, hepatic, pancreatic, esophageal and cervical carcinomas [8–10]. However, in endometrial cancer, many studies attempted to find similar association and prove that GLUT1 phenotype could be utilized as a diagnostic and prognostic tissue marker, but the findings were inconsistent and need further confirmation [11–22]. Therefore, this manuscript will describe the immunohistochemistry phenotype of GLUT1 in a panel of endometrial carcinomas compared to normal tissues, and analyse its relationship with clinicopathological features, to determine its clinical value and its role in endometrial cancer. Furthermore, this study will evaluate a GLUT1 expression as a diagnostic marker and predictor of survival in patients with endometrial carcinoma.

Objectives

This study will investigate the phenotype of Glucose transporter 1 (GLUT1) in endometrial cancer and the association of its expression with tumor's clinicopathological factors.

MATERIAL AND METHODS

Two groups of tissue samples were included in this study; the first group is 71 specimens related to patients with histologically confirmed endometrial carcinomas. The second group is 30 tissue samples from curetted patients for noncancerous conditions (4 endometrial polyps, 16 proliferative endometrium, and 10 secretory endometrium), as a control. The mean age of second group individuals was 36 (ranged 22–50). All ethical rules and regulations adapted by author institution have been followed.

This study will utilize GLUT1 monoclonal antibody using immunohistochemistry staining standard protocol to identify the location and expression pattern of GLUT1, which will be graded with respect to the estimated fraction of malignant cells with positive and relative intense stain.

All recruited tissue specimens were paraffin-embedded tissue blocks and were collected along with their clinicopathological data from the Department of Pathology (Tab. 1). All paraffin blocks were cut (4 μm thickness), Hematoxylin-and-Eosin (H&E) stained and reevaluated for diagnosis and grading confirmation by two pathologists. Later, tissue microarray (TMA) was built using all paraffin-embedded specimens of both groups (carcinomas and controls) in the same way as was stated by Al-Maghrabi et al. [23]. Next, TMA Blocks were cut into 4 μm slices, placed on coated slides and used later in immunohistochemistry (IHC) to detect GLUT1 using BenchMark autostainer (Ventana, Arizona, USA), anti-GLUT1 polyclonal antibody and UltraView Universal diaminobenzidine (DAB) Detection Kit (Ventana Medical Systems, USA). A slide with trisaminomethane (tris) buffer instead of anti- GLUT1 polyclonal antibody were included as a negative control in every staining procedure performed as well as positive tissue control of colorectal carcinoma as indicated by the manufacturer.

Two pathologists analyzed the quality of GLUT1 expression and approximated the percentage of positive neoplastic cells. The estimations of GLUT1 positive cells were determined by semi-quantitative procedure in 3 microscopic fields using 40 × lenses. All cases with brown color in less than 5% of neoplastic cells were counted negatively stained. Grades of 0, 1, 2, and 3 were assigned for negative, weak, modest and strong stain respectively. These scores are displayed in this report as high (2 and 3), and low (0 and 1). The lowest grade recorded by any pathologist was taken into account if a disparity occurred.

Statistical Analysis

The data were analysed by using version 21 of International Business Machines-Statistical Package for the Social Sciences (IBM-SPSS). All results were displayed as incidences and percentages. The relationship between clinical factors of ECs and GLUT1 immunoexpression was investigated by Fisher and chi-square tests. Assessment of survival distributions for several GLUT1 IHC staining scores were calculated by using a Log Rank test. The significance level was considered at $p < 0.05$.

RESULTS

Clinicopathological factors of all ECs cases with the expression of GLUT1 was presented in Table 1. Transformed epithelium of sixty four endometrial cancer cases (90.1%) showed high scores GLUT1 IHC staining, and 7 (9.9%) sam-

Table 1. Distribution of various clinicopathological variables with Glucose transporter 1 (GLUT1) immunostaining in transformed endometrial cells

		Gut 1 in Epithelial cells				p-value
		Low		High		
		n	[%]	n	[%]	
Group	**Control**	18	60.0	12	40.0	**0.000**
	Endometrial Cancer	7	9.9	64	90.1	
GLUT1 staining location	**Negative**	3	100	0	0.0	0.0001
	Cytoplasmic	4	40.0	6	60.0	
	Cytoplasmic and membranous	0	0.0	58	100.0	
Diagnosis	**Clear cell carcinoma**	0	0.0	1	100.0	0.695
	Endometrioid adenocarcinoma	7	11.9	52	88.1	
	MMMT	0	0.0	2	100.0	
	Serous carcinoma	0	0.0	9	100.0	
Grade	**I**	5	12.5	35	87.5	0.999
	II	2	8.7	21	91.3	
	III	0	0.0	6	100.0	
	Ungraded	0	0.0	2	100.0	
Stage	**I**	6	15.4	33	84.6	**0.000**
	II	0	0.0	5	100.0	
	III	0	0.0	9	100.0	
	IV	0	0.0	3	100.0	
	Unstaged	1	6.7	14	93.3	
Differentiation	**M**	2	10.0	18	90.0	0.884
	NA	0	0.0	2	100.0	
	P	0	0.0	8	100.0	
	W	5	12.2	36	87.8	
Recurrence	**No**	6	10.7	50	89.3	0.999
	Yes	1	6.7	14	93.3	
Alive	**No**	0	0.0	17	100.0	0.185
	Yes	7	13.0	47	87.0	

GLUT1 — Glucose transporter 1

ples revealed negative or weak staining. High scores of GLUT1 IHC staining in stromal cells of ECs were found in the total of only 21 out of 71 cases of ECs. High scores of GLUT1 staining were found more frequently in cancer cases, it was recognized in 64 (90%) endometrial cancers compared to 12 (40%) control cases. Staining of the normal endometrial epithelium, if present, was much lower than observed in tumor cells from the same patient.

Biologic behavior tissue type (cancer versus non-cancerous) was obviously associated with GLUT1 immunohistochemistry staining ($p = 0.000$). Significant association between strong GLUT1 staining of malignant epithelial cells and stage of tumor ($p = 0.000$) was observed, advanced disease stages were more prevalent with high GLUT1 staining in malignant epithelial cells. There is also a significant association between high GLUT1 staining scores and cytoplasmic and membranous expression locations in malignant epithelium ($p = 0.000$), 100 percent of cases (58) with cytoplasmic and membranous expression showed high GLUT1 staining scores. The remaining cases were 3 negative and ten cases revealed cytoplasmic staining only of which 60% were of strong staining.

Most positive GLUT1 cases showed a brown color in greater than 50% of the transformed cells (Fig. 1 A, B, C and D). Substantial variability was identified in GLUT1 staining, for instance, some neoplasms exhibited positive stain in selected glands or cells and others showed identical stain in all glandular or cellular parts. No significant associations were analysed between GLUT1 immunostaining and neoplasm diagnosis, grade, recurrence and alive/deceased status.

The log rank test was used to compare survival distributions among cases of low and high GLUT1 staining scores. Table 2 defines the average survival times of tumor patients with different clinical risk factors varied for

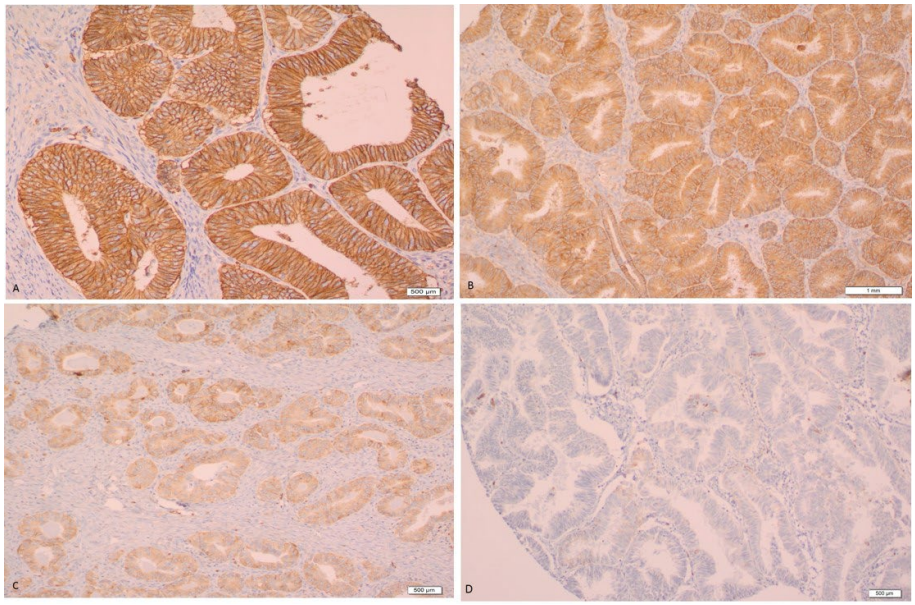

Figure 1. Glucose transporter 1 (GLUT1) immunostaining pattern in endometrial cancer; A — strong staining in endometrial tissue; B — moderate staining in endometrial cancer; C — weak staining in endometrial cancer; D — negative staining in endometrial cancer

Table 2. Comparison of survival distribution patterns by various clinicopathological variables in positive Glucose transporter 1 (GLUT1) immunostained endometrial cancers

		n	No. of Events	Mean	S.E	p-value[a]
Grade	**I**	40	4	105.016	7.000	0.000
	II	21	6	95.970	13.932	
	III	6	5	20.154	7.048	
	Ungraded	2	1	11.187	5.401	
Stage	**I**	38	2	108.154	6.788	0.000
	II	5	1	59.644	12.330	
	III	8	7	26.801	14.952	
	IV	3	3	22.198	14.932	
	Unstaged	15	3	97.449	10.617	
Differentiation	**W**	41	4	106.066	6.523	0.002
	M	18	6	87.111	16.245	
	P	8	5	45.745	15.734	
	NA	2	1	11.187	5.401	
Recurrence	**No**	55	7	102.797	6.065	0.000
	Yes	14	9	45.930	14.736	
Diagnosis	**Endometrioid adenocarcinoma**	59	8	114.965	7.348	0.000
	MMMT	2	1	11.187	5.401	
	Serous carcinoma	8	7	25.741	6.823	

a — Log-Rank test adjusted for GLUT1 immunostaining

GLUT1 staining. Considerable varied survival models were observed with neoplasm diagnosis, grade, stage, differentiation and recurrence (p-values 0.000, 0.000, 0.000, 0.002, and 0.000 respectively). Survival estimates are considerably healthier in positive GLUT1 staining cases of endometrial carcinoma, which have endometrioid adenocarcinoma type, low grade, low stage, well differentiation or no recurrence. On the other hand, positive neoplasms with high grade, high stage, poor differentiation or recurrence displayed poorer survival estimations. Kaplan Meier survival curves

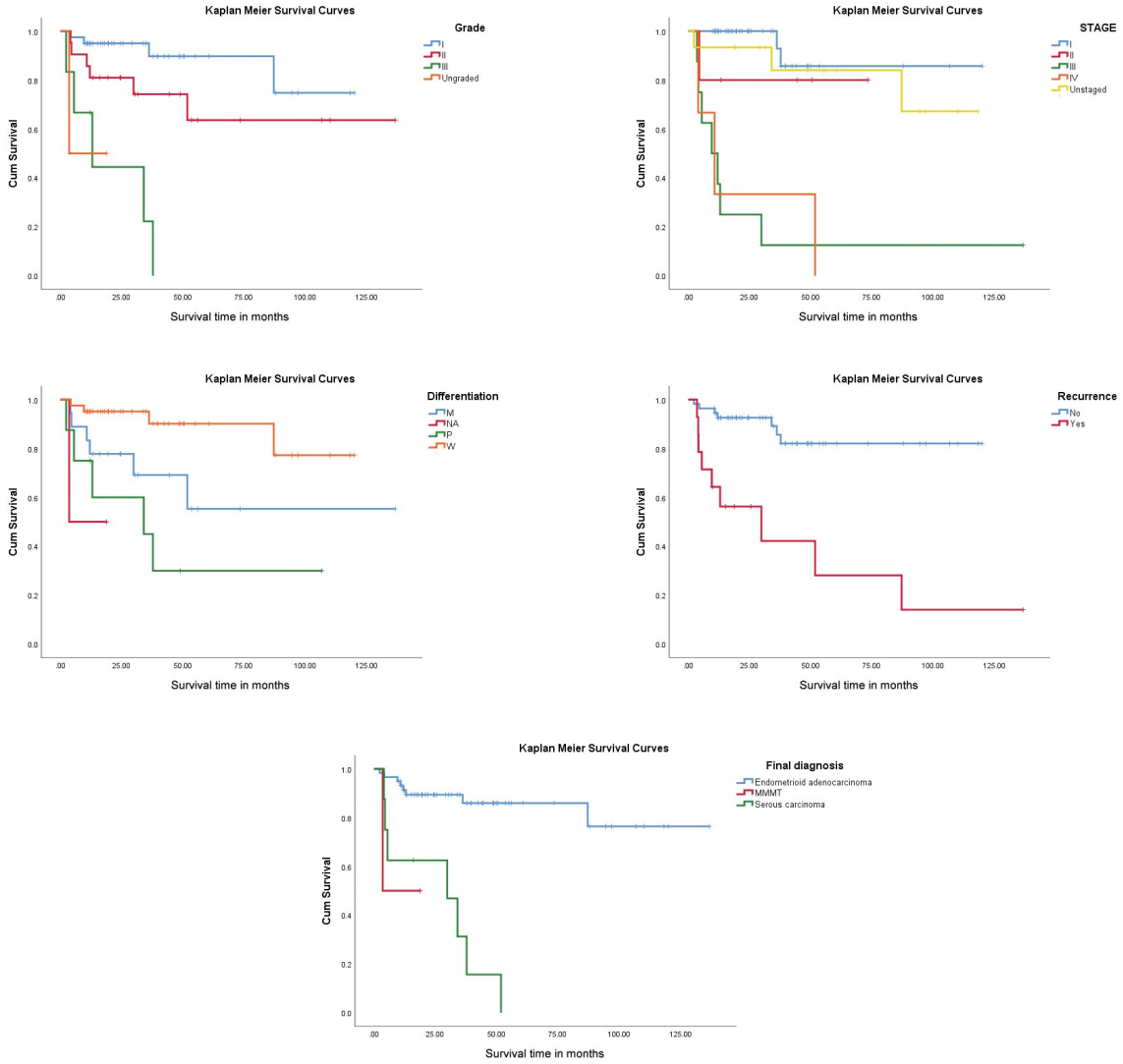

Figure 2. Kaplan Meier Survival Curves by various clinicopathological variables with Glucose transporter 1 (GLUT1) immunostaining in endometrial cancer

exhibited significant improved survival experience in cases of endometrioid adenocarcinoma type, low grade, low stage well differentiation or no recurrence (Fig. 2).

DISCUSSION

Glucose transporters have become one of the core subjects in cancer biology since it has been found that neoplastic cells show higher glucose metabolism in comparison with normal tissue. The resultant big growth in glucose necessity indicates a demand for a consistent rise in the transportation of glucose through the cell membrane. The greater part of tumors show increased expression of GLUT1 that has been existed in relevant normal counterpart tissues in non-cancerous states. Furthermore, because of the need for power to serve unrestrained proliferation, neoplastic cell frequently expresses GLUT1 that would not be expressed in the cells in ordinary circumstances [24–25]. The level and membranous location of GLUT1 expression could be an appropriate biomarker of glucose metabolism that might be assessed easily and economically as part of the histologic assessment practice of neoplasms [19]. Since increased expression of GLUT1 is already known in many neoplasms, its relationship with prognostic parameters has been studied [8–10]. The earliest and the most striking study on this subject to date is the one that was conducted on colon cancer. In addition to indicating GLUT1 as a good marker to determine aggressive biological behavior of colorectal carcinomas, it also showed a direct correlation between lymph node metastases and GLUT1 expression [26].

In endometrial neoplasms, nevertheless, many studies [11–22] tried to find a comparable association and verify that the IHC GLUT1 phenotype could be utilized as a diagnostic and prognostic tissue marker, but the findings were inconsistent (Tab. 3). In agreement with the majority of literature data, our

Table 3. Correlation between high level of Glucose transporter 1 (GLUT1) immunoreactivity and clinicopathological parameters of endometrial cancer in the current study compared to studies of the literature

Previous studies	GLUT1 in endometrial cancer	GLUT1 in control group	GLUT1 staining location	Grade	Stage	Recurr-ence	Alive/ Deceased status	Survival
The current study	90% p = 0.0001	40%	CM p = 0.0001	NS	0.000	NS	NS	p = 0.005
Nemejcova et al. 2017 [11]	90%	33%	M					
Anagnostou et al. 2017 [20]	63%		M	NS				
Al-Sharaky et al. 2016 [12]	98.5% p = 0.008	88.9%	CM	p = 0.003	p = 0.004			
Canpolat et al. 2016 [13]	95%	31.9%	M	p = 0.007	NS			NS
Ma et al. 2015 [14]	70% p < 0.05	14%	N	p < 0.05	p < 0.05			
Sadlecki et al. 2014 [15]	100%		CM	NS	NS	NS		NS
Xiong et al. 2010 [16]	71%	0%	M					
Wahl et al. 2010 [22]	53%	0%	M					
Ashton-Sager et al. 2006 [17]	90%	17%	M					
Goldman et al. 2006 [21]	present	present	C	p < 0.002				
Sebastiani et al. 2004 [18]	43%		M					NS
Wang et al. 2000 [19]	100%	0%	M					

GLUT1 — Glucose transporter 1; C — cytoplasmic; M — membranous; CM — cytoplasmic and membranous; N — nuclear; NS — not significant

results were capable statically to show increased cytoplasmic and/or membranous expression of GLUT1 in ECs compared to normal endometrium [11–22]. Decreased GLUT1 expression in normal endometrium as well as its weak expression in non-cancerous lesions and overexpression in endometrial cancer suggests that this molecule might be involved in endometrial carcinogenesis as the findings of this study and others [12, 14] showed significant association with tumor stage. On the other hand, some studies including the present one could not demonstrate any significant relationship between GLUT1 expression and other prognostic parameters of ECs [11, 15–20, 22]. While, few studies found that the association between GLUT1 phenotype and clinical data, i.e. increasing grade and stage, is statistically significant [12, 14]. Goldman et al. (2006) [21] and later Canpolat et al. (2016) [13] reported that among clinical characteristics, only grade was found to be significantly correlated to GLUT1 expression. According to Xiong et al. [16], the expression of GLUT1 can be used to distinguish between benign endometrial lesions and endometrial cancer but has no prognostic value in women with this malignancy. This is opposite to the present investigation which showed significant differences in the expression of GLUT1 associated with clinical stage or prognosis in endometrial cancer patients.

The present investigation showed that the impact of GLUT1 phenotype on the survival estimates of endometrial cancer was modified significantly by some clinical factors, including the type of tumor, grade, stage and recurrence. This finding is in line with the recent analyses of numerous studies which have reported paradoxical evidence of the relationship between GLUT1 expression and prognosis in solid human tumors [8, 27].

The differences between the previous studies and the current one could be clarified by method sensitivity, people's difference, and variations in the size of samples. The present report and previous similar ones which evaluated the diagnostic and prognostic power of GLUT1 immunoreactivity in endometrial malignancy had weak points such as the relatively small sample size involved in these studies and the semi-quantitative interpretation of immunostaining. However, greater inclusive studies are undoubtedly of great value for estimating the diagnostic and prognostic values of GLUT1 immunoreactivity in endometrial malignancy.

CONCLUSIONS

Our results showed increased expression of GLUT1 in endometrial tumors. IHC staining of GLUT1 can be a supportive mean in predicting prognosis and survival estimates of endometrial tumors with specific clinical factors.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018; 68(1): 7–30, doi: 10.3322/caac.21442, indexed in Pubmed: 29313949.
2. Alrawaji AI, Al-Shahrani ZS, Alzahrani W, et al. Cancer Incidence Report Saudi Arabia 2015, Saudi Cancer Registry 2018. https://nhic.gov.sa/eServices/Pages/TumorRegistration.aspx.
3. Piulats JM, Guerra E, Gil-Martín M, et al. Molecular approaches for classifying endometrial carcinoma. Gynecol Oncol. 2017; 145(1): 200–207, doi: 10.1016/j.ygyno.2016.12.015, indexed in Pubmed: 28040204.
4. Sohaib SA, Houghton SL, Meroni R, et al. Recurrent endometrial cancer: patterns of recurrent disease and assessment of prognosis. Clin Radiol. 2007; 62(1): 28–34; discussion 35, doi: 10.1016/j.crad.2006.06.015, indexed in Pubmed: 17145260.
5. Odagiri T, Watari H, Hosaka M, et al. Multivariate survival analysis of the patients with recurrent endometrial cancer. J Gynecol Oncol. 2011; 22(1): 3–8, doi: 10.3802/jgo.2011.22.1.3, indexed in Pubmed: 21607089.
6. Żyła MM, Wilczyński JR, Kostrzewa M, et al. The significance of markers in the diagnosis of endometrial cancer. Prz Menopauzalny. 2016; 15(3): 176–185, doi: 10.5114/pm.2016.63500, indexed in Pubmed: 27980530.
7. Noguchi Y, Saito A, Miyagi Y, et al. Suppression of facilitative glucose transporter 1 mRNA can suppress tumor growth. Cancer Letters. 2000; 154(2): 175–182, doi: 10.1016/s0304-3835(00)00392-x.
8. Wang Ji, Ye C, Chen C, et al. Glucose transporter GLUT1 expression and clinical outcome in solid tumors: a systematic review and meta-analysis. Oncotarget. 2017; 8(10): 16875–16886, doi: 10.18632/oncotarget.15171, indexed in Pubmed: 28187435.
9. Yu M, Yongzhi H, Chen S, et al. The prognostic value of GLUT1 in cancers: a systematic review and meta-analysis. Oncotarget. 2017; 8(26): 43356–43367, doi: 10.18632/oncotarget.17445, indexed in Pubmed: 28498810.
10. Zhang Bo, Xie Z, Li B. The clinicopathologic impacts and prognostic significance of GLUT1 expression in patients with lung cancer: A meta-analysis. Gene. 2019; 689: 76–83, doi: 10.1016/j.gene.2018.12.006, indexed in Pubmed: 30552981.
11. Němejcová K, Rosmusová J, Bártů M, et al. Expression of Glut-1 in Normal Endometrium and Endometrial Lesions: Analysis of 336 Cases. Int J Surg Pathol. 2017; 25(5): 389–396, doi: 10.1177/1066896916683510, indexed in Pubmed: 28381136.
12. Al-Sharaky DR, Abdou AG, Wahed MM, et al. HIF-1α and GLUT-1 Expression in Atypical Endometrial Hyperplasia, Type I and II Endometrial Carcinoma: A Potential Role in Pathogenesis. J Clin Diagn Res. 2016; 10(5): EC20–EC27, doi: 10.7860/JCDR/2016/19576.7805, indexed in Pubmed: 27437226.
13. Canpolat T, Ersöz C, Uğuz A, et al. GLUT-1 Expression in Proliferative Endometrium, Endometrial Hyperplasia, Endometrial Adenocarcinoma and the Relationship Between GLUT-1 Expression and Prognostic Parameters in Endometrial Adenocarcinoma. Turk Patoloji Derg. 2016; 32(3): 141–147, doi: 10.5146/tjpath.2015.01352, indexed in Pubmed: 27562387.
14. Ma X, Hui Y, Lin Li, et al. Clinical significance of COX-2, GLUT-1 and VEGF expressions in endometrial cancer tissues. Pak J Med Sci. 2015; 31(2): 280–284, doi: 10.12669/pjms.312.6604, indexed in Pubmed: 26101475.
15. Sadlecki P, Bodnar M, Grabiec M, et al. The role of Hypoxia-inducible factor-1 α , glucose transporter-1, (GLUT-1) and carbon anhydrase IX in endometrial cancer patients. Biomed Res Int. 2014; 2014: 616850, doi: 10.1155/2014/616850, indexed in Pubmed: 24745019.
16. Xiong Y, Xiong YY, Zhou YF. Expression and significance of beta-catenin, Glut-1 and PTEN in proliferative endometrium, endometrial intraepithelial neoplasia and endometrioid adenocarcinoma. Eur J Gynaecol Oncol. 2010; 31(2): 160–164, indexed in Pubmed: 20527231.
17. Ashton-Sager A, Paulino AFG, Afify AM. GLUT-1 is preferentially expressed in atypical endometrial hyperplasia and endometrial adenocarcinoma. Appl Immunohistochem Mol Morphol. 2006; 14(2): 187–192, doi: 10.1097/01.pai.0000162003.43334.c7, indexed in Pubmed: 16785788.
18. Sebastiani V, Visca P, Botti C, et al. Fatty acid synthase is a marker of increased risk of recurrence in endometrial carcinoma. Gynecol Oncol. 2004; 92(1): 101–105, doi: 10.1016/j.ygyno.2003.10.027, indexed in Pubmed: 14751145.
19. Wang BY, Kalir T, Sabo E, et al. Immunohistochemical staining of GLUT1 in benign, hyperplastic, and malignant endometrial epithelia. Cancer. 2000; 88(12): 2774–2781, indexed in Pubmed: 10870060.
20. Anagnostou E, Miliaras D, Meditskou S, et al. Immunohistochemical investigation of metabolic markers fatty acid synthase (FASN) and glucose transporter 1 (GLUT1) in normal endometrium, endometrial hyperplasia, and endometrial malignancy. Hippokratia. 2017; 21(4): 169–174, indexed in Pubmed: 30944506.
21. Goldman NA, Katz EB, Glenn AS, et al. GLUT1 and GLUT8 in endometrium and endometrial adenocarcinoma. Mod Pathol. 2006; 19(11): 1429–1436, doi: 10.1038/modpathol.3800656, indexed in Pubmed: 16892013.
22. Wahl H, Daudi S, Kshirsagar M, et al. Expression of metabolically targeted biomarkers in endometrial carcinoma. Gynecol Oncol. 2010; 116(1): 21–27, doi: 10.1016/j.ygyno.2009.10.040, indexed in Pubmed: 19878980.
23. Al-Maghrabi J, Emam E, Gomaa W, et al. c-MET immunostaining in colorectal carcinoma is associated with local disease recurrence. BMC Cancer. 2015; 15: 676, doi: 10.1186/s12885-015-1662-6, indexed in Pubmed: 26459369.
24. Warburg O. On the Origin of Cancer Cells. Science. 1956; 123(3191): 309–314, doi: 10.1126/science.123.3191.309.
25. Liberti MV, Locasale JW, Liberti MV, et al. The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci. 2016; 41(3): 211–218, doi: 10.1016/j.tibs.2015.12.001, indexed in Pubmed: 26778478.
26. Haber RS, Rathan A, Weiser KR, et al. GLUT1 glucose transporter expression in colorectal carcinoma: a marker for poor prognosis. Cancer. 1998; 83(1): 34–40, doi: 10.1002/(sici)1097-0142(19980701)83:1<34::aid-cncr5>3.0.co;2-e, indexed in Pubmed: 9655290.
27. Zhao ZX, Lu LW, Qiu J, et al. Glucose transporter-1 as an independent prognostic marker for cancer: a meta-analysis. Oncotarget. 2018; 9(2): 2728–2738, doi: 10.18632/oncotarget.18964, indexed in Pubmed: 29416806.

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Body mass composition and dietary habits in adolescents with polycystic ovary syndrome

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ABSTRACT

Objectives: The aim of the present study is to assess differences in body composition between female participants in the study group who suffer from PCOS versus a healthy control group.

Material and methods: The study included 85, 14–22-year-old, female participants. Participants belonged to one of two groups. Thirty seven participants with a diagnosis of PCOS were in the clinical group, and 48 participants were in the healthy control group with no prior diagnosis of PCOS.

Results: A statistically significant difference between groups was found in their answer regarding diet. A correlation was found between the body fat index and the use of dieting among participants; participants with a lower body fat index (in kilograms) were less likely to be on a diet.

Conclusions: The young female participants with PCOS were shown to have similar body composition to age-matched healthy controls. However, the clinical group with PCOS reported more frequent use of dieting, with less use of exercise.

Key words: PCOS; body mass composition; dietary habits; young females

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder that is diagnosed in 3–6% of adolescent females. It is estimated that 16% of women who report to a gynecological clinic with a menstrual disorder receive a final diagnosis of PCOS. The etiology of the syndrome is not fully understood; however, it is known that genetics play a significance role in determining risk [1, 2].

The most commonly used criteria for the diagnosis of polycystic ovary syndrome are The Rotterdam Criteria proposed at the Rotterdam conference with the participation of the European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproduction Medicine (ASRM). Determining the diagnosis based on these criteria, however, causes difficulties in the group of adolescents and women in the perimenopausal period. In adolescents, the presence of clinical and biochemical symptoms of hyperandrogenism should be particularly taken into account. Some of the symptoms that meet the criteria for polycystic ovary syndrome may be the result of physiological changes in the body of an adolescent woman. Therefore, in girls, diagnosis is based modified Rotterdam criteria, i.e. all three criteria must be met (and not 2 out of 3 as for adult women):

1. Oligo- or anovulation (adolescent's cycle lasting > 45 days, or amenorrhea > 90 days);
2. Biochemical exponents of hyperandrogenism or hirsutism;
3. The volume of one of the ovaries > 12 mL or > 24 vesicles in the ovary in the ultrasound examination of the pelvis.

Menstrual disorders should be confirmed no earlier than two years after the menarche [1–3].

In 2018, the latest recommendations were issued based on international evidence-based guidelines for the assessment and management of polycystic ovary syndrome. It was found that ultrasound should not be used in the diagnosis of PCOS in girls of gynecological age < 8 years (*i.e.* < 8 years after the first menstruation), due to the high incidence of polycystic ovaries at this stage of life [4].

Characteristic for PCOS are the coexistence of pre-diabetic conditions (abnormal fasting glucose levels and

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glucose intolerance), type 2 diabetes, hyperinsulinaemia, insulin resistance and obesity. Attention is drawn to the following sequence of events: the results of low birth weight are successively: accelerated weight gain, premature adrenarche and PCOS. An increase in body mass is most likely the key predictor of the severity of the syndrome in early puberty. The therapeutic priority for women with PCOS is therefore to maintain a normal weight, or lose weight in cases where the woman is overweight. Studies show that the loss of 10% of one's body weight at diagnosis aids in the return of regular cycles, fertility as well as other metabolic parameters; such weight loss also increases insulin sensitivity in tissues [1, 5–6].

However, the calculation of body mass from a scale does not provide any important information about the amount of adipose tissue and muscle in the body or the overall hydration and condition of the body. As such, body mass index (BMI) is not a sufficient measure to determine the efficiency of human metabolism. In turn, a body composition analysis using the bioimpedance method can generate this critical information. The bioimpedance analysis (BIA) measures the impedance (the type of resistance) of tissues through low-voltage electric current [7].

Objectives

The aim of the present study is to assess differences in body composition between female participants in the study group who suffer from PCOS versus a healthy control group. The plan is to evaluate the individual body mass elements in association with the level of Anti-Mullerian Hormone (AMH) and leptin, in an attempt to determine whether lifestyle has an effect on the body composition of females diagnosed with PCOS versus healthy controls.

MATERIAL AND METHODS

Participants

The study included 85, 14–22-year-old, recruits. Participants belonged to one of two groups. Thirty seven participants with a diagnosis of PCOS were in the clinical group, and 48 participants were in the healthy control group with no prior diagnosis of PCOS. The female participants were patients recruited from the Department of Pediatric Endocrinology of the Upper Silesian Children's Health Center John Paul II in Katowice.

Inclusion criteria for all participants in the study included the following: being 14–22 years old and having experienced menarche for at least two years prior to the study. Exclusion criteria included the use of any pharmacotherapy (*e.g.*, hormonal therapy, contraceptives, NSAIDs) and the presence of any systemic disease (*e.g.*, cardiovascular disease, diabetes, endocrine disease, autoimmune disorders). Participants included in the clinical group were rated according to the Rotterdam Criteria (3/3 criteria) to determine the presence of PCOS. Only women who formally consented to participate in the study, or girls whose guardians consented to their participation, were included in the study.

All participants in the study were informed, in detail, about the purpose and methodology. Consent was obtained from all participants. Ethics approval for the study was obtained from the Bioethical Commission of the Medical University of Silesia in Katowice. Throughout the implementation of the research, researchers strictly abided by the ethical principles outlined in the Declaration of Helsinki.

Methods

Participants were required to answer background questions about their demographics, gynecological history, general medical history including relevant diseases, and lifestyle using the HBSC Questionnaire — a mandatory, standardized questionnaire in Poland in 2013/2014. HBSC is an international study on the health behavior of school children, which are carried out periodically. The questionnaire consists of mandatory questions used by all countries, as well as optional questions. The following indicators were used: meals, physical activity, dietary habits, self-esteem. Based on participant medical history, the length of the menstrual cycle was determined. A regular menstrual cycle (eumenorrhea) was defined as one that cycled every 21–45 days with a length of menstrual bleeding of 3–7 days, and an estimated blood loss of 5–80 mL.

Subsequently, anthropometric measurements (i.e., height, weight, and BMI) were taken for each participant. The measurements were performed in the morning after overnight fasting. The weight (without shoes, in light clothing) was measured using the RADWAG certified electronic scale. The height (in an upright standing position, without shoes) was measured using a stadiometer. The weight was obtained with an accuracy of 0.1 kg, while the height was obtained with an accuracy of 0.5 cm.

The body composition analysis was performed with the use of the BodystatQuadscan 4000. The measurement was performed 10 minutes after obtaining the supine position. The electrodes in accordance to the tetra polar system were placed in the mid-dorsal line of the hands and feet.

The intensity of participants' hirsutism was assessed in accordance with the Ferriman–Gallwey score (the quantitative assessment of body hair in nine different parts of the body: upper lip, chin, upper and lower abdomen, chest, upper arms, as well as thighs, upper and lower back). The severity of hirsutism in each part of the body was rated on a scale from 0 to 4 points. Hirsutism was diagnosed with a score of 8 or more. A transvaginal ultrasound of the pelvis was performed on sexually active women and a transab-

dominal ultrasound (with a full bladder) was performed on not sexually active women.

Subsequently, a series of laboratory procedures were completed. The blood was collected in the morning after overnight fasting, between the 2nd and the 5th day of the cycle in the early follicular phase of the menstrual cycle. Then the level of total and free testosterone, androstenedione, leptin, AMH in serum, insulin, glucose, and the mathematical model of insulin resistance HOMA-IR were measured. Insulin resistance was diagnosed at HOMA-IR ≥ 2.5.

The diagnosis of PCOS was made based on the criteria set by the European Society for Human Reproduction (ESHRE) and the American Society of Reproductive Medicine (ASRM): 1) oligoovulation or anovulation occurred at least 2 years after the menarche), 2) biochemical exponents of hyperandrogenism and hirsutism, 3) the volume of one of the ovaries > 12 mL in a pelvic ultrasound scan. PCOS in the clinical group of participants was recognized if all of the three criteria were met.

RESULTS

The healthy control and clinical groups were compared on the variables investigated in this study. The mean age of female participants in the clinical group was 19.4 ± 2.4 years, while the mean age of participants in the control group was 20.0 ± 2.2 years. The groups were matched on age, with no significant difference between them (p = 0.32). Also there were no significant differences between groups on measures of weight, height and BMI (Tab. 1).

A statistically significant difference between groups was found in their answer regarding diet (p = 0.02). When asked, "Are you following any diet or doing anything to lose your weight?", 54.7% of participants in the control group answered that they did not need to adhere to any specific diet because their weight is where it is supposed to be, whereas only 21.21% of participants in the clinical group answered in this way. In the clinical group, 27.27% of participants reported that they currently use or have used in the past some dietary plan, while only 14.29% of participants in the control group provided this answer. However, no statistical significance was found between the quality of the meals that each group consumed. The girls from both groups consumed fruits and vegetables as well as sweets and sweet drinks with equal frequency. The results of this analysis are presented in Table 2. The participants in the clinical group reported significantly less engagement in sports, defined as participation in physical activity lasting at least 60 minutes per week (p = 0.02). There was no statistical significance between the self-assessment measure of participants in the control versus clinical group in terms of physical appearance (Fig. 1). Furthermore, when asked about the number of hours spent watching movies, playing games, using computers and social networks during their free time, there were no significant differences between groups in their answers.

Table 3 presents the distribution of individual body mass components in the clinical versus control groups. An association was noted between the leptin level and body composition. A statistically significant correlation was found between the mass of adipose tissue (described in kilograms and percentage) and the content of water in the body (Tab. 4).

Table 1. Basic characteristic of anthropometric measurements amongst the study and control group

Statistical parameter	Study group [average ± SD]	Control group [average ± SD]	Mann–Whitney U test
Height [cm]	165.9 ± 6.5	165.5 ± 6.1	p = 0.49
Weight [kg]	64.3 ± 12.4	61.8 ± 9.9	p = 0.31
BMI [kg/m^2]	23.3 ± 4.1	22.6 ± 3.5	p = 0.55

BMI — body mass index

Table 2. The frequency of consumption of particular products by females from the test and control groups

Frequency of consumption	Products							
	Fruits		Vegetables		Sweets (candy, chocolate)		Coca-cola or other sweet drinks	
	Study group	Control group	Study group	Control group	Study group	Control group	Study group	Control group
Never	0%	0%	2.94%	0%	2.94%	2.27%	29.41%	22.73%
Less than once a week	6.06%	2.27%	0%	9.09%	11.76%	13.64%	17.65%	47.73%
Once a week	21.21%	15.91%	0%	9.09%	17.65%	13.64%	20.59%	15.91%
2–4 days a week	18.18%	36.36%	41.18%	20.45%	32.35%	34.09%	14.71%	11.36%
5–6 days a week	18.18%	15.91%	20.59%	20.45%	8.82%	11.36%	8.82%	0%
One time every day	18.18%	11.36%	8.82%	22.73%	11.76%	15.91%	2.94%	2.27%
More than once every day	18.18%	18.18%	26.47%	18.18%	14.71%	9.09%	5.88%	0%
Yates's chi-squared test	p = 0.60		p = 0.10		p = 0.98		p = 0.10	

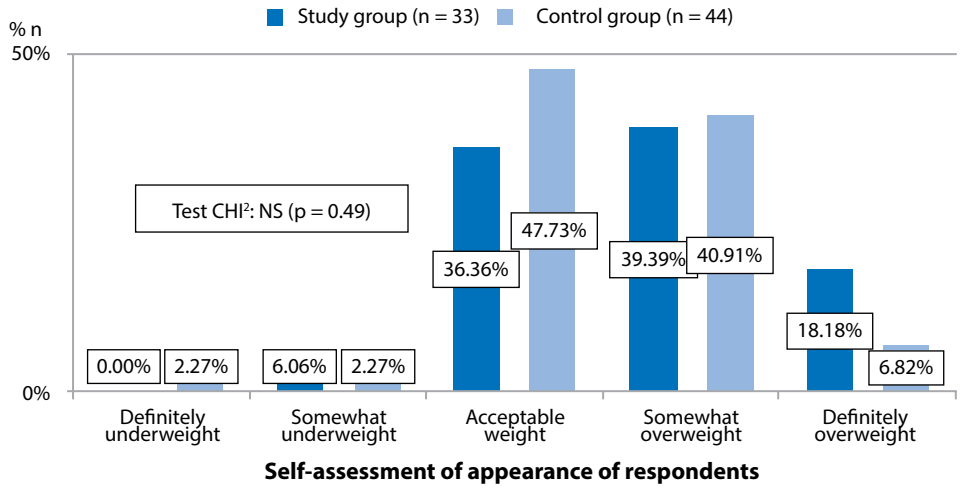

Figure 1. Self-assessment of appearance among study and control groups of females

Table 3. Distribution of body mass components amongst the study and control groups

Body mass components	Study group			Control group			Chi-squared test
	Below the norm	Norm	Above the norm	Below the norm	Norm	Above the norm	
Body fat index [%]	1 (3.45%)	6 (20.69%)	22 (75.86%)	2 (4.26%)	18 (38.30%)	27 (57.45%)	p = 0.28
Body fat [kg]	1 (3.45%)	5 (17.24%)	23 (79.31%)	7 (14.89%)	13 (27.66%)	27 (57.45%)	p = 0.15
FFM [kg]	6 (20.69%)	23 (79.31%)	0 (0.00%)	20 (42.55%)	27 (57.45%)	0 (0.00%)	p = 0.06
Water content [%]	22 (75.86%)	7 (24.14%)	0 (0.00%)	25 (53.19%)	21 (44.68%)	1 (2.13%)	p = 0.17
Water content [kg]	22 (75.86%)	7 (24.14%)	0 (0.00%)	26 (55.32%)	20 (42.55%)	1 (2.13%)	p = 0.23

FFM — fat-free mass

Table 4. Leptin level [ng/mL] in association with the body mass components amongst the study and control groups

Body mass component	Category	Study group		Control group	
		Average ± standard deviation.	U Mann-Whitney test	Average ± standard deviation	U Mann-Whitney test
Body fat index [%]	Below the norm	8.11	p = 0.008	4.98 ± 2.96	p = 0.004
	Norm	6.20 ± 2.84		9.11 ± 6.30	
	Above the norm	16.40 ± 11.18		16.90 ± 16.04	
Body fat [kg]	Below the norm	8.11	p = 0.03	5.54 ± 2.23	p = 0.001*
	Norm	6.39 ± 3.13		10.65 ± 6.69	
	Above the norm	15.92 ± 11.17		16.78 ± 16.14	
FFM [kg]	Below the norm	19.14 ± 17.40	p = 0.61	10.00 ± 6.73	p = 0.07
	Norm	12.67 ± 8.19		15.93 ± 16.30	
	Above the norm	—		—	
Water content [%]	Below the norm	16.40 ± 11.18	p = 0.006	17.46 ± 16.53	p = 0.003
	Norm	6.47 ± 2.69		8.89 ± 6.06	
	Above the norm	—		7.07	
Water content [kg]	Below the norm	16.40 ± 11.18	p = 0.006	17.10 ± 16.30	p = 0.003

*Kruskal–Wallis H test was applied to 3 categories; FFM — fat-free mass

However, there was no correlation between the level of AMH and individual body mass components. See Table 5 for findings.

A correlation was found between the body fat index and the use of dieting among participants; participants with a lower body fat index (in kilograms) were less likely to be on

Table 5. Spearman's correlationof the body mass componentswith the value of AMH among the study and control groups

Correlations	AMH	
	Study group	Control group
Body fat index [%]	R = –0.16 p = 0.40	R = –0.08 p = 0.61
Body fat [kg]	R = –0.24 p = 0.21	R = 0.03 p = 0.87
FFM [kg]	R = 0.08 p = 0.68	R = 0.08 p = 0.61
Water content [%]	R = 0.10 p = 0.60	R = –0.01 p = 0.95
Water content [kg]	R = –0.10 p = 0.59	R = 0.0 p = 0.91

FFM — fat-free mass

a diet (Fig. 2). The same association was found between the measure of Fat-free mass (FFM) and the likelihood of being on a diet (Fig. 3). Finally, participants with a higher body fat index were more likely to see themselves as 'somewhat, or definitely too fat' (Fig. 4).

DISCUSSION

The incidence of PCOS increases rapidly between 12 and 14 years of age, peaking between 15 and 24 years of age and gradually decreasing until the individual reaches menopause. For this reason, research in this area with young females is so important. It appears that research on the etiopathogenesis of PCOS, which remains unclear, should focus on this age group [8].

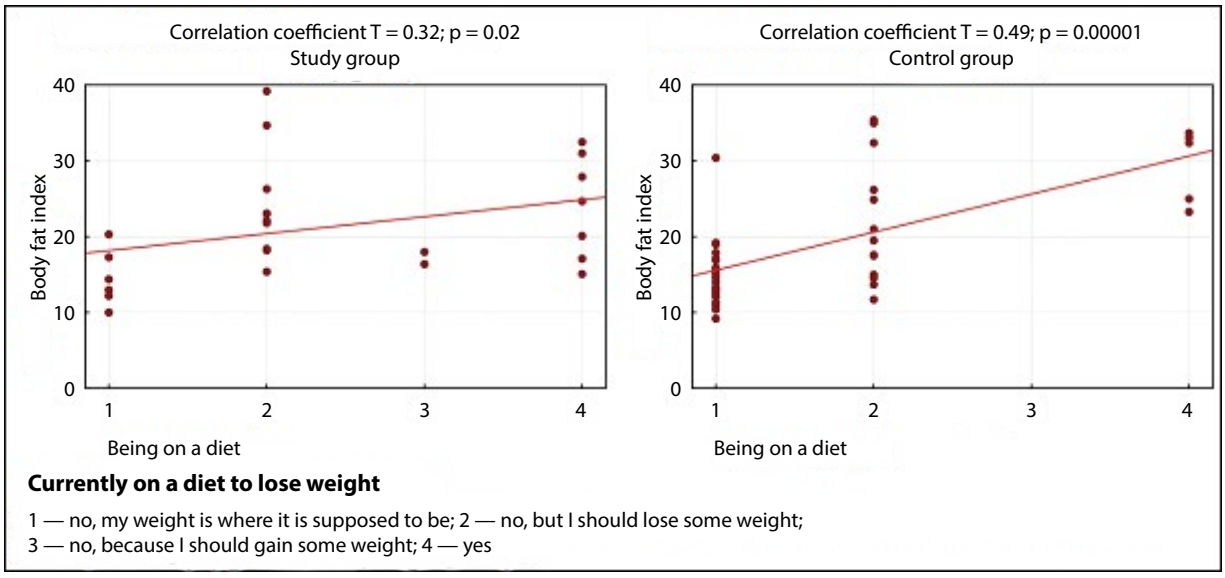

Figure 2. Correlation between body fat index (kg) and current diet among study and control groups

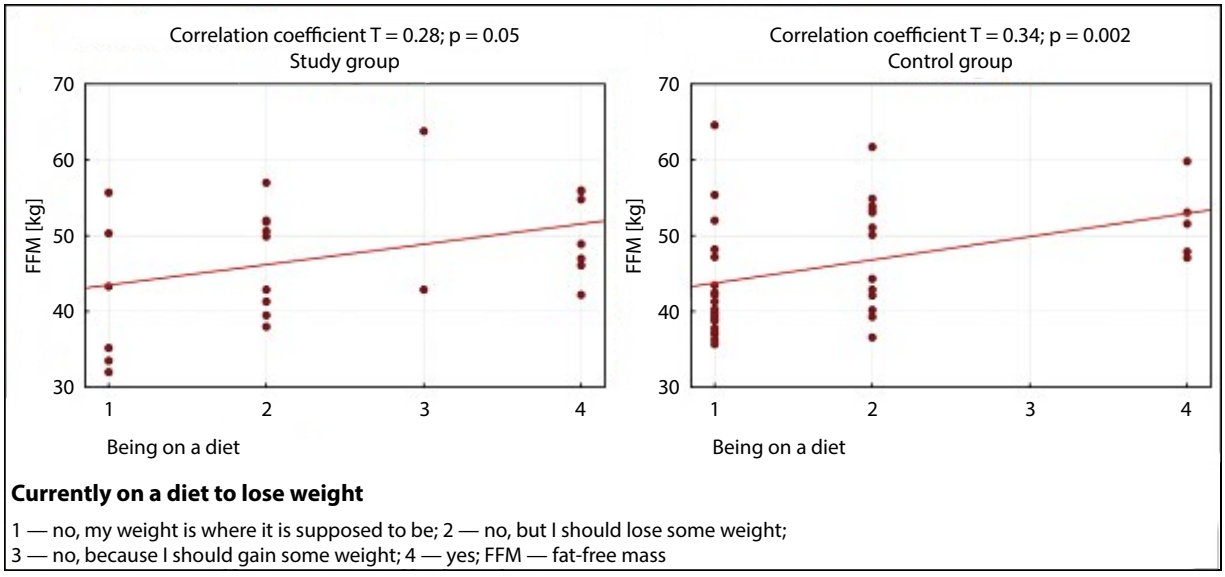

Figure 3. Correlation betweenFat-free mass (kg) and current diet among study and control groups

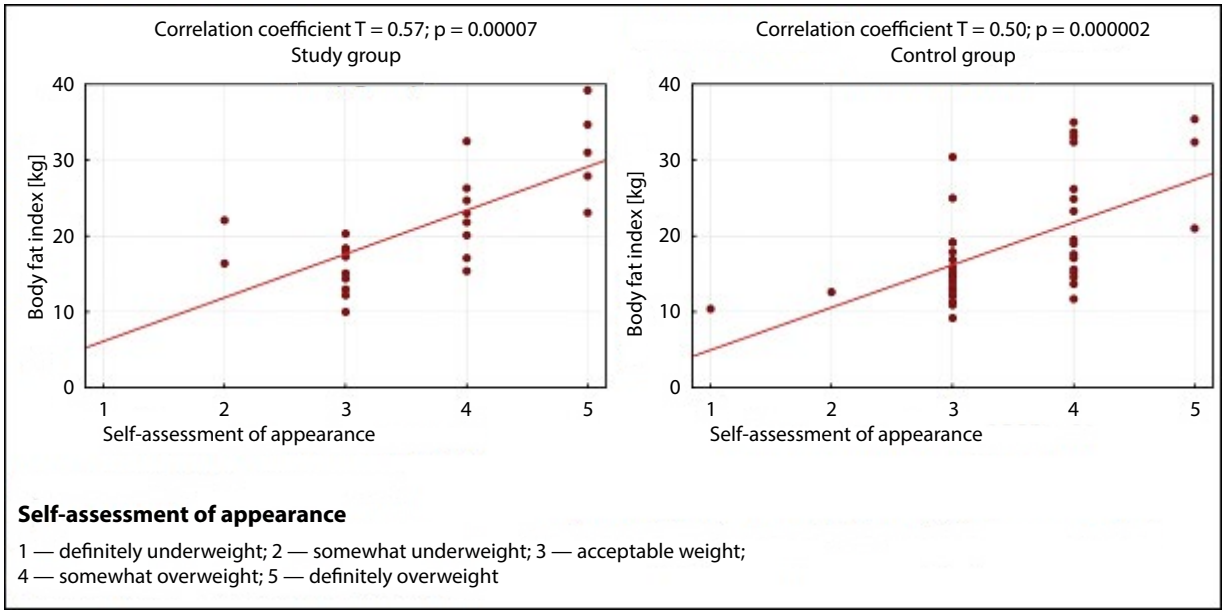

Figure 4. Correlation between body fat index (kg) and self-assessment of appearance among study and control groups

The common features of PCOS, including as hyperinsulinaemia, metabolic dysfunction and hyperandrogenemia, are associated with the accumulation of excess adipose tissue. Regardless of BMI, adipose tissue distribution along with proportionately greater abdominal obesity may further exacerbate metabolic abnormalities associated with PCOS [9].

Our study did not show statistically significant differences between the body mass composition of female participants in a healthy control versus clinical group (participants diagnosed with PCOS). The absence of a statistically significant difference in the body mass composition between healthy and clinical groups of women, measured using the bioimpedance method, was previously reported by Geronikolou et al. [10]. Furthermore, Attlee et al. [11] used BIA analysis to discover that FFM and percentage of adipose tissue did not significantly differ between young females with and without PCOS. This finding expands on other studies that did not find a difference between anthropometric measurements including weight, BMI, waist and hip circumference, in women with PCOS versus women without PCOS [12]. In turn, Ezeh et al. [13] studied a group of adult female patients with PCOS and found an unfavorable body composition, characterized by an increased fat-to-muscle ratio. In the studies comparing only BMI between groups, women with PCOS had a significantly higher BMI compared to the control group (without PCOS) [14].

According to the research, 30–70% of the population around the world with PCOS is overweight or obese [15]. The increasing number of metabolic and reproductive dysfunctionsassociated with adipose tissue disorders, such as altered cytokine secretion, chronic low grade inflammation and oxidative stress, is related to an increased occurrence of PCOS [15].

Adipose tissue is not only the body's energy storage but also the largest endocrine and paracrine organ that synthesizes the biologically active substances. These substances are called adipokines and they act both at autocrine/paracrine and at endocrine levels. One of these adipokines is called leptin. The concentration of the leptin in plasma is directly proportional to the amount of adipose tissue. In obese individuals, prolonged existence of high concentrations of leptin leads to leptin resistance and, as a consequence, to increased resistance of peripheral tissues to insulin. According to some studies, concentrations of leptin in young females with PCOS are directly proportional to BMI, body fat index, waist circumference and HOMA-IR index, and are higher than those in healthy young females. Our study confirmed that the relation between leptin and body fat index exists in both the clinical and control groups. It is commonly believed that the concentration of leptin in the plasma of young females is a determining factor in the onset of puberty. The delay in puberty onset in females with low body fat index and the occurrence of secondary amenorrhea are most likely associated with a decrease in leptin levels [16–19].

It has been found that lifestyle changes can improve body mass composition, symptoms of hyperandrogenism and insulin resistance in women with PCOS, although there is no evidence of a positive effect of these changes on the glycemic profile and lipid profile in participants. Nevertheless, lifestyle changes are recommended as a first-line treatment in overweight ad obese adolescents with suspected PCOS. In our study, young females from the clinical group reported significantly more adherence to a diet and less participation in sports. Meanwhile, it was that yoga classes

were more effective than conventional physical exercises in improving glucose, lipids and insulin values. No difference was noted between groups in the time that participants reported to use the computer or television. Participants with a higher body fat index were more likely to be on a diet, or only declared it, across groups [8].

Psychological interventions are also an important consideration in this group of patients. An Endocrine Society Clinical Practice Guideline indicate an increased incidence of depression and anxiety in women with PCOS. It is suggested that women with PCOS be screened for depression and anxiety. Obesity was indicated as the main factor causing depression and emotional stress among young females with PCOS. It is stated that the main psychological and behavioral interventions that improve mental health in young female with PCOS are: good sleep, lifestyle changes (healthy diet and prevention of sedentary lifestyles) and regular exercise. These strategies are designed to reduce stress and depression through, among others, weight loss [8, 20].

One of the limitations of the present study is a small sample size in the clinical group. However, due to the occurrence of PCOS and the range of age of participants in the study, collecting a larger sample size would require a much longer time frame. Finally, body mass composition was measured using BIA, which is less sensitive and accurate than other methods such as DXA or MRI. However, BIA has been shown to be significantly more cost-effective and provides comparable results. Unfortunately, in the literature there are no such studies conducted on a group of young PCOS girls regarding body composition and dietary habits.

CONCLUSIONS

The young female participants with PCOS were shown to have similar body composition to age-matched healthy controls. However, the clinical group with PCOS reported more frequent use of dieting, with less use of exercise. There is a need for further studies in this area of research with a larger sample size in order to confirm these results. Although PCOS is considered primarily to be a gynecological condition, it is related to many systems in the human body. This can result in an increased risk of infertility, insulin resistance, metabolic syndromes, type 2 diabetes, and other health concerns. Therefore, a greater understanding of the etiopathogenesis of PCOS is of utmost importance.

REFERENCES

1. Skrzypulec-Plinta V, Drosdzol-Cop A. Pierwotny i wtórny brak miesiączki. In: Drosdzol-Cop A. ed. Ginekologia dziecięca i dziewczęca. PZWL, Warszawa 2017: 107–109.
2. Chung PW, Chan SSC, Yiu KW, et al. Menstrual disorders in a Paediatric and Adolescent Gynaecology Clinic: patient presentations and longitudinal outcomes. Hong Kong Med J. 2011; 17(5): 391–397, indexed in Pubmed: 21979477.
3. Rackow BW. Polycystic ovary syndrome in adolescents. Curr Opin Obstet Gynecol. 2012; 24(5): 281–287, doi: 10.1097/GCO.0b013e32835669ff, indexed in Pubmed: 22781076.
4. Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018; 110(3): 364–379.
5. Panidis D, Balaris C, Farmakiotis D, et al. Serum parathyroid hormone concentrations are increased in women with polycystic ovary syndrome. Clin Chem. 2005; 51(9): 1691–1697, doi: 10.1373/clinchem.2005.052761, indexed in Pubmed: 16037412.
6. Diamanti-Kandarakis E. PCOS in adolescents. Best Practice & Research Clinical Obstetrics & Gynaecology. 2010; 24(2): 173–183, doi: 10.1016/j.bpobgyn.2009.09.005.
7. Lewitt A, Mądro E, Krupienicz A. Podstawy teoretyczne i zastosowania analizy impedancji bioelektrycznej (BIA). Endokrynologia, Otyłość, Zaburzenia Przemiany Materii. 2007; 3(4): 79–84.
8. Wang FF, Pan JX, Wu Y, et al. American, European, and Chinese practice guidelines or consensuses of polycystic ovary syndrome: a comparative analysis. J Zhejiang Univ Sci B. 2018; 19(5): 354–363, doi: 10.1631/jzus.B1700074, indexed in Pubmed: 29732746.
9. Goss AM, Chandler-Laney PC, Ovalle F, et al. Effects of a eucaloric reduced-carbohydrate diet on body composition and fat distribution in women with PCOS. Metabolism. 2014; 63(10): 1257–1264, doi: 10.1016/j.metabol.2014.07.007, indexed in Pubmed: 25125349.
10. Geronikolou SA, Bacopoulou F, Cokkinos D. Bioimpedance Measurements in Adolescents with Polycystic Ovary Syndrome: A Pilot Study. Adv Exp Med Biol. 2017; 987: 291–299, doi: 10.1007/978-3-319-57379-3_26, indexed in Pubmed: 28971467.
11. Attlee A, Nusralla A, Eqbal R, et al. Polycystic ovary syndrome in university students: occurrence and associated factors. Int J Fertil Steril. 2014; 8(3): 261–266, indexed in Pubmed: 25379154.
12. Aydin K, Cinar N, Aksoy DY, et al. Body composition in lean women with polycystic ovary syndrome: effect of ethinyl estradiol and drospirenone combination. Contraception. 2013; 87(3): 358–362, doi: 10.1016/j.contraception.2012.07.005, indexed in Pubmed: 22898361.
13. Ezeh U, Pall M, Mathur R, et al. Association of fat to lean mass ratio with metabolic dysfunction in women with polycystic ovary syndrome. Hum Reprod. 2014; 29(7): 1508–1517, doi: 10.1093/humrep/deu096, indexed in Pubmed: 24813197.
14. Roe AH, Prochaska E, Smith M, et al. Using the androgen excess-PCOS society criteria to diagnose polycystic ovary syndrome and the risk of metabolic syndrome in adolescents. J Pediatr. 2013; 162(5): 937–941, doi: 10.1016/j.jpeds.2012.11.019, indexed in Pubmed: 23260096.
15. Jafari-Sfidvajani S, Ahangari R, Hozoori M, et al. The effect of vitamin D supplementation in combination with low-calorie diet on anthropometric indices and androgen hormones in women with polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. J Endocrinol Invest. 2018; 41(5): 597–607, doi: 10.1007/s40618-017-0785-9, indexed in Pubmed: 29110281.
16. Hausman DB, DiGirolamo M, Bartness TJ, et al. The biology of white adipocyte proliferation. Obes Rev. 2001; 2(4): 239–254, indexed in Pubmed: 12119995.
17. Leal Vd, Mafra D. Adipokines in obesity. Clin Chim Acta. 2013; 419: 87–94, doi: 10.1016/j.cca.2013.02.003, indexed in Pubmed: 23422739.
18. Jürimäe J. Adipocytokine and ghrelin responses to acute exercise and sport training in children during growth and maturation. Pediatr Exerc Sci. 2014; 26(4): 392–403, doi: 10.1123/pes.2014-0162, indexed in Pubmed: 25372374.
19. Kedikova SE, Sirakov MM, Boyadzhieva MV. Leptin levels and adipose tissue percentage in adolescents with polycystic ovary syndrome. Gynecol Endocrinol. 2013; 29(4): 384–387, doi: 10.3109/09513590.2012.752455, indexed in Pubmed: 23350621.
20. Sadeeqa S, Mustafa T, Latif S. Polycystic Ovarian Syndrome-Related Depression in Adolescent Girls: A Review. J Pharm Bioallied Sci. 2018; 10(2): 55–59, doi: 10.4103/JPBS.JPBS_1_18, indexed in Pubmed: 29962792.

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The relationship between IGF1 and the expression spectrum of miRNA in the placenta of preeclampsia patients

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ABSTRACT

Objectives: Pre-eclampsia (PE) affects many women worldwide and remains the leading cause of morbidity and mortality in neonatal and maternal settings. Abnormal expression of placental microRNAs (miRNAs) may be associated with PE.

Material and methods: This study was conducted to the relationship between IGF1 and the expression spectrum of miRNA in the placenta of preeclampsia patient. The expression of miRNA in placental tissue was compared between pre-eclampsia (n = 6) and normal pregnant women (n = 5) miRNA targets were studied by computer simulation and functional assays. The role of miRNA was verified in trophoblast cell lines by apoptosis assay and invasion assay.

Results: There was a significant increase in miRNAs in the placenta of women with pre-eclampsia compared with patients with normal pregnancy. Luciferase assay confirmed direct regulation of miRNA.

Conclusions: The expression of IGF1 and miRNA was significantly increased in the placenta of patients with pre-eclampsia.

Key words: preeclampsia; IGF1; miRNA; placenta; expression spectrum

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INTRODUCTION

Preeclampsia (PE), eclampsia and gestational hypertension are unique during pregnancy diseases, which falls within the category of gestational hypertension (hypertensive disorders in pregnancy), morbidity [1–2]. It is about 10% in China, especially in preeclampsia. PE is mainly manifested within 20 weeks after pregnancy [3–4]. Hypertension, proteinuria, can cause multiple organ dysfunction as well as functional failure in the whole body. This is leading to maternal and the main cause of perinatal mortality has been the focus of pathological obstetric research, the pathogenesis is unknown.

Because most preeclampsia conditions can quickly relieve or even heal themselves after placental delivery, some scholars believe that the placenta is the root cause of its disease. In the process of embryo implantation and placenta formation, if various factors lead to trophoblastic dysfunction, it will cause uterine spiral artery recasting disorder, placental blood supply insufficientcy, which results in preeclampsia disease. With the gradual deepening of microRNA (miRNA) function research, more and more studies have confirmed that miRNA plays an important regulatory role in the development of PE. This provides molecular biology clues that reveal the pathogenesis of PE [5, 6].

MiRNAs in eukaryotic organisms, about 18-25 bases in length, by pairing with the target gene mRNA base to induce the silent Complex (RISC) to degrade mRNA or inhibit its translation, thus achieving negative regulation of the expression level of the target gene at the post-transcription level. The discovery of MiRNAs is a milestone in the field of gene expression regulation, which regulates about 30% of human protein-coded genes, and is related to assorted physiological and pathological processes. For instance, embryonic development, organ formation and the occurrence of diseases, through negative regulation of the level of target gene expression.

MATERIAL AND METHODS

Patient samples

All patients received written informed consent. The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Nanchang University. Six

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patients with pre-eclampsia placenta and 5 patients who underwent caesarean section were enrolled in our study. Patients who underwent caesarean section served as the control group. The 11 patients were used for microarray analysis of miRNAs. All of the patients were further evaluated. Patients with pre-eclampsia were confirmed by clinical diagnosis by the professional doctor in our hospital. The placenta tissues from patients were immediately snap-frozen in liquid nitrogen after abscission. The detailed information of samples were shown in Table 1 and Table 2.

Table 1. Sample Description

Sample Number	Sample Name	Group
1	34	T
2	42	T
3	86	T
4	90	T
5	95	T
6	114	T
7	32	N
8	63	N
9	50	N
10	71	N
11	81	N

Table 2. Hybridizations to be performed

Slide no.	Hy3	Hy5
Slide 1	34	
Slide 2	42	
Slide 3	86	
Slide 4	90	
Slide 5	95	
Slide 6	114	
Slide 7	32	
Slide 8	63	
Slide 9	50	
Slide 10	71	
Slide 11	81	

Methods

Microarray analysis of miRNAs

The miRCURYTM LNA array (7th generation) (version 18.0, Exiqon) includes 3,100 capture probes embodying the whole annotations in miRBase 18.0. the miRNAs of mice and rats, and the miRNAs of all viruses associated with them. Furthermore, the array covers the probes for miLlusTM human miRNAs [7].

Extraction of total RNA

Total RNA of patients was extracted by using total RNA isolation kits (Invitrogen) and miRNeasy mini kits (QIAGEN) under the conditions recommended by the manufacturer. The purity of extracted RNA was determined by using the spectrophotometer (ND-1000, Nanodrop Technologies). In addition, we evaluated the RNA integrity by gel electrophoresis [8].

RNA labeling

RNA labeling was performed by using the miRCURYTM Hy3TM / Hy5TM Power Labeling Kits (Exiqon, Vedbaek, Denmark). Furthermore, the sample (1 μg) was performed 3'-end-labeling by using a Hy3TM fluorescent label. We also mixed 2.0 μL of RNA in water with 1.0 μL LCIP buffer and CIP (Exiqon). The mixture was incubated at 37°C for 30 min and at 95°C for 5 min. In addition, we added labeling buffer (3.0 μL), labeling enzyme (2.0 μL), fluorescent label (Hy3TM) (1.5 μL), and DMSO (2.0 μL) into the mixture, which was incubated at 16°C for 1 h and at 65°C for 15 min [9].

Array hybridization

The miRCURYTM LNA array (Section 18.0) (Exiqon) was used for array hybridization. Firstly, hybridization buffer [25 (mu)L] was added into the above sample [25 (mu)L]. Next, the mixture was incubated at 95 (deg.) C for 2 min in order to denaturate, followed by incubation at 4°C for 2 min. After that, the mixture was hybridized to the microarray at 56 (deg.) C. for 16–20 h. Bay Hybridization Systems (Hybridization System — Nimblegen Systems, Inc., Madison, WI, USA) provided effective mixing and invariant incubation temperature. Finally, slides were prepared and washed three times with a wash buffer kit (Exiqon). After washed, slides were dried by centrifugation (400 rpm/min) for 5 min. Finally, slides were scanned using Axon GenePix 4000B microarray scanner (Axon Instruments, Foster City, CA) [10].

Array Information

Exiqon's miRNA arrays feature Tm-standardized and LNATM enhanced capture probes, which have outstanding specificity and sensitivity even for AT-rich miRNAs. They also have excellent repeatability. The correlation between arrays was 99%. The dynamic range was more than 5 orders of magnitude.

miRCURY LNA™ microRNA Array, 7th gen — hsa, mmu & rno

The 7th generation miRNA array included 3100 capture probes, which covered the whole of miRNAs in humans, mice and rats annotated in miRBase 18.0, and viral miR-

NAs. Moreover, there were capture probes for miLlusTM human microRNAs in the array, which were proprietary microRNAs not found in miRBase [11].

Data analysis

The scanned image was imported into GenePix Pro 6.0 software (Axon) for grid alignment and data extraction. The replicated miRNAs were averaged and the miRNAs more than 50 intensities were selected for calculation of normalization factors. The expressed data was normalized by using median normalization. Moreover, miRNAs, with obviously different expression, were discerned by using Volcano Plot filtration. MEV software (v4.6,TIGR) was used for hierarchical clustering.

RESULTS

Low intensity filtering and data normalization

The scanned image was imputed into GenePix Pro 6.0 software (Axon) to do grid alignment and to extract data. The repetitive miRNAs were average while miRNAs which were more than 50 intensities were chosen to calculate querynormalization. The median normalization was used to normalize the data of miRNA. The miRNAs were selected for differential expression. The following list represents only a subset of the overall results. The entire results can be found in the expression "expression matrix" in the miRNA expression profiling Data.xls file (Tab. 3).

miRNA purity

The box plot is an intuitive method in order to effectively visualize the level of disaggregation of data sets. It is an effective way to compare the distribution of miRNAs. In addition, the log2 ratio distribution of each sample was basically identical (Left: data with non-normalized log2-ratio distribution; right: data with median normalized log2-ratio distribution) (Fig. 1).

Correlation Matrix and scatter plot

A scatter plot is a visualization that can be used to evaluate the repetitiveness of chips. Its axis was the normalized signal value (scale scale) (Fig. 2).

Differentially expressed miRNAs screening

Differentially expressed miRNAs with statistically significant differences were identified. We performed Volcano Plot filtration on both groups in the experiment. The threshold, a fold change more than 1.5 and a P value less than 0.05, was used to screen for up-regulation or down-regulation of miRNA. The following list represents only a portion of the differentially expressed miRNAs in the T to N results (Tab. 4).

Heat map and hierarchical clustering

It was displayed the bidirectional hierarchical clustering of samples and miRNAs in the heat map. The abscissa represented miRNA and the ordinate represented samples. On the

Table 3. The results of miRNA Expression

ID	Name	Fold Change	P-value	ForeGround-BackGround		Normalized	
		T vs N	T vs N	...	Mean of N group	...	Mean of N group
13138	Hy3	0.996336378	0.989417	...	333.4	...	1.148126
42638	hsa-miR-23a-5p	1.568977638	0.07266	...	117.3	...	0.390772
42888		1.14788641	0.665814	...	1586.9	...	5.350498
17519	ebv-miR-BART1-3p	1.097571346	0.730312	...	17.2	...	0.059658
17278		1.160911656	0.5042	...	19.4	...	0.066363
46507	hsa-miR-921	1.586768402	0.200918	...	42.2	...	0.140007
17928	hsa-miR-181a-2-3p	1.510173499	0.312414	...	17.2	...	0.059737
42826		0.783954977	0.240246	...	590.1	...	2.016165
17537		1.008976924	0.970497	...	171.2	...	0.585387
42722		1.739150162	0.078576	...	17	...	0.058376
42645		0.560113342	0.294479	...	22.2	...	0.081096
46636		1.195298862	0.508479	...	31.1	...	0.104871
11134	hsa-miR-502-5p	0.931910856	0.855179	...	252.6	...	0.832847
17295	hsa-miR-583	0.927580386	0.667943	...	503.8	...	1.70745
32825	hsa-miR-620	1.204907579	0.413182	...	40.6	...	0.14099
46276		1.87952483	0.05826	...	6	...	0.021253

ID — Contains the miRNA ID number constituted by Exiqon; Name — miRNA name; P-value — T-test result between samples in different groups; Foldchange — Ratio of normalized intensities between two conditions; ForeGround-BackGround — The intensities of the miRNAs before Median normalization; Normalized — The normalized values of the miRNAs after Median normalization

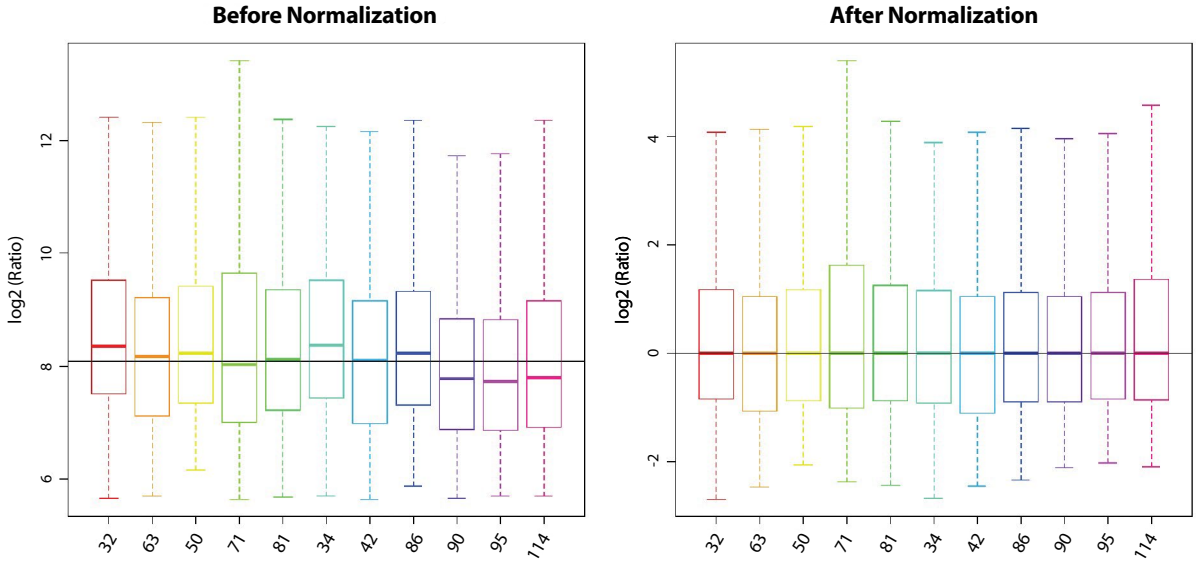

Figure 1. The box plots of before and after normalization

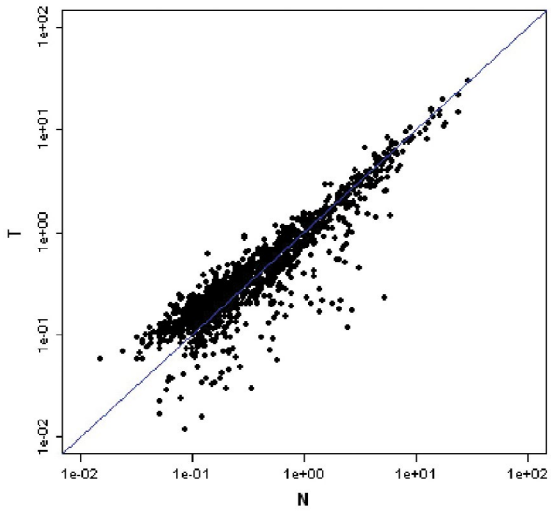

Figure 2. Scatter-plot is for Group-T vs Group-N

left is the miRNA cluster tree, and on the top is the sample cluster tree. According to their expression levels, samples and miRNAs were divided into different groups by cluster analysis, which help us to evaluate their correlation.

Hierarchical clustering based on "Differentially expressed miRNAs in T vs N passed Volcano Plot" was carried out. The results of hierarchical clustering showed a distinguishable miRNA expression profile between samples. The relationship between IGF1 and the expression spectrum of miRNA was showed in Figure 3 and Figure 4.

DISCUSSION

The MicroRNA (miRNA) gene is a new class of non-coding single-stranded RNA molecules 19 to 25 nucleotides in length that are widely found in eukaryotes and have no open reading frame (ORF) [12]. The miRNAs are characterized by diversity of species expression, high conservation, tissue specificity, and clustering of genes. The miRNAs do not directly encode proteins. It is believed that mature miRNAs cause complete or incomplete complementary pairing with targeted messenger RNAs (mRNAs), causing degradation or translational inhibition of target mRNAs and regulating expression of target genes. More and more researches believe

Table 4. T vs N 1.5 fold up regulated miRNAs

ID	Name	Fold change	P-value	ForeGround-BackGround			Normalized		
		T vs N	T vs N	32	...	...	32	...	...
148635	hsa-miR-933	1.52065878	0.0283	34	...	...	0.103976	...	...
168910	hsa-miR-4735-5p	1.63602691	0.029116	52	...	...	0.159021	...	...
168569	hsa-miR-5088	3.22345834	0.038633	39	...	...	0.119266	...	...

ID — Contains the miRNA ID number constituted by Exiqon; Name — miRNA name; Foldchange — Ratio of normalized intensities between two conditions; P-value — P-value calculated from t-test; ForeGround-BackGround — Intensities of the miRNAs in each sample before Median normalization; Normalized — Normalized values of the miRNAs in each sample after Median normalization

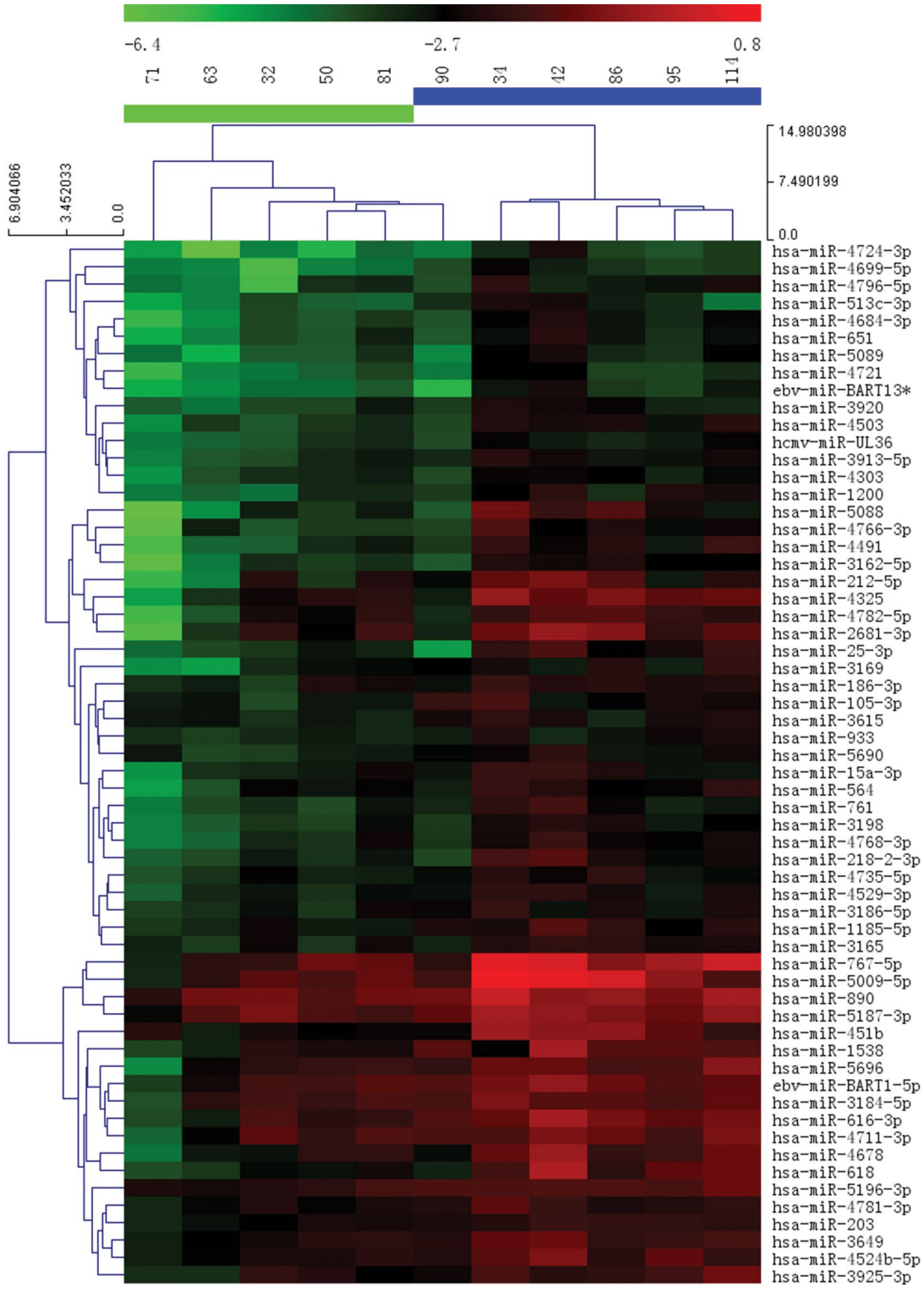

Figure 3. Hierarchical clustering for Differentially expressed miRNAs in T vs N passed Volcano Plot. Red indicates high relative expression, and green indicates low relative expression

that miRNAs play a role in all levels of life. They play an important role in development, cell differentiation, apoptosis, proliferation and tumorigenesis. They have become one of the new research hotspots in life sciences in recent years.

The miRNA genes belong to a highly conserved family of non-coding genes, and various miRNAs can find homologs in other lines [13]. Therefore, miRNAs are involved in various important processes in life processes, such as early embry-

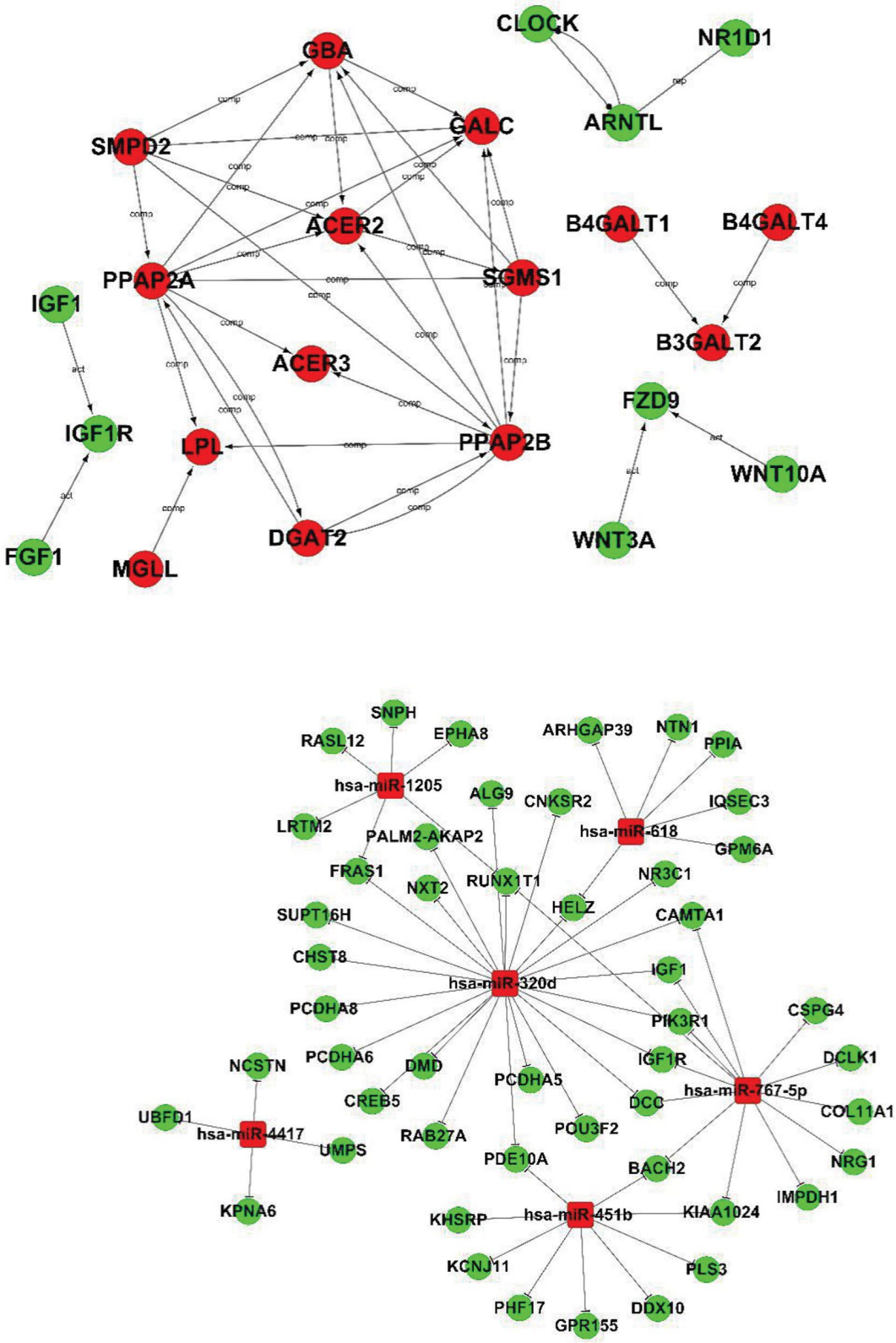

Figure 4. Signal target network diagram

onic development, cell differentiation regulation, apoptosis, cell cycle, wound healing, and the body's immune system. The diversity of a miRNA sequence structure and expression mode plays a very important role in the field of gene expression regulation.

Certain miRNAs are abnormally expressed in mouse and human placental tissues and are differentially expressed in placenta of PE patients. A growing number of studies have demonstrated partial differentially expressed miRNAs in the placenta of PE patients in cell adhesion, immunity, signaling, and cell cycle cardiovascular development. They play an important role in the pathways that play a major role in the pathogenesis of pre-eclampsia.

Insulin-like growth factor-1 (IGF-1) is located on the short arm of chromosome 12 and contains 70 amino acids with a relative molecular mass of 7.6 kD. It is a member of the insulin-like growth factor family and has a structure similar to insulin with insulin-like metabolic effects [14]. Most of the IGF-1 in the body's circulation is derived from the liver, kidneys, thymus and fat. A small amount of IGF-1 can also be detected in tissues. Studies have found that IGF-1 can also be produced in the endometrium and placenta [15]. In human and animal blood and body fluids, IGF-1 rarely exists in a free state, mainly by binding to proteins to form IGF-1 binding protein (IGFBP) to prolong its half-life and ensure its stability in vivo.The biological effects of IGF-1 are mainly exerted by binding to the IGF-1 receptor (IGF-1R), and IGF binding proteins 1 to 6 (IGFBP1 to 6) play an important regulatory role in this process. IGF-1 has a wide range of biological functions, in addition to promoting insulin metabolism, such as gluconeogenesis and glycolysis, hypoglycemic and hypolipidemic. It also has the effects of relaxing blood vessels, promoting cell differentiation and mitosis.In recent years, its research on the mechanism of action in cell differentiation, apoptosis, proliferation and carcinogenesis has become a hot topic.

Because IGF-1 may be involved in the regulation of embryo implantation, revascularization as well as placental fetal growth and development. Abnormal expression of IGF-1 may lead to pathological pregnancy such as PE, which directly affects pregnancy outcomes [16–17]. Studies have confirmed that the incidence of PE is associated with decreased levels of IGF-1 expression.Some researchers have pointed out that the use of immunohistochemistry and radioimmunoassay found that IGF-1 levels in peripheral blood of patients with preeclampsia decreased compared with normal pregnancy While the expression level of IGFBP-1 in placenta of preeclampsia increased, presumed eclampsia Low levels of IGF-1 in the maternal blood and elevated expression of IGFBP-1 in the placenta may lead to placental dysplasia and fetal growth restriction. In addition, some researchers also compared the expression levels of IGF-1 in the pre-eclampsia and normal maternal serum, found that the serum levels of IGF-1 in pre-eclampsia were significantly lower than those in the control group. There was also a significant difference in blood IGF-1 levels between mild pre-eclampsia and severe pre-eclampsia.The more severe the condition, the lower the level of IGF-1 in the maternal serum. It was shown that the pre-eclampsia condition can be assessed by detecting the expression level of IGF-1 in maternal serum [18].

CONCLUSIONS

Expression of IGF1 and miRNA was significantly increased in the placentas of patients with preeclampsia.

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REFERENCES

1. Ramos JG, Sass N, Costa SH. Preeclampsia. Rev Bras Ginecol Obstet. 2017; 39(9): 496–512, doi: 10.1055/s-0037-1604471, indexed in Pubmed: 28793357.
2. Guedes-Martins L. Superimposed Preeclampsia. Adv Exp Med Biol. 2017; 956: 409–417, doi: 10.1007/5584_2016_82, indexed in Pubmed: 27722963.
3. Wu W, Yang H, Feng Y, et al. Polymorphisms in complement genes and risk of preeclampsia in Taiyuan, China. Inflamm Res. 2016; 65(10): 837–845, doi: 10.1007/s00011-016-0968-4, indexed in Pubmed: 27405496.
4. Liu J, Yang H, Yin Z, et al. Remodeling of the gut microbiota and structural shifts in Preeclampsia patients in South China. Eur J Clin Microbiol Infect Dis. 2017; 36(4): 713–719, doi: 10.1007/s10096-016-2853-z, indexed in Pubmed: 27988814.
5. Liu S, Xie X, Lei H, et al. Identification of Key circRNAs/lncRNAs/miRNAs/mRNAs and Pathways in Preeclampsia Using Bioinformatics Analysis. Med Sci Monit. 2019; 25: 1679–1693, doi: 10.12659/MSM.912801, indexed in Pubmed: 30833538.
6. Biró O, Fóthi Á, Alasztics B, et al. Circulating exosomal and Argonaute-bound microRNAs in preeclampsia. Gene. 2019; 692: 138–144, doi: 10.1016/j.gene.2019.01.012, indexed in Pubmed: 30659946.
7. Ye Y, Yang J, Lv W, et al. Screening of differentially expressed microRNAs of essential hypertension in Uyghur population. Lipids Health Dis. 2019; 18(1): 98, doi: 10.1186/s12944-019-1028-1, indexed in Pubmed: 30975221.
8. Landolt L, Marti HP, Beisland C, et al. RNA extraction for RNA sequencing of archival renal tissues. Scand J Clin Lab Invest. 2016; 76(5): 426–434, doi: 10.1080/00365513.2016.1177660, indexed in Pubmed: 27173776.
9. George JT, Srivatsan SG. Posttranscriptional chemical labeling of RNA by using bioorthogonal chemistry. Methods. 2017; 120: 28–38, doi: 10.1016/j.ymeth.2017.02.004, indexed in Pubmed: 28215631.
10. Wang N, Lu Y, Chen Z, et al. Multiplexed PCR-Free Detection of MicroRNAs in Single Cancer Cells Using a DNA-Barcoded Microtrough Array Chip. Micromachines (Basel). 2019; 10(4), doi: 10.3390/mi10040215, indexed in Pubmed: 30934734.
11. Lagatie O, Batsa Debrah L, Debrah A, et al. Plasma-derived parasitic microRNAs have insufficient concentrations to be used as diagnostic biomarker for detection of Onchocerca volvulus infection or treatment monitoring using LNA-based RT-qPCR. Parasitol Res. 2017; 116(3): 1013–1022, doi: 10.1007/s00436-017-5382-5, indexed in Pubmed: 28111713.
12. Guan B, Mu L, Zhang L, et al. MicroRNA-218 inhibits the migration, epithelial-mesenchymal transition and cancer stem cell properties of prostate cancer cells. Oncol Lett. 2018; 16(2): 1821–1826, doi: 10.3892/ol.2018.8877, indexed in Pubmed: 30008871.

13. Schanza LM, Seles M, Stotz M, et al. MicroRNAs Associated with Von Hippel-Lindau Pathway in Renal Cell Carcinoma: A Comprehensive Review. Int J Mol Sci. 2017; 18(11), doi: 10.3390/ijms18112495, indexed in Pubmed: 29165391.
14. Wang Y, Zhang He, Cao M, et al. Analysis of the value and correlation of IGF-1 with GH and IGFBP-3 in the diagnosis of dwarfism. Exp Ther Med. 2019; 17(5): 3689–3693, doi: 10.3892/etm.2019.7393, indexed in Pubmed: 30988753.
15. Luo Lu, Wang Q, Chen M, et al. IGF-1 and IGFBP-1 in peripheral blood and decidua of early miscarriages with euploid embryos: comparison between women with and without PCOS. Gynecol Endocrinol. 2016; 32(7): 538–542, doi: 10.3109/09513590.2016.1138459, indexed in Pubmed: 27174569.
16. Monte APO, Barros VRP, Santos JM, et al. Immunohistochemical localization of insulin-like growth factor-1 (IGF-1) in the sheep ovary and the synergistic effect of IGF-1 and FSH on follicular development in vitro and LH receptor immunostaining. Theriogenology. 2019; 129: 61–69, doi: 10.1016/j.theriogenology.2019.02.005, indexed in Pubmed: 30822644.
17. Liao S, Vickers MH, Taylor RS, et al. Maternal serum IGF-1, IGFBP-1 and 3, and placental growth hormone at 20weeks' gestation in pregnancies complicated by preeclampsia. Pregnancy Hypertens. 2017; 10: 149–154, doi: 10.1016/j.preghy.2017.07.148, indexed in Pubmed: 29153669.
18. Niu ZR, Han T, Sun XL, et al. MicroRNA-30a-3p is overexpressed in the placentas of patients with preeclampsia and affects trophoblast invasion and apoptosis by its effects on IGF-1. Am J Obstet Gynecol. 2018; 218(2): 249.e1–249.e12, doi: 10.1016/j.ajog.2017.11.568, indexed in Pubmed: 29155142.

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The absence of fetal nasal bones in ultrasound examination between 11 + 0 and 13 + 6 weeks of gestation versus the occurrence of trisomies 21, 18, and 13

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ABSTRACT

Objectives: One part of the ultrasound examination of fetuses in the first trimester of gestation is visualization of the nasal bones. Numerous studies have demonstrated a correlation between the absence of nasal bones and abnormal fetal karyotype. Aim: To assess the utility of ultrasound visualization of nasal bones during the first trimester of pregnancy as a marker of the most common chromosomal trisomies.

Material and methods: Ultrasound visualization of nasal bones was carried out in 941 fetuses from a high-risk group between 11 + 0 and 13 + 6 weeks of gestation. Amniocentesis was performed to determine karyotype in all 941 cases.

Results: Normal fetal karyotype was observed in 847 cases, trisomy 21 in 45 cases, trisomy 18 in 16 cases and trisomy 13 in 10 cases. Other abnormal karyotypes were detected in the remaining 23 cases. The absence of nasal bones demonstrated 27% sensitivity, 97% specificity and a positive predictive value of 35% as an indicator of trisomy 21 in the study group, and 12% sensitivity, 97% specificity and 12% positive predictive value for trisomies 18 and 13.

Conclusions: The absence of nasal bones in ultrasound examination in the first trimester of pregnancy is characterized by low sensitivity and high specificity as a marker of the most common trisomies. Visualization of fetal nasal bone is a poor marker of aneuploidy and should not be taken into account in risk calculation algorithms.

Key words: nasal bone; ultrasound trisomy marker; chromosomal trisomies

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INTRODUCTION

Visualization of the nasal bones is one of the main stages of the fetal ultrasound examination between 11 + 0 and 13 + 6 weeks of pregnancy. Numerous studies have reported a correlation between the absence of nasal bone in the first trimester of pregnancy and abnormal fetal karyotype. This has been attributed to the nasal bone ossification process being delayed in fetuses with chromosomal aberrations such as trisomies 21, 18 or 13.

In 1866, Langdon Down observed that patients with trisomy 21, the most commonly occurring chromosomal aberration, were characterized by a typical facial appearance including a small, short nose [1]. In terms of frequency, Down's syndrome is followed by Edwards syndrome, i.e. trisomy 18, with an incidence rate of up to 1/6,000 live births [2]. Following this, trisomy 13 (Patau syndrome) is observed in 1/8,000 to 1/12,000 live births. Trisomies 18 and 13 are characterized by a much more severe clinical presentation than Down's syndrome, with less than 10% of children surviving the first year [3]. These trisomies are associated with the presentation of an abnormal profile together with multiple other serious defects [4]. It has been found that ultrasound scans performed between 11 + 0 and 13 + 6 weeks of gestation indicate an absence of nasal bones in more than 50% of fetuses with trisomy 18, as well as in about 30% of fetuses with trisomy 13 [5].

Aim

To assess the value of ultrasound visualization of nasal bones during the first trimester of pregnancy as a marker of the most common chromosomal trisomies.

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MATERIAL AND METHODS

Nasal bone visualization was performed in 941 fetuses between 11 + 0 and 13 + 6 weeks of gestation (ultrasound scan + biochemical markers: PAPP-A + free βhCG) by certified researchers at two independent referral centers. Voluson E8 and Philips iU22 ultrasound scanners and transabdominal probes were used for the examination. The procedure was performed according to the Fetal Medicine Foundation standards of assessing the presence of nasal bones.

The presence of nasal bones was recorded in ultrasound scan summaries. The risk of fetal chromosomal aberrations was calculated on the basis of maternal age, ultrasound parameters (crown-lump length CRL, nuchal translucency NT) and biochemical analyses (PAPP-A, free βhCG) using Astraia software.

Following the combined non-invasive first trimester screening, comprising ultrasound and biochemical testing, an invasive diagnostic procedure was performed in all 941 cases to assess fetal karyotype and identify any high risk of fetal aneuploidies.

RESULTS

In total, 941 fetal karyotype results were analyzed. A normal fetal karyotype was observed in 847 cases. Of the remainder, trisomy 21 was observed in 45 cases, trisomy 18 in 16 cases, trisomy 13 in 10 cases, and other karyotype anomalies in the remaining 23 cases.

The nasal bones were observed to be absent in 39 fetuses, including 22 with a normal karyotype; however, the nasal bones were visualized in 33 out of 45 T21 patients. In addition, they were observed in all fetuses with trisomy 18, and in seven of ten fetuses with trisomy 13 (Tab. 1).

Two study groups were defined for the purposes of statistical analysis: the group of fetuses with T21 syndrome (n = 45) and the combined group of fetuses with T18 and T13 syndromes (n = 26). The control group consisted of 847 fetuses with a normal karyotype. The frequencies of absence of nasal bones in the T21 group and the T18 and T13 group were compared with that of the control group.

In the group of T21 patients, the nasal bones were found to be absent in 12 cases (Tab. 2). The absence of nasal bones demonstrated 27% sensitivity and 97% specificity as a marker of T21, with a positive predictive value of 35%.

In the combined group of T18 and T13 patients, nasal bones were found to be absent in only three cases (Tab. 3). Lack of nasal bones was found to offer the 12% sensitivity and 97% specificity in the detection of trisomies 18 and 13, with a positive predictive value of 12%.

DISCUSSION

Current non-invasive risk assessment of fetal chromosomal aberrations is based on maternal age, ultrasound markers (NT, FHR) and biochemistry (PAPP-A, free βhCG). This algorithm allows the detection of 85–90% of fetuses with trisomies 21, 18, or 13, with some publications recording a detection rate of 90–95% [6–8]. More sophisticated algorithms incorporating additional markers such as the presence of nasal bones, analysis of ductus venous or tricuspid valve flow have been proposed to reduce the false positive rate (FPR); however, these are the subject of ongoing research.

Many reports have proposed the absence of nasal bones as a potential marker of fetal chromosomal defects. Our present results obtained by ultrasound examination of fetuses with Down's syndrome support those of previous studies indicating that the absence of nasal bones may be a promising marker of fetal trisomy 21. This has been confirmed in many reports, including data from multicenter studies [9, 10]

A paper published in The Lancet in 2001 reported the absence of nasal bones in 43 out of 59 (73%) ultrasound scans in the first trimester of pregnancy; however, while the study also reports that nasal bones were found to be absent in only 0.5% of examined fetuses with normal karyotype [9]. This figure was found to be 2.6% in the present study. Węgrzyn et al. [10] (2016) reported nasal bones to be absent in 64.8% of fetuses with trisomy 21, but only 4.3% of fetuses with normal karyotype. Similarly, a higher rate of absence was also confirmed among fetuses with trisomy 21 (70%) in studies conducted in

Table 1. Presence of nasal bones vs. fetal karyotype: results of the analysis

	NB +	NB –
Normal karyotype	825	22
T21	33	12
T18	16	0
T13	7	3
Other karyotypic anomaly	21	2
Total	902	39

Table 2. Presence of nasal bones in T21 fetuses (n = 45) and controls

	Nasal bones present, NB+	Nasal bones absent, NB -
Normal karyotype	825 (97.4%)	22 (2.6%)
T21	33 (73%)	12 (27%)

Table 3. Absence of nasal bones in T18 and T13 fetuses (n = 26) compared to controls

	Nasal bones present, NB +	Nasal bones absent, NB –
Normal karyotype	825 (97.4%)	22 (2.6%)
T18, T13	23 (88.5 %)	3 (11.5 %)

much smaller study groups [11], and elsewhere, absence was noted in 70% of Down's syndrome fetuses scanned in the first trimester of pregnancy [12]. Interestingly, while the authors of the mentioned article reported the absence of nasal bones in 80% of fetuses with trisomy 18, this was observed in only 11.5% of fetuses from the combined group of trisomy 18 or 13 cases identified in the present study: the vast majority (88.5%) of fetuses tested between 11 + 0 and 13 + 6 weeks of pregnancy were found to possess nasal bones. In addition, another ultrasound study found nasal bones to be absent in 52.8% of fetuses with trisomy 18, 45% of fetuses with trisomy 13, and only 59.8 % of fetuses with trisomy 21 [13]. Sepulveda et al. [14] reported nasal bone hypoplasia in 53% of a group of 53 fetuses with trisomy 18, while Wagner et al. [15] reported a lack of nasal bones in 60% of fetuses with trisomy 18 and 69% of fetuses with trisomy 13.

Our data on the absence of nasal bones in ultrasound examination acquired in T21 syndrome fetuses between 11 + 0 and 13 + 6 weeks of pregnancy are similar to those obtained by other authors. As an ultrasound marker of trisomy 21, ultrasound identification of a lack of nasal bones is characterized by low sensitivity (27%) and high specificity (97%). Likewise, Sieroszewski et al. [16] found such an approach to be characterized by low sensitivity (40%) but very high specificity (100%) in assessing the risk of chromosomal aneuploidies.

Our present findings indicate a much lower percentage of fetuses presenting an absence of nasal bones among the combined group of fetuses with less common aberrations, i.e. those with trisomies 13 or 18; in addition, the presence of nasal bones was detected in all fetuses with trisomy 18. The sensitivity and specificity of the absence of nasal bones as a predictive factor of these trisomies were 12% and 97%, respectively.

CONCLUSIONS

1. The absence of nasal bones between 11 + 0 and 13 + 6 weeks of gestation, identified by ultrasound examination, can be used as a marker of the most common trisomies with low sensitivity and high specificity.
2. Visualization of the fetal nasal bone is a poor marker of aneuploidy and should not be taken into account in risk calculation algorithms.

REFERENCES

1. Langdon Do. Observations on an ethnic classification of idiots. Clinical Lectures and Reports, London Hospital. 1866; 3: 259–262.
2. Cereda A, Carey JC. The trisomy 18 syndrome. Orphanet J Rare Dis. 2012; 7: 81, doi: 10.1186/1750-1172-7-81, indexed in Pubmed: 23088440.
3. Rasmussen SA, Wong LYC, Yang Q, et al. Population-based analyses of mortality in trisomy 13 and trisomy 18. Pediatrics. 2003; 111(4 Pt 1): 777–784, doi: 10.1542/peds.111.4.777, indexed in Pubmed: 12671111.
4. Bruns DA, Campbell E. Nine children over the age of one year with full trisomy 13: a case series describing medical conditions. Am J Med Genet A. 2014; 164A(12): 2987–2995, doi: 10.1002/ajmg.a.36689, indexed in Pubmed: 25323598.
5. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol. 2004; 191(1): 45–67, doi: 10.1016/j.ajog.2004.03.090, indexed in Pubmed: 15295343.
6. Kagan KO, Etchegaray A, Zhou Y, et al. Prospective validation of first-trimester combined screening for trisomy 21. Ultrasound Obstet Gynecol. 2009; 34(1): 14–18, doi: 10.1002/uog.6412, indexed in Pubmed: 19526452.
7. Kagan KO, Wright D, Maiz N, et al. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol. 2008; 32(4): 488–492, doi: 10.1002/uog.6123, indexed in Pubmed: 18726925.
8. Wright D, Syngelaki A, Bradbury I, et al. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther. 2014; 35(2): 118–126, doi: 10.1159/000357430, indexed in Pubmed: 24356462.
9. Cicero S, Curcio P, Papageorghiou A, et al. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet. 2001; 358(9294): 1665–1667, doi: 10.1016/S0140-6736(01)06709-5, indexed in Pubmed: 11728540.
10. Węgrzyn P, Czuba B, Serafin D, et al. Nasal bone in screening for T21 at 11-13 + 6 weeks of gestation - a multicenter study. Ginekol Pol. 2016; 87(11): 751–754, doi: 10.5603/GP.2016.0082, indexed in Pubmed: 27958633.
11. Otaño L, Aiello H, Igarzábal L, et al. Association between first trimester absence of fetal nasal bone on ultrasound and Down syndrome. Prenat Diagn. 2002; 22(10): 930–932, doi: 10.1002/pd.431, indexed in Pubmed: 12378580.
12. Zoppi MA, Ibba RM, Axiana C, et al. Absence of fetal nasal bone and aneuploidies at first-trimester nuchal translucency screening in unselected pregnancies. Prenat Diagn. 2003; 23(6): 496–500, doi: 10.1002/pd.628, indexed in Pubmed: 12813765.
13. Kagan KO, Cicero S, Staboulidou I, et al. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol. 2009; 33(3): 259–264, doi: 10.1002/uog.6318, indexed in Pubmed: 19248005.
14. Sepulveda W, Wong AE, Dezerega V. First-trimester sonographic findings in trisomy 18: a review of 53 cases. Prenat Diagn. 2010; 30(3): 256–259, doi: 10.1002/pd.2462, indexed in Pubmed: 20112232.
15. Wagner P, Sonek J, Hoopmann M, et al. First-trimester screening for trisomies 18 and 13, triploidy and Turner syndrome by detailed early anomaly scan. Ultrasound Obstet Gynecol. 2016; 48(4): 446–451, doi: 10.1002/uog.15829, indexed in Pubmed: 26611869.
16. Sieroszewski P, Baś-Budecka E, Perenc M. [Nasal bone (NB) length measurement in the first trimester of pregnancy in Polish population and its validity as fetal aneuploidy indicator]. Ginekol Pol. 2007; 78(8): 616–620, indexed in Pubmed: 18050610.

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Early potential metabolic biomarkers of primary postpartum haemorrhage based on serum metabolomics

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ABSTRACT

Objectives: Postpartum hemorrhage (PPH) is the leading cause of maternal death, accounting for 1/4 of maternal deaths worldwide. Determining sensitive biomarkers in the peripheral blood to identify postpartum haemorrhage (PPH) is essential for the early diagnosis and management of PPH. The purpose of this study is to identify predictive serum metabolic biomarkers of PPH. Thirty healthy pregnant women and 30 cases of postpartum hemorrhage were studied for our research.

Material and methods: The serum metabolites of all pregnant were detected by liquid chromatography-quadruple time-of-flight mass spectrometry (LC-QTOFMS) and the corresponding biomarkers were identified.

Results: 34 significantly altered metabolites in PPH-pre-group were identified. They were mainly involved in fatty acid, and glycerophospholipid metabolism.

Conclusions: The LysoPCs, PCs, PGs, PIs were effective biomarkers for identifying PPH. The disturbed signaling pathways, mTOR signaling, acute phase response signaling, AMPK signaling and eNOS signaling pathways might be related to the etiopathogenesis of PPH. Our study provided a valuable attempt to screen early diagnostic markers of PPH and to further understand its pathogenesis.

Key words: biomarker; metabolomics; primary postpartum haemorrhage

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INTRODUCTION

Postpartum haemorrhage is the leading cause of death during pregnancy and preterm birth, accounting for 1/4 of maternal deaths worldwide [1]. It is defined as blood loss of more than 500 mL after the birth of the genital tract (or > 1000 mL after a caesarean section). It is estimated that 140,000 women die of PPH each year [2]. Of postpartum deaths, 45% occur within the first 24 hours and 66% occur within the first week. The prevalence of PPH worldwide is between 6% and 10% [3]. The reason for deaths of PPH is that is not easy to notice immediately or earlier which is important to early diagnosis and intervention for PPH. Women who have prolonged childbirth, multiple pregnancies, amniotic fluid, and fetal macrosomia, obesity or fever during childbirth are at increased risk. Previous studies have identified risk factors associated with the PPH [4, 5], such as history of PPH, overdistended uterus, nullipara or low parity, multiple birth, high blood pressure, ante-partum hemorrhage and increased maternal BMI have been significantly associated with PPH [5]. While these associations are not the sensitive predictive biomarkers, women with these risk factors are not suffering from postpartum hemorrhage, while some pregnant women without these risk factors may be suffering from postpartum hemorrhage.

Metabolomics is a discipline that studies the changes of metabolites induced by physiological stimuli or genetic modifications in living systems [6]. Until now, metabolomics has been applied to identify early diagnostic markers of various diseases and study pathogenesis of them. Furthermore, metabolomics is a rapid method for qualitative and semiquantitative analysis of metabolites in cells, biological fluids and tissues. Due to its unique advantages and significant efficiency, metabolomics has been widely used in studying diseases of obstetrics and gynecology in various

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aspects. For example, a series of experimental investigations had been conducted on gestational diabetes mellitus [7, 8], preeclampsia [9, 10] and cancers [11, 12], and to identify the potential markers in maternal serum, plasma, amniotic fluid (AF), cord blood, urine, feces, trophocyte, or tissues. While until now, there is no metabolomics study of PPH for the early diagnostic biomarker identification before childbirth.

In our study, we first employed a LC-MS-based metabolomics study to characterize the metabolic profiles of PPH-pre, and then used bioinformatics analysis software to identify corresponding pathogenesis of PPH. Our aim is to search for effective biomarkers and further understanding the pathogenesis of PPH.

MATERIAL AND METHODS

Patients

Pregnant women with no previous history of pregnancies were included in our study. The subjects were assessed for their health status. Their weight and height information was also recorded, and body mass index was calculated to be kg/m^2. These women were asked to fast for one hour prior to serum collection to prevent the effects of diet on metabolism. All subjects signed informed consent when they were admitted to this study. This study was approved by the research ethics committee of Tongren Hospital, School of Medicine, Shanghai Jiaotong University. Collection of pregnant women's serum within 24 hours prior to the production as the study subjects. After childbirth, calculation of the amount of bleeding in pregnant women with parturition. Women with total bleeding volume more than 500 mL following vaginal delivery were included as the PPH, and these serums collected within 24 hours prior to the production were included as PPH-pre-group. The serum collected within 24 hours before the production from women with total bleeding volume less than 500 mL was included as control group. Lastly, 30 cases of postpartum hemorrhage and 30 women in control group were collected. The patient's age, gestational age, total blood loss during PPH were recorded.

Serum Sample Collection

Venous blood was drawn into a non-heparin tube, placed for 20 minutes at 25°C, and then centrifuged for 10 minutes at 3000 rpm. One Hundred µL serum samples were added with 300 µL methanol with internal standard (2-chlorobenzene alanine, 0.2 mg/mL) and then the sample was vortexed and placed for 1 min then the supernatant was collected after a 12,000 rpm centrifuge for 10 mins at 4°C.

LC-Q-TOF/MS

A 3 µL aliquot of sample was injected into Agilent 1290 LC-Agilent 6545 QTOF/MS (Agilent Technologies, Santa Clara, CA, USA). All samples were separated with a HSS T3 C18 column (100 mm × 2.1 mm, 1.7 µm, Waters, USA) and the column was heated at 45°C. The following gradient program was used water and acetonitrile. The elution procedure was 5% acetonitrile for 0–2 min; 2–95% acetonitrile 0.1% formic acid for 2–13 min; elution with 95% acetonitrile 0.1% formic acid for 13–15 min; post-time step for 3 min. The flow rate was 0.3 mL/min. Mass detection was operated with an electrospray source operating in either positive or negative ion mode. The mass spectrum parameters are set as follows: drying gas (N2) flow rate was set at 7 L/min; gas temperature was set at 330°C; pressure of nebulizer gas was 35 psig; Vcap was 4200V; fragmentor was 165V; skimmer was 60V; scan range (m/z) was 80–1000. The MS/MS detection was used the targeted MS2 mode with collision energy 10EV, 20EV and 40EV. Serum samples were run randomly, and the quality control (QC) sample was analyzed every 10 serum samples. The analysis should be stopped when the QC sample was abnormal. Then rinse the column and ion source and correct the mass axis until the QC test was normal.

Data Processing and Statistical Analysis

The Agilent Mass Hunter Qualitative Analysis Software 7.0 (Agilent Technologies, Palo Alto, CA, USA) was used for peak extraction, deconvolution and peak matching from the UPLC-QTOFMS ESI+ and ESI- raw data. After that a list of each peak detected was created including the retention time, m/z and ion intensity information of each ion. And then the SIMCA-P13.0 software (Umetrics AB, Umea, Sweden) was used for Principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). The default 7-fold cross-validation was applied for guard against PLS-DA model over-fitting. Those significantly changed ions were identified and those with the VIP (variable importance in the projection)value more than 1 obtained from the PLS-DA model, as well as $P < 0.05$ obtained from the Student's t test, were selected as potential biomarkers. Those ions were identified by comparison of exact molecular weight and fragment ion acquired in targeted MS/MS mode with the Human Metabolome Database (http://www.hmdb.ca/) and Metlin (https://metlin.scripps.edu/). Pathway analysis of the markers was used the Web-based software, MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/).

IPA Analysis

To systematically understand the biomarkers of PPH, we uploaded the excel file with the deferentially expressed metabolites (with HMDB IDs and KEGG IDs) and the fold changes information onto an online software Ingenuity Pathway Analysis (IPA) server (IPA, Ingenuity® Systems, http://www.ingenuity.com). Then the bioinformatics analysis was per-

formed in accordance with operational guidelines, and the pathways analysis and networks analysis were carried out based on the knowledge sorted in the database of IPA.

RESULTS

Identification of the Differential Metabolites

After removing those peaks with missing value in the two groups, a total of 1070 peaks of ESI+ and 670 peaks of ESI- were obtained from the acquired data. The PCA score plot showed a separation tendency between the control samples and PPH-pre-individuals, and the QC samples were clustered together illustrating the stability of the method through the whole run (Fig. 1). The PLS-DA score also showed the same separation trend between the control samples and PPH-pre-individuals (Fig. 2A, B), The permutation test of the model also showed that there was no overfitting, which proved that the model was reliable as shown in Figure 2C, D. Thirty-four metabolites with VIP > 1 and $p < 0.05$ calculated from the PLS-DA model and the Student's t test ($p < 0.05$) were identified showed in Table 1. Some of metabolites showed increased concentration in PPH patients, such as LysoPC [20:4 (5Z, 8Z, 11Z, 14Z)], while several lipids, such as PI (O-20:0/0:0), PG (18:0/0:0), octadecanedioic acid and PC (15:0/0:0) were observed in decreased levels in the PPH-pre group.

Metabolic Pathway Analysis with IPA

To study the relationship between metabolites, we used IPA software to conduct molecular pathways and networks analysis. The interaction Network Analysis of differential metabolites between the PPH-pre and control was shown in Figure 3. These metabolites were correlated with mTOR signaling, acute phase response signaling, AMPK signaling and eNOS signaling. IPA revealed that Myo-inositol Biosynthesis (1/9), Urate Biosynthesis (1/22), Guanosine Nucleotides Degradation (1/22), Maturity Onset Diabetes of signaling (1/25), and Adenosine Nucleotides Degradation (1/27) (as shown in Tab. 2) ,which were the top five significantly changed pathways in PPH-pre-compared to control. The metabolic functions analysis by IPA analysis were summarized in Table 2. The most significantly perturbed biological functions

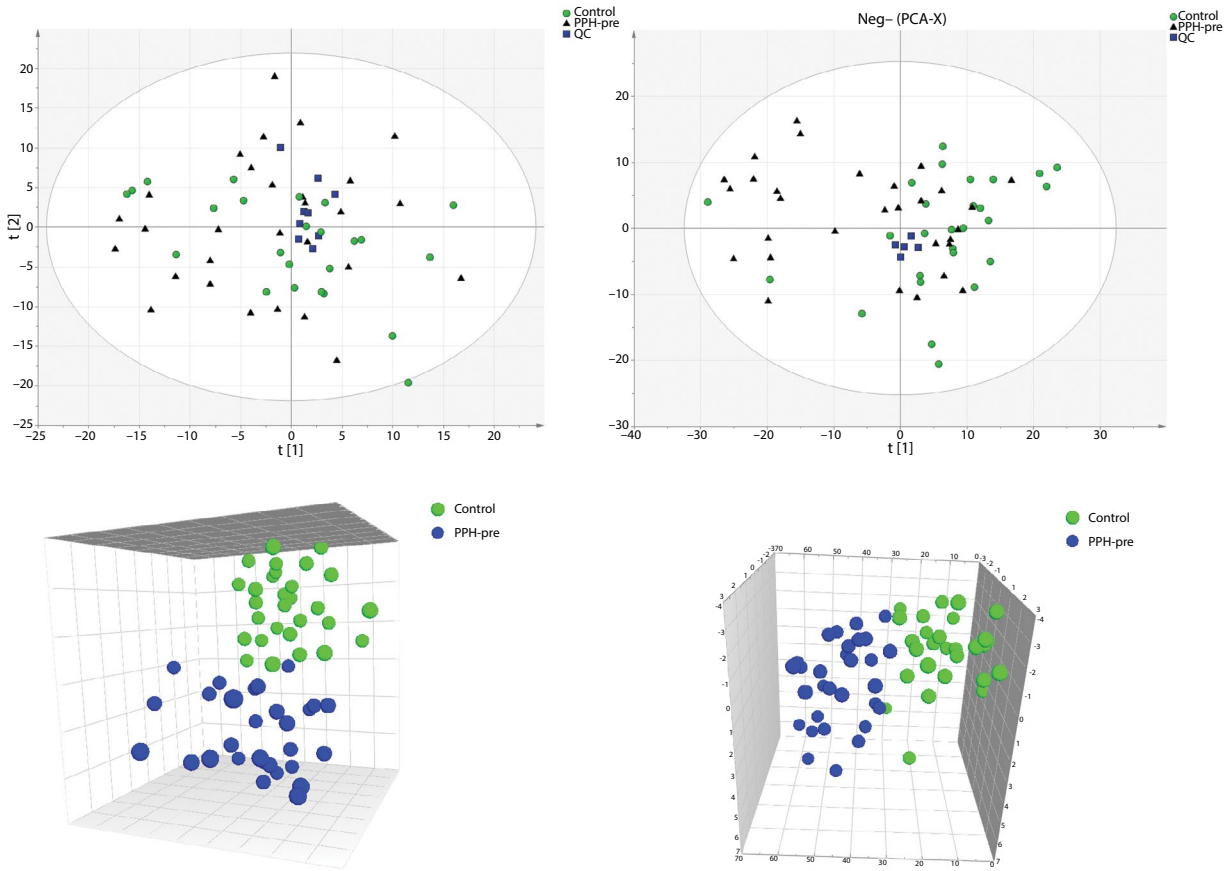

Figure 1. Principle component analysis (PCA) scores plot and 3D scores plot discriminating the metabolic profiles in serum of PPH-pre and those in healthy control. (A), POS (two components model: R2X = 0.34, Q2 = 0.2). (B), NEG (two components model: R2X = 0.41, Q2 = 0.2); ■ — QC group; ● — Healthy Control group; ▲ — PPH-pre group; PPH — Primary Postpartum haemorrhage; QC — Quality Control

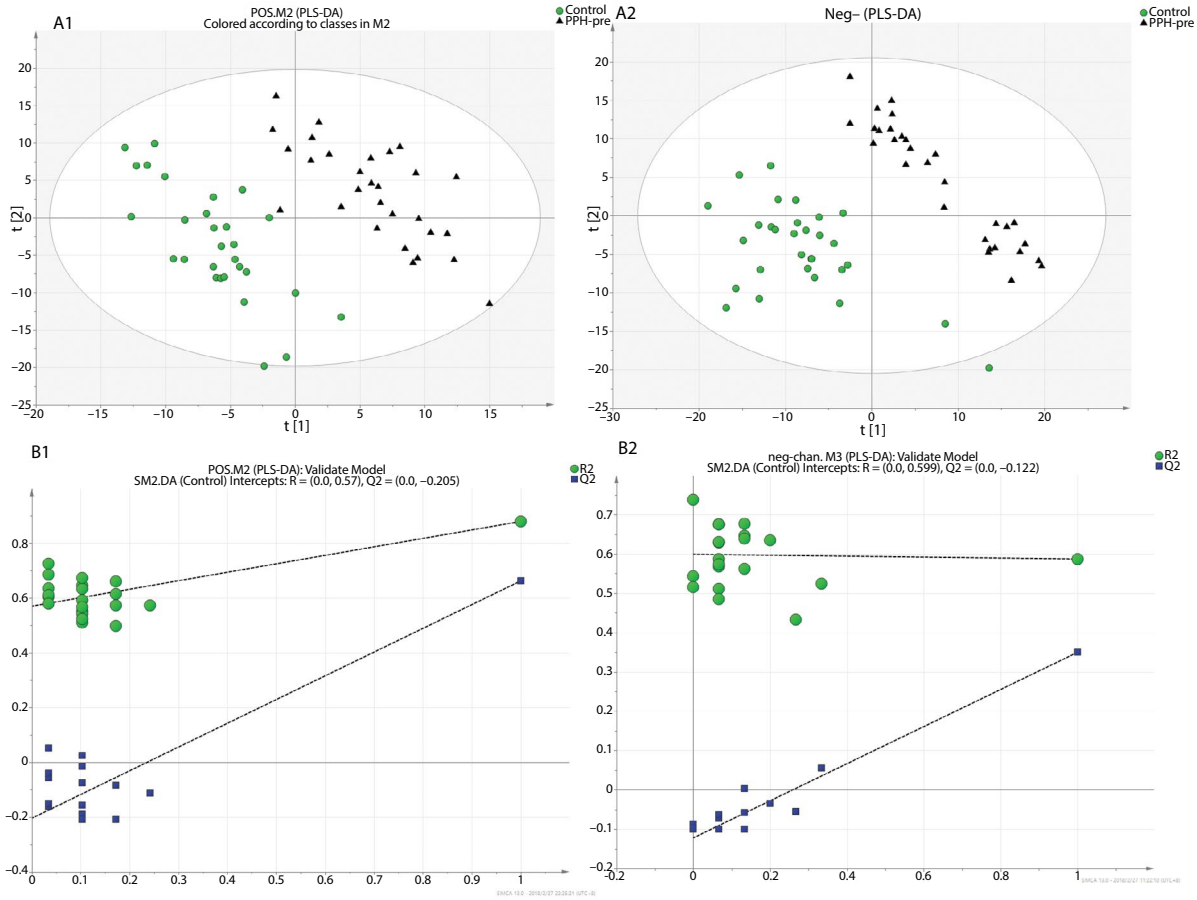

Figure 2. Partial least squares-discriminant analysis (PLS-DA) scores plot and permutation test for the model discriminating serum samples from PPH-pre group and healthy control; ● — Healthy Control group; ▲ — PPH-pre group; (A1) POS-PLS-DA scores plot. The model parameters were: R2Xcum = 0.34, R2Ycum = 0.87, Q2 = 0.89; (A2) NEG-PLS-DA scores plot. The model parameters were: R2Xcum = 0.44, R2Ycum = 0.89, Q2 = 0.87; (B1) A 999-times permutation test for the corresponding model. The Y-axis intercepts were: R2 (0, 0.55), Q2 (0, –0.13). (B2) A 999-times permutation test for the corresponding model. The Y-axis intercepts were: R2 (0, 0.52), Q2 (0, –0.12); PPH — Primary Postpartum haemorrhage

were connective Tissue Disorder (p = 6.02e-04-8.6e-05), Developmental Disorder (p = 8.60e-05-8.60e-05), Hematological Diseases (p = 3.78e-02-8.60e-05), Hereditary Disorder (p = 8.60e-05-8.60e-05) and Inflammatory Diseases (p = 1.21e-02-8.60e-05) (Tab. 2). The top 5 significant molecular and cellular functions were Free Radical Scavenging, Lipid metabolism, Small molecule biochemistry, Cellular Movement and Cell death and survival (Tab. 2). Then we performed pathway enrichment analysis of significantly changed metabolites in PPH group, As shown in Figure 3 and Table 3, Glycerophospholipid metabolism, D-Glutamine and D-glutamate metabolism, Purine metabolism, Linoleic acid metabolism, Citrate cycle (TCA cycle), Vitamin B6 metabolism.

DISCUSSION

PPH causes approximately 14 million deaths each year and the prevalence of PPH worldwide is between 6% and 10%. The major reason for deaths of PPH is that related medical emergencies are not easy to early diagnosis even intervene immediately. Until now the diagnosis of PPH has been dependent on the estimate the amount of bleeding with low predictive ability and accuracy. Metabolomics is a relatively new tool for biomarkers identification for diseases like genomics, transcriptomics, and proteomics. We firstly applied metabolomics study on PPH using LC/MS to screen the predictive biomarkers of PPH. In our study, vaginal delivery pregnant women without PPH and pregnant women with PPH were recruited, then we detected the metabolites of serum obtained prior to childbirth. In our study, 34 significantly altered metabolites in PPH-pre-group were identified. They were mainly involved in fatty acid, and glycerophospholipid metabolism.

Fatty acids, especially the unsaturated derivatives, affect many physiological functions and a wide range of mechanisms [13]. In addition to the source of energy, polyunsaturated fatty acids (PUFA) also endow cell mem-

Table 1. The potential biomarkers of PPH detected by UPLC-Q-TOF/MS and their variation tendency

RT	Mass	Name	HMDB	KEGG	vip	p	PPH/Control
0.58	801.7194	SM (d17:1/24:0)	HMDB0011695	–	1.72	0.01	0.47
0.93	173.0046	Quinaldic acid	HMDB0000842	C06325	2.00	0.00	0.24
13.63	750.5649	PG (16:0/18:0)	HMDB0010572	–	2.17	0.00	0.49
14.24	757.5686	PC [20:2(11Z, 14Z)/14:0]	HMDB0008328	C00157	1.83	0.02	1.60
0.56	145.989	oxoglutarate	HMDB0000208	C00026	1.86	0.02	1.13
12.43	281.2718	Oleamide	HMDB0002117	C19670	1.73	0.03	1.32
4.14	145.0527	N-Butyrylglycine	HMDB0000808	–	1.88	0.02	0.89
10.11	543.3333	LysoPC [20:4 (5Z, 8Z, 11Z, 14Z)]	HMDB0010395	C04230	1.57	0.04	1.58
11.28	547.3641	LysoPC [20:2 (11Z, 14Z)]	HMDB0010392	C04230	1.70	0.03	1.84
9.65	517.3172	LysoPC [18:3 (6Z, 9Z, 12Z)]	HMDB0010387	C04230	2.34	0.00	1.30
2.26	119.0739	L-Threonine	HMDB0000167	C00188	1.44	0.04	0.89
1.45	161.0509	Indole-3-carboxylic acid	HMDB0003320	C19837	1.54	0.04	1.39
8.32	248.1988	hexadecatetraenoic acid	–	–	1.77	0.00	0.24
12.20	413.3507	Heptadecanoyl carnitine	HMDB0006210	–	1.70	0.03	0.44
9.40	184.1462	hendecenoic acid	–	–	1.63	0.05	0.37
10.68	196.146	dodecadienoic acid	–	–	1.83	0.02	0.38
10.21	519.3365	1-Linoleoylglycerophosphocholine	HMDB0010386	C04230	2.14	0.01	0.80
1.24	168.0287	Uric acid	HMDB0000289	C00366	1.32	0.04	2.00
9.39	379.2497	Sphingosine-1-phosphate	–	–	1.75	0.00	0.83
12.72	464.351	SM (d18:1/0:0)	HMDB0006482	C03640	1.36	0.03	0.59
9.65	539.324	PS (19:0/0:0)	–	–	1.89	0.00	2.17
7.46	614.3683	PI (O-20:0/0:0)	–	–	1.94	0.00	0.76
11.91	586.3609	PI (O-18:0/0:0)	–	–	1.50	0.02	0.81
10.61	558.3299	PI (O-16:0/0:0)	–	–	1.41	0.03	0.79
8.08	510.2843	PG [18:1 (9Z)/0:0]	–	–	1.48	0.02	0.70
7.62	512.3004	PG (18:0/0:0)	–	–	1.30	0.04	0.81
6.70	482.2532	PG [16:1 (9Z)/0:0]	–	–	1.53	0.01	0.41
10.61	481.3185	PC (15:0/0:0)	–	–	1.80	0.00	0.73
10.20	555.3109	Pateamine	–	–	2.44	0.00	0.71
7.73	368.1668	PA (6:0/6:0)	–	–	1.84	0.00	3.96
10.24	450.263	PA [19:1 (9Z)/0:0]	–	–	1.32	0.04	0.77
11.50	314.2455	Octadecanedioic acid	HMDB0000782	–	1.24	0.05	0.55
0.66	60.0211	Urea	HMDB0003344	C00266	1.38	0.03	0.71
0.73	260.0293	D-Glucose 6-phosphate	HMDB0001401	C00092	1.27	0.04	2.08

branes with unique structural and functional properties, modulate cellular and intercellular communication and gene expression [14]. Certain fatty acids regulate transcription by peroxisome proliferator activated receptor family (PPARs). PPARs may play a role in placental metabolism, fetal development and preeclampsia [15, 16]. During pregnancy, fatty acids accumulated in developing tissues [17] and the supply of maternal PUFA is crucial for mothers and neonates [18, 19]. The γ-3 fatty acid can inhibit the thrombosis and reduce the incidence and mortality of cardiovascular diseases. Other research suggested that γ-3 fatty acid also could reduce the blood pressure, triglyceride concentration, and risk of vascular endothelial dysfunction [20, 21]. In our study, the fatty acids were decreased in PPH-pre, this may be the warning signal for PPH. Most importantly is that we may also give the pregnant women ω-3 fatty acids supplemental diet for prevent PPH.

Phospholipids are hydrolyzed to produce lysophospholipids and free fatty acids. Lysophospholipids exists in biological fluids and plays an important role in cell proliferation, migration and survival [22, 23]. Lysophos-

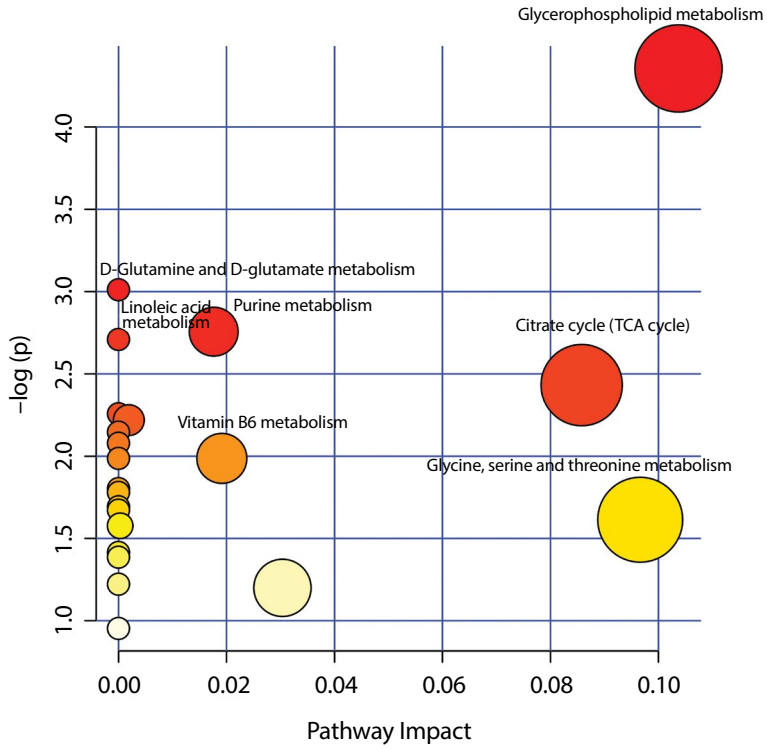

Figure 3. Summary of the pathway analysis of biomarkers of Primary Postpartum haemorrhage

Table 2. Summary of Biomarkers Function Analysis

Top canonical pathways				**p-value**	**Overlap**
Myo-inositol Biosynthesis				7.74e-04	11.1%,1/9
Urate Biosynthesis				1.89e-03	4.5%, 1/22
Guanosine Nucleotides Degradation				1.89e-03	4.5%, 1/22
Maturity Onset Diabetes of signaling				2.15e-03	4%, 1/25
Adenosine Nucleotides Degradation				2.32e-03	3.7%, 1/27
Diseases and bio functions				**p-value range**	**Molecules**
Connective Tissue Disorder				6.02e-04-8.6e-05	1
Developmental Disorder				8.60e-05-8.60e-05	1
Hematological Diseases				3.78e-02-8.60e-05	1
Hereditary Disorder				8.60e-05-8.60e-05	1
Inflammatory Diseases				1.21e-02-8.60e-05	1
Molecular and cellular functions				**p-value range**	**Molecules**
Free Radical Scavenging				8.58e-03-6.57e-05	2
Lipid metabolism				1.22e-02-1.26e-04	2
Small molecule biochemistry				3.98e-02-1.26e-04	2
Cellular Movement				2.08e-02-1.72e-04	1
Cell death and survival				2.92e-02-2.58e-04	1
ID	**Molecules in Network**	**Score**	**Focus**	**Top Diseases and Functions**	
1	APOA1, ASB7, cholesterol sulfate, COL4A1, Collagentype VII, CPB2, CPN1, F13A1, F13B, factor XIII, FGA, FGB, FGG, Fibrin, Fibrinogen, glycosylphosphatidylinositol, GPLD1, HDL, Hmgn3, HSPG2, ITIH4, LAMA3, LAMB3, LPA, miR-18a-5p (and other miRNAs w/seed AAGGUGC), NID1, P38 MAPK,PDGF-DD, Pzp, SERPINF2, Stat3-Stat3, Tgf beta, TGFB1, TLL1, TLL2	27	10	Organismal Injury and Abnormalities, Hematological System Development and Function, Developmental Disorder	
2	ABCB4, ACOX1, AIRE, C1QA, choline, CKMT1A/CKMT1B, CXCR6, CYCS,D-glucose, FST, FTL, G6PC, Gm15807/Hmgn5, GPD1, Hbb-b2,Hmgn3, IFNG, IL16, LAMA4, LAMB2, LTB, MAFK, Mbl1, MLXIPL, Mup1(includes others), NR1I2, NRF1, PEPCK, PLAC8, RETNLB, RORA, RORC, SCD, SMARCB1, TP53	9	4	Inflammatory Response, Cancer, Organismal Injury and Abnormalities	

Table 3. Pathway analysis of biomarkers of Primary Postpartum haemorrhage

Pathway Name	Match Status	p	-log (p)	Holm p	FDR	Impact
Glycerophospholipid metabolism	2/39	0.012832	4.3558	1.0	0.9181	0.1037
D-Glutamine and D-glutamate metabolism	1/11	0.049237	3.0111	1.0	0.9181	0.0
Purine metabolism	2/92	0.06351	2.7566	1.0	0.9181	0.01763
Linoleic acid metabolism	1/15	0.066588	2.7092	1.0	0.9181	0.0
Citrate cycle (TCA cycle)	1/20	0.087871	2.4319	1.0	0.9181	0.08577
Alanine, aspartate and glutamate metabolism	1/24	0.10458	2.2578	1.0	0.9181	0.0
Sphingolipid metabolism	1/25	0.10871	2.219	1.0	0.9181	0.00191
Valine, leucine and isoleucine biosynthesis	1/27	0.11693	2.1462	1.0	0.9181	0.0
alpha-Linolenic acid metabolism	1/29	0.12507	2.0789	1.0	0.9181	0.0
Lysine biosynthesis	1/32	0.13716	1.9866	1.0	0.9181	0.0
Vitamin B6 metabolism	1/32	0.13716	1.9866	1.0	0.9181	0.01914
Inositol phosphate metabolism	1/39	0.16479	1.8031	1.0	0.9181	0.0
Butanoate metabolism	1/40	0.16867	1.7798	1.0	0.9181	0.0
Histidine metabolism	1/44	0.18402	1.6927	1.0	0.9181	0.0
Ascorbate and aldarate metabolism	1/45	0.18782	1.6723	1.0	0.9181	0.0
Glycine, serine and threonine metabolism	1/48	0.19912	1.6139	1.0	0.9181	0.09661
Glyoxylate and dicarboxylate metabolism	1/50	0.20657	1.5771	1.0	0.9181	0.0
Starch and sucrose metabolism	1/50	0.20657	1.5771	1.0	0.9181	3.1E-4
Pyrimidine metabolism	1/60	0.2429	1.4151	1.0	0.99993	0.0
Arachidonic acid metabolism	1/62	0.24998	1.3864	1.0	0.99993	0.0
Aminoacyl-tRNA biosynthesis	1/75	0.29457	1.2223	1.0	1.0	0.0

phatidylcholine (LPC) is a bioactive lysophospholipids, mainly produced by the action of phospholipase A2 (PLA2) enzyme on the plasma membrane. After cell uptake, the free LPC reaction produces PC or deacylation to produce FA and choline. These endogenous lysophosphatidates regulate the PPAR gamma function required for vascular wall pathology and metabolic related diseases. The imbalance of PPAR gamma can induce cell function changes, including ROS production, NOS and cytokines expression. In our study, the level of PCs, PSs, PIs were decreased in the PPHand the lysoPCs were increased in the PPH, in which the latter may be cause the coagulation dysfunction in the PPH. According to our study, it may be useful to monitor the changes of lipids to assess the risk of PPH, especially for the lysoPCs.

Although the occurrence of PPH is associated with a variety of causes, in most cases, PPH is caused by bleeding from the site of the placenta, which is caused by uterine inertia [24, 25]. Due to high uterine artery blood flow at the end of pregnancy, uterine inertia can lead to severe bleeding. The PPH stepwise initiative management scheme can improve the prognosis. So far, there is no unified conclusion about the pathogenesis of PPH. In addition, we need to find markers for PPH risk prediction and find new precise mechanisms. According to our pathway analysis with IPA, these potential makers found in our study were related to mTOR signaling, acute phase response signaling, AMPK signaling and eNOS signaling, as shown in Figure 4. These signaling pathways may provide guidance for further studies on the pathogenesis of postpartum hemorrhage.

CONCLUSIONS

In our study, we found the LysoPCs, PCs, PGs, PIs were effective biomarkers for predicating PPH. The disturbed signaling pathways, mTOR signaling, acute phase response signaling, AMPK signaling and eNOS signaling might be related to the etiopathogenesis of PPH. Our study provided a valuable attempt to screen early diagnostic markers of PPH and to further understand its pathogenesis.

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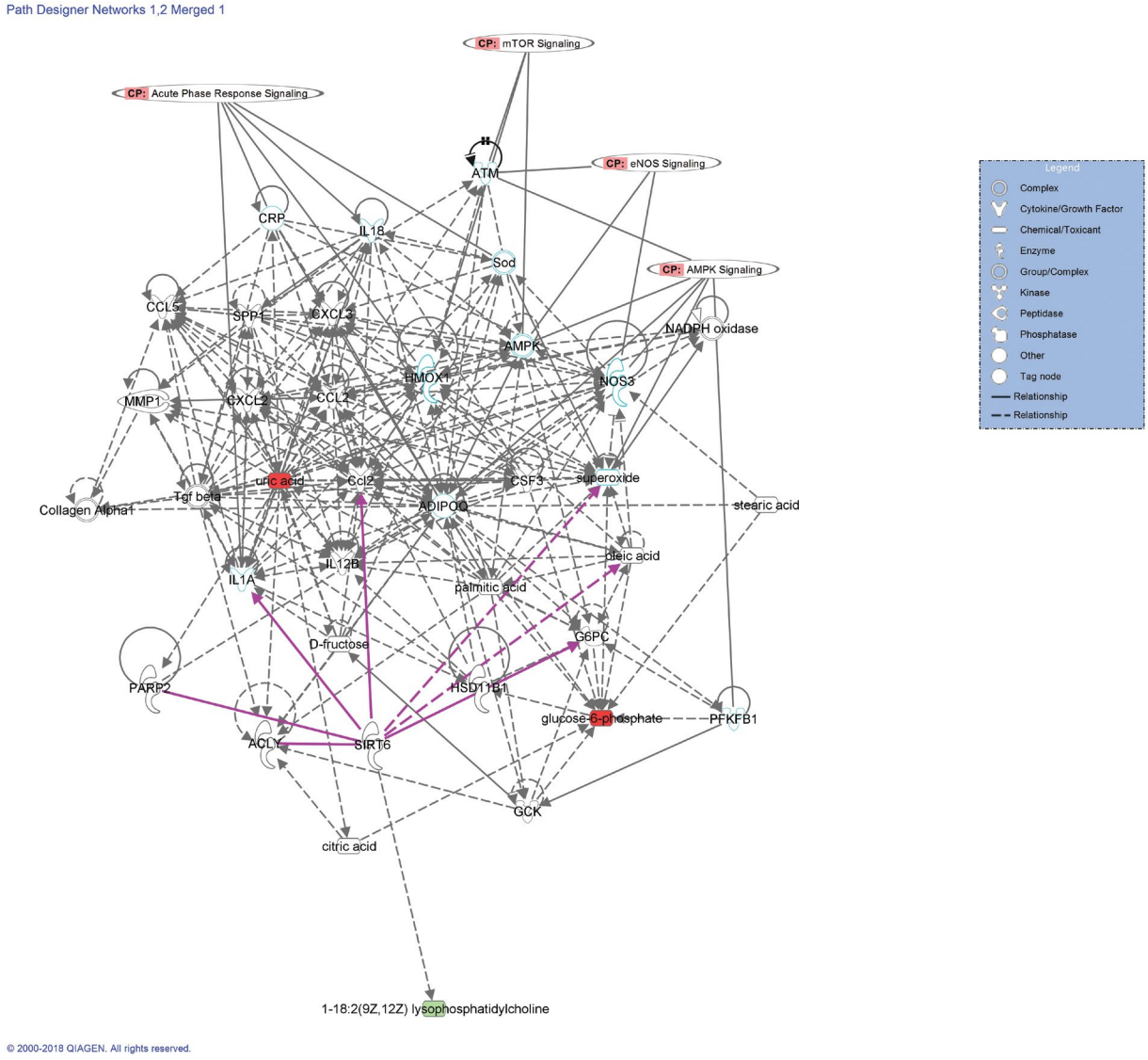

Figure 4. Biological network, canonical pathways and functions related to the identified metabolites. In the network, molecules are represented as nodes, and the biological relationship between two nodes is represented as a line. Red symbols represent up-regulated metabolites; blue symbols represent down-regulated metabolites; while the green symbols represent canonical pathways that are related to the identified specific metabolites. Solid lines between molecules show a direct physical relationship between molecules, while dotted lines show indirect functional relationships

REFERENCES

1. AbouZahr C. Global burden of maternal death and disability. Br Med Bull. 2003; 67: 1–11, doi: 10.1093/bmb/ldg015, indexed in Pubmed: 14711750.
2. Nour NM. An introduction to maternal mortality. Rev Obstet Gynecol. 2008; 1(2): 77–81, indexed in Pubmed: 18769668.
3. Burke C. Active versus expectant management of the third stage of labor and implementation of a protocol. J Perinat Neonatal Nurs. 2010; 24(3): 215–28; quiz 229, doi: 10.1097/JPN.0b013e3181e8ce90, indexed in Pubmed: 20697238.
4. Magann EF, Evans S, Hutchinson M, et al. Postpartum hemorrhage after cesarean delivery: an analysis of risk factors. South Med J. 2005; 98(7): 681–685, doi: 10.1097/01.SMJ.0000163309.53317.B8, indexed in Pubmed: 16108235.
5. Al-Zirqi I, Vangen S, Forsen L, et al. Prevalence and risk factors of severe obstetric haemorrhage. BJOG. 2008; 115(10): 1265–1272, doi: 10.1111/j.1471-0528.2008.01859.x.
6. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999; 29(11): 1181–1189, doi: 10.1080/004982599238047, indexed in Pubmed: 10598751.
7. Liu T, Li J, Xu F, et al. Comprehensive analysis of serum metabolites in gestational diabetes mellitus by UPLC/Q-TOF-MS. Anal Bioanal Chem. 2016; 408(4): 1125–1135, doi: 10.1007/s00216-015-9211-3, indexed in Pubmed: 26677023.
8. Enquobahrie DA, Denis M, Tadesse MG, et al. Maternal Early Pregnancy Serum Metabolites and Risk of Gestational Diabetes Mellitus. J Clin Endocrinol Metab. 2015; 100(11): 4348–4356, doi: 10.1210/jc.2015-2862, indexed in Pubmed: 26406294.
9. Austdal M, Tangerås LH, Skråstad RB, et al. First Trimester Urine and Serum Metabolomics for Prediction of Preeclampsia and Gestational Hypertension: A Prospective Screening Study. Int J Mol Sci. 2015; 16(9): 21520–21538, doi: 10.3390/ijms160921520, indexed in Pubmed: 26370975.
10. Chen T, He P, Tan Y, et al. Biomarker identification and pathway analysis of preeclampsia based on serum metabolomics. Biochem Biophys Res Commun. 2017; 485(1): 119–125, doi: 10.1016/j.bbrc.2017.02.032, indexed in Pubmed: 28188789.
11. Turkoglu O, Zeb A, Graham S, et al. Metabolomics of biomarker discovery in ovarian cancer: a systematic review of the current literature. Metabolomics. 2016; 12(4), doi: 10.1007/s11306-016-0990-0, indexed in Pubmed: 28819352.

12. Slupsky CM, Steed H, Wells TH, et al. Urine metabolite analysis offers potential early diagnosis of ovarian and breast cancers. Clin Cancer Res. 2010; 16(23): 5835–5841, doi: 10.1158/1078-0432.CCR-10-1434, indexed in Pubmed: 20956617.
13. Mozurkewich E, Berman DR, Chilimigras J. Role of omega-3 fatty acids in maternal, fetal, infant and child wellbeing. Expert Review of Obstetrics & Gynecology. 2014; 5(1): 125–138.
14. Jump DB. The biochemistry of n-3 polyunsaturated fatty acids. J Biol Chem. 2002; 277(11): 8755–8758, doi: 10.1074/jbc.R100062200, indexed in Pubmed: 11748246.
15. Lendvai Á, Deutsch MJ, Plösch T, et al. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. Am J Physiol Endocrinol Metab. 2016; 310(10): E797–E810, doi: 10.1152/ajpendo.00372.2015, indexed in Pubmed: 26860983.
16. McCarthy FP, Drewlo S, English FA, et al. Evidence implicating peroxisome proliferator-activated receptor-γ in the pathogenesis of preeclampsia. Hypertension. 2011; 58(5): 882–887, doi: 10.1161/HYPERTENSIONAHA.111.179440, indexed in Pubmed: 21931072.
17. Innis SM. Essential fatty acid transfer and fetal development. Placenta. 2005; 26 Suppl A: S70–S75, doi: 10.1016/j.placenta.2005.01.005, indexed in Pubmed: 15837071.
18. De Vriese SR, Matthys C, De Henauw S, et al. Maternal and umbilical fatty acid status in relation to maternal diet. Prostaglandins Leukot Essent Fatty Acids. 2002; 67(6): 389–396, doi: 10.1054/plef.2002.0446, indexed in Pubmed: 12468259.
19. Susanne KE, Rania S, Cristina C, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr. 2007; 85(5): 1392–1400.
20. Diekman C, Hornstra G, Koletzko BV, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk. Ann Intern Med. 2014; 161(6): 456–457, doi: 10.7326/L14-5018-8, indexed in Pubmed: 25222400.
21. Manuela C, Davide S, Catapano AL, et al. Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, double blind, placebo controlled trials. Atherosclerosis. 2013; 14(2): 243–251.
22. Tigyi G. Aiming drug discovery at lysophosphatidic acid targets. Br J Pharmacol. 2010; 161(2): 241–270, doi: 10.1111/j.1476-5381.2010.00815.x, indexed in Pubmed: 20735414.
23. Choi JiW, Herr DR, Noguchi K, et al. LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol. 2010; 50: 157–186, doi: 10.1146/annurev.pharmtox.010909.105753, indexed in Pubmed: 20055701.
24. Rizvi F, Mackey R, Barrett T, et al. Successful reduction of massive postpartum haemorrhage by use of guidelines and staff education. BJOG. 2004; 111(5): 495–498, doi: 10.1111/j.1471-0528.2004.00103.x, indexed in Pubmed: 15104617.
25. Alamia V, Meyer BA. Peripartum hemorrhage. Obstet Gynecol Clin North Am. 1999; 26(2): 385–398, indexed in Pubmed: 10399768.

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