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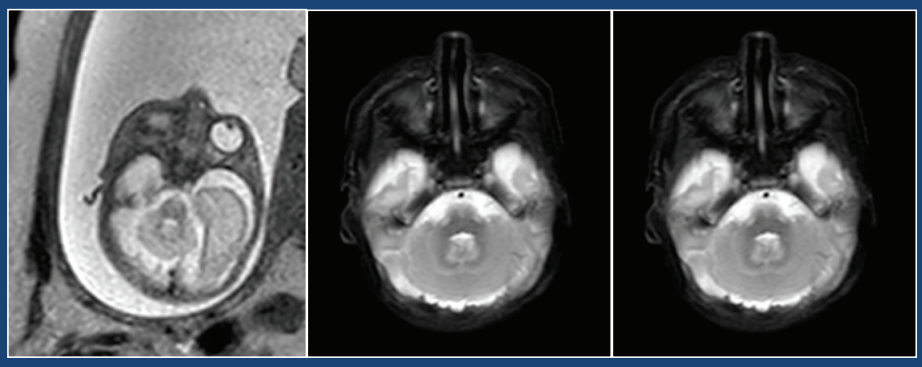

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Cover Picture: Şeker et al. Fetal posterior fossa anomalies

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December. The publication language of the journal is English. Manuscripts are reviewed in accordance with "double-blind peer review" process for both reviewers and authors.

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Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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Ertan AK, Tanriverdi HA, Schmidt W. Doppler Sonography in Obstetrics. In: Kurjak A, Chervenak FA, editors. Ian Donald School Textbook of Ultrasound in Obstetrics and Gynecology. New Delhi, India: Jaypee Brothers; 2003. p. 395-421.

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Kohler G; Egelkraut H. In Kohler G and Egelkraut H (edts).Munchener Funktionelle Entwicklungsdiagnostik im zweitem und drittem Lebensjahr. Handanweisung. Munchen: Uni Munchen, Institut fur Soziale Paediatrie und Jugendmedizin; 1984.

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the first issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2023. This issue is consisted of seven articles and three reviews that we hope you will read with interest. Also you may have the oppurtunity to watch the latest video here (http://www.jtgga.org/video). Here we share some of our favorite articles that were published in this issue of the journal.

Preeclampsia caused by placental dysfunction remains the leading cause of maternal and neonatal mortality worldwide. You will read an article determining the incidence of raised intra cranial pressure (ICP) as evident by enlarged optic nerve sheath diameter (ONSD) by ocular ultrasound among patients with preeclampsia and its relationship to severity of disease.

Adolescence is the period of life in which an individual matures physically and begins to transition psychologically from childhood to adulthood. Adolescent gynecology is gaining more and more importance as a field where special knowledge and expertise are required. You will also read a comprehensive review which discusses the etiology, clinical characteristics, treatment and prognosis of a number of diseases in prepubertal and adolescent girls.

You will also have the opportunity to read a review about the genes involved in non-syndromic cleft lip and/or palate which is one of the most common congenital malformations with a prevalence of 1:700.

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Sincerely,

Prof. Cihat Ünlü, M.D.
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Regional differences in the sex ratio at birth in Mexico

Victor Grech

Department of Pediatrics, Mater Dei Hospital, Malta

Abstract

Objective: The sex ratio at birth, defined as males/total (M/T) approximates 0.515 but is affected by many factors. Acute and chronic stress have been shown to lower M/T, and both regional and racial differences exist. This study analysed regional differences in M/T in Mexico.

Material and Methods: Live births by sex and year were available for 1994-2020. Regional births were available for 2010-2020 for five regions: North, Centre, West, East and South.

Results: There were 68,423,415 births for 1994-2020 and 25,436,687 for 2010-2020 (M/T=0.5060, 95% confidence interval: 0.5058-0.5062). M/T was <0.515 ($p<0.0001$). Live births correlated negatively with year ($p<0.0001$). M/T fell for 1994-2003 then rose to 2020 ($p<0.0001$). M/T was highest in North followed by West, South, Centre and East ($p<0.0001$).

Conclusion: Chronic stress with socioeconomic deprivation may reduce M/T and may explain the low M/T found in this study from Mexico.

(J Turk Ger Gynecol Assoc 2023; 24: 1-4)

Keywords: Epidemiology, Mexico, sex ratio at birth

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Introduction

The sex ratio at birth, calculated as male number divided by males/total (M/T) is approximately 0.515 (1) but is influenced by various factors (2). Acutely stressful events have been shown to reduce M/T (3) and this accords with the Triver-Willard hypothesis which posits that evolution has favoured parents with the capacity to influence M/T according to periconceptual and gestational conditions (4). This is because in polygynous species (where males have multiple mating opportunities), a robust son conceived under favourable conditions has more reproductive opportunities than a daughter who is naturally constrained by pregnancy and lactation. On the other hand, a male foetus will be less likely to be survive pregnancy to term, and if he does so, a frail male may not survive to reproductive age. Even if he survives, he would contend inadequately with stronger males for mating privileges. However, a frail female is likelier to survive and reproduce. Hence, under unfavourable conditions, the parental passage of genes is favoured if less males ensue through the culling of weaker males (4).
Regional differences in M/T have also been observed (5). This study was carried out in order to ascertain whether there were any regional differences in M/T in Mexico.

Material and Methods

Ethical approval was not applied for as these are anonymous and freely available datasets from the Instituto Nacional de Estadística y Geografía, the National Institute of Statistics and Geography (6,7). For this reason, informed consent was not obtained. Sex and year as well as region were available for the period January 2010-December 2020 (6,7). These were grouped into five regions, as per Table 1 (6,7).

Statistical analysis

The binomial equations of Fleiss were used to calculate confidence intervals (CI) for proportions (8). Bespoke Excel

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sheets were used to perform chi test, chi tests for trends and Spearman correlation (9,10). A p-value <0.05 was taken to represent a statistically significant result.

Results

There were a total of 68,423,415 births for the period 1994-2020 and 25,436,687 for 2010-2020. Live births correlated negatively and significantly with year [Spearman rho: -0.94, p<0.0001 (Figure 1)]. M/T fell significantly from 1994 to 2003 (chi for trend: 267.3, p<0.0001) then rose significantly to 2020 [chi for trend: 723.3, p<0.0001 (Figure 1)].

For 2010-2020, there were 2,520 births with sex unspecified ("no especificado") and 104,320 non-Mexicans ("extranjero") and these were excluded to yield an M/T of 0.5060 (95% CI 0.5058-0.5062). Live births by sex and region are displayed in Table 1. There were significant regional differences, with M/T highest in the North, followed by the West, South, Centre and East, as shown in Figure 2 (chi for trend: 223.1, p<0.0001). No geographical gradient was present.

Discussion

Fertility has been dwindling in South America for decades (11). Mexico is no exception, as shown in this study, with a fertility decline initiated by a government sponsored contraception campaign 40 years ago that was deliberately intended to replace its former nation-building policy (12). Broader changes, such as more women in work and education, and a rise in housing prices, also reduced fertility (12). Indeed, currently circa 40% of married Mexican women undergo sterilisation, possibly due to the lack of other contraceptive measures in some areas of the country and the strict laws against abortion, except in Mexico's capital (12). Moreover, the introduction of emergency contraception in Latin America has further impacted fertility (13).

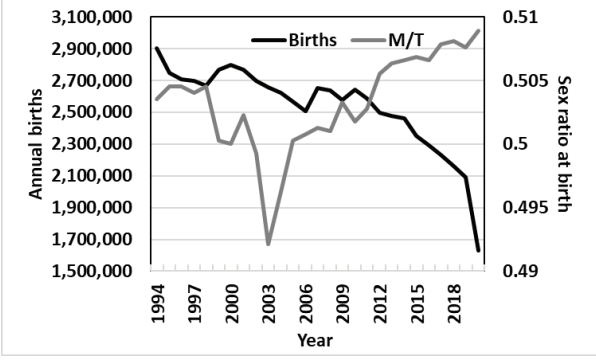

Figure 1. Total births and sex ratio at birth by year, 1994-2020

Acute stress reduces M/T. This includes all types of stress, both due to natural events like earthquakes, smog and floods (14) and man-made events, such as terrorist attacks (3), and contracting economies (15). The M/T was reduced in Mexico in the present study, as noted in previous studies (5) and this country's ratio is significantly less than the expected value of 0.515 based on a world reference range that included 88,875,750 births (chi: 5935.8, p<0.0001) (1). It had been mooted that M/T may be lower in certain ethnicities due to innate physiological differences (16,17). However, chronic stress has also been hypothesised to reduce M/T, in accordance with the Trivers-Willard hypothesis (18). This is supported by the finding that race in the United States is the principal determinant of socio-economic status and the most significant variable associated with wealth inequality and stress (19). Indeed, in the US, M/T is highest in Asian births, followed by White, American Indian/Alaska Native, and Black/African American births (20). This is further supported by the analysis of a United Nations dataset that showed that M/T was lowest in least developed countries (21). Additional results supporting the hypothesis include a multivariate analysis of global M/T with health and socioeconomic indicators which found a significant positive correlation of M/T with these indicators (22).

Mexico has been one of the principal targets of the "War on Drugs", first mooted by US President Richard Nixon in 1971 (23). The Nixon administration attempted to justify the escalation

Table 1. Live births by sex and region, 2010-2020

Region	1: North	2: Centre	3: West	4: East	5: South	Total
M	2,713,563	3,872,332	2,230,794	1,995,633	2,006,317	12,871,350
F	2,626,505	3,796,968	2,165,086	1,962,723	1,961,182	12,564,073
Total	5,340,068	7,669,300	4,395,880	3,958,356	3,967,499	25,435,423
UCI	0.5086	0.5053	0.5079	0.5046	0.5062	0.5062
M/T	0.5082	0.5049	0.5075	0.5042	0.5057	0.5060
LCI	0.5077	0.5046	0.5070	0.5037	0.5052	0.5058

Region 1 (North): Baja California, Baja California Sur, Chihuahua, Coahuila, Durango, Nuevo León, Sinaloa, Sonora, Tamaulipas. Region 2 (Centre): Mexico City, Mexico State, Guanajuato, Morelos, Querétaro, San Luis Potosí, Zacatecas. Region 3 (West): Aguascalientes, Colima, Guerrero, Jalisco, Michoacan, Nayarit. Region 4 (East): Hidalgo, Puebla, Tlaxcala, Veracruz. Region 5 (South): Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco, Yucatan
M: Male, F: Female, M/T: Males/total, UCI: Upper confidence interval, LCI: lower confidence interval

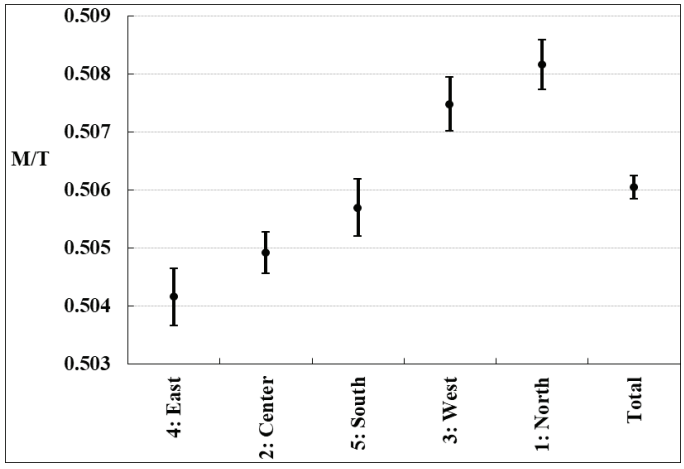

Figure 2. Ranked M/T gradient (per Table 1, regions in table 1 footnotes)
M/T: Males/total

of repressive measures against drug users to counter the increasing accommodation and normalisation of cannabis use as a gateway to more addictive drugs, such as opiates (23). However, the pursuit of a total prohibitionist policy has created a parallel economy fuelled by diverse, extensive and opposing criminal networks which resort to unbridled violence to protect their markets with often lethal effects, especially among young males (24). Governments have responded through police and/or military/paramilitary forces that react in similar fashion, to the extent that in Mexico, the escalation in homicides since the deployment of military forces against drug traffickers in 2006 has resulted in a reduction in national life expectancy (25). These policies have also led to the excessive use of incarceration with lengthy sentences for minor, non-violent, drug-related offences (25). Drug use and injection in prisons is common, and this circulates conditions such as HIV and hepatitis, often complicated by co-infection with tuberculosis (25). Unsurprisingly, this has resulted in a negative effect on population mental health, particularly vis-à-vis communication used by criminal organisations (narcomessages), the viciousness of gang executions, and the violent confrontations between law forces and criminal groups (26). Furthermore, thousands of women in Mexico, and indeed, Central America have been displaced from their countries by violence (27).

Terrorist acts have been linked to acute and transient drops in M/T in already pregnant women, 3-5 months after the event (3,28) and this has been confirmed with a systematic review and meta-analysis (29). The impact of violence varies hugely and depends on many factors; this has shifted the drug market to some extent to different parts of Mexico and to other Central American countries (30).

Finally, the COVID-19 pandemic has caused global disruptions and stress (31) and it has been speculated that this may affect M/T (32). Indeed, there are indications that this happened in Japan (33) South Africa (34) and in some large cities (35). These effects may completely subsume other, relatively smaller stressors and this will be an interesting topic of study, as may be the current war in Ukraine and its global effects.

Study Limitations

This study is limited by several factors. There were significant numbers of births that were of unspecified sex and for non-Mexicans, and these were excluded. In addition, no other data was available that might have allowed comparison, such as maternal or paternal factors. This is typical of most online datasets, preventing any form of multivariate analysis. Furthermore, this is an ecological study and it is therefore impossible to identify what caused these differences.

Conclusion

Mexico has a baseline low M/T, possibly due to chronic stress (associated with the country's underdeveloped status). The M/T has varied over the study period and also varied significantly in different geographical regions of the country.

Ethical Committee Approval: *Ethical approval was not applied for as these are anonymous and freely available datasets from The Instituto Nacional de Estadística y Geografía, The National Institute of Statistics and Geography.*

Informed Consent: *Informed consent was not obtained.*

Peer-review: *Externally peer-reviewed.*

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Optic nerve sheath diameter measurements using ultrasonography to diagnose raised intracranial pressure in preeclampsia: an observational study

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Abstract

Objective: To estimate the incidence of raised intracranial pressure (ICP) as evident by enlarged optic nerve sheath diameter (ONSD) by ocular ultrasound among patients with preeclampsia and its relationship to severity of disease.

Material and Methods: Sixty pregnant mothers with preeclampsia were compared to 30 normotensive, uncomplicated pregnant controls. For ONSD measurement, a 7-MHZ linear probe was used and three values from each optic nerve were taken and the mean of six values of both eyes was recorded. All study subjects were followed until seven days after delivery.

Results: Two cut off values (5.8 mm and 4.6 mm) were used to compare ONSD in severe and non-severe preeclampsia with that of healthy pregnant individuals. The incidence of raised ICP among severe preeclampsia above 5.8 mm and 4.6 mm cut-off were 43.3% and 90%, respectively, before delivery. ONSD was significantly elevated among preeclampsia subjects at both cut-off values at pre-delivery ($p=0.004$ for ONSD >5.8 mm and $p<0.001$ for ONSD >4.6 mm) compared to controls. There a significant association between presence of neurological manifestations and enlarged ONSD ($p<0.001$ for ONSD >5.8 mm and $p=0.04$ for ONSD >4.6 mm) before delivery.

Conclusion: Severe preeclampsia with neurological features was associated with increased ONSD, reflecting raised ICP. Further studies are needed to compare ONSD values with invasive ICP monitoring for better understanding of this relationship. (J Turk Ger Gynecol Assoc 2023; 24: 5-11)

Keywords: Preeclampsia, optic nerve sheath diameter, intracranial pressure, ultrasonography

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Introduction

Preeclampsia is a pregnancy specific syndrome and poses a reproductive disadvantage unique to humans. Preeclampsia occurs in 7-8% of pregnancies and is complicated with eclampsia in up to 0.9% of cases (1-3). It is a potentially serious disease being a common cause of maternal morbidity and mortality in low resource countries, whereas poor neonatal outcome due to iatrogenic premature delivery is its most significant consequence in resource rich countries (1). Preeclampsia is characterised by multisystem involvement and central nervous system (CNS) manifestations are well documented (4). Signs of cerebral oedema have been found in magnetic resonance images (MRI) in up to 70-100% of mothers with severe preeclampsia (5-7). Early recognition of increased intracranial pressure (ICP) and prompt treatment aimed at reduction has been found to improve clinical outcome (8). However, the exact incidence of raised ICP in preeclampsia is not known and clinical signs of increased ICP are often difficult to interpret (9).

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The gold standard method to measure ICP is by invasive procedures (10). A part of the CNS, 3 mm behind the ocular globe optic nerve, is surrounded only by fat and its dural sheath is distensible when pressure in the cerebrospinal fluid (CSF) is elevated (9). Several clinical studies confirmed the utility of non-invasive ultrasound measurement of optic nerve sheath diameter (ONSD) to diagnose raised ICP in conditions incluing head injuries, intracranial haemorrhage and hydrocephalus (11-13). However, there is little published data about ONSD in preeclampsia worldwide, and even fewer in India. This prompted the present prospective observational study that included pregnant mothers with both severe and non-severe preeclampsia to estimate the incidence of raised ICP measured by ocular ultrasound examination when compared with uncomplicated pregnancies. A further aim of this study was to study preeclampsia-related pregnancy and neonatal outcomes in relation to the magnitude of raised ICP.

Material and Methods

This was a prospective cohort study conducted over a period of one year from October 2020 to November 2021 in the department of obstetrics and gynecology in a tertiary care centre, after approval from the Calcutta National Medical College Institutional Ethics Committee (approval number: CNMC-GYN-228, date: 07.10.2019). A total of 90 subjects participated and they were divided into three groups, each having an equal number of participants with severe preeclampsia, non-severe preeclampsia and uncomplicated term pregnancy after admission. Preeclampsia was defined as an association of a blood pressure elevation (systolic pressure >140 mmHg or diastolic pressure >90 mmHg) and a proteinuria >0.3 g per day in a pregnant woman after 20 weeks of gestation (14).

Severe preeclampsia was determined by presence of one or more of the following features (15):

- Systolic BP ≥160 mm of Hg,
- Diastolic BP ≥110 mm of Hg,
- Proteinuria ≥300 mg (0.3 gm) per day or ≥1+ on dipstick test,
- Presence of headache,
- Upper abdominal pain or epigastric pain,
- Visual disturbances,
- Pulmonary edema,
- Oliguria (urinary output ≤400 mL/day),
- Thrombocytopenia (platelets count <100,000/mm^3)
- Elevated serum transaminase (alanine aminotransferase and/or aspartate aminotransferase) levels of twice the upper limit of normal,
- Elevated serum creatinine (>1.1 mg/dL or doubling of baseline),
- Intrauterine growth restriction of fetus.

Patients with preeclampsia but not meeting these criteria were classified as having non-severe preeclampsia. Women with uncomplicated singleton pregnancy at term (gestational age 37 weeks or more) served as the control group. Informed consent was obtained from study subjects.

Exclusion criteria included unwillingness to participate in the study, prior ocular injury, prior ocular surgery, prior optic nerve disease, including optic neuritis and optic atrophy.

ONSD measurements were done before delivery prenatally (day P) and on day 4 and day 7 after delivery with the HD 7 ultrasonography (USG) machine (Philips) using a high frequency (7 MHz) linear transducer. Each patient was placed in the supine position and the probe placed over closed lids after applying a USG gel on the lid. The gel serves as a coupling fluid to prevent sound wave attenuation caused by air. ONSD was measured 3 mm behind the optic globe using an electronic calliper and an axis perpendicular to the optic nerve. For each optic nerve, three ONSD measurements were taken in the vertical transverse plane at low gain settings (16). The recorded ONSD was the mean of six values taken, three from each eye.

Demographic information including age, body mass index (BMI), parity, period of gestations at study measurement was obtained for each subject. Data regarding obstetric and neonatal outcomes in terms of mode of delivery, fetal growth restriction, sick new-born care unit (SNCU) admission and neonatal deaths were recorded.

Statistical analysis

All categorical data were represented as frequency (percentage) and continuous data as median (interquartile range). Comparisons of means were done with Mann-Whitney U test (between two groups) or Kruskal-Wallis test (more than two groups) and comparison of proportions with chi-squared test or Fisher's exact test. To determine optimal cut off values for the optic sheath measurements, receiver operating characteristic (ROC) curves were drawn and analysed. All statistical tests were performed using SPSS, version 21 (IBM Corp., Armonk, NY, USA).

Results

A total of 90 patients were included, consisting of 30 patients each with severe and non-severe preeclampsia and 30 subjects with uncomplicated pregnancies. Baseline comparisons of the groups are given in Table 1. Patients in the preeclampsia group were matched in terms of age with the non-preeclampsia group. Hypertension (100% vs. 0% respectively, $p<0.001$), micro-albuminuria [49 (81.7%) vs. 0% respectively, $p<0.001$] and thrombocytopenia [29 (48.3%) vs. 1 (3.3%) respectively, $p<0.001$] were more frequent in the preeclampsia group. Patients in the preeclampsia group had higher BMI compared

to the non-preeclampsia subjects and the prevalence of obesity tended to be higher in the preeclampsia group [19 (31.7%) vs. 4 (13.3%), respectively, p=0.06] although this did not reach significance.

Optic nerve sheath diameter measurements

Descriptive data of ONSD are given in Table 2. There was a variable period between first ONSD measurement (day P) and delivery (1-5 days) among the study subjects including healthy controls. ONSD was measured again on the fourth day post-partum (day 4) and then on seventh post-partum day (day 7).

ROC curve analysis was performed to identify optimal cut-off values for ONSD to distinguish preeclampsia from controls, which were (Figure 1): ≥4.25 [sensitivity 81.7%, specificity 83.3%, area under curve (AUC): 0.89, 95% confidence interval (CI): 0.821-0.954] for day P, ≥4.05 (sensitivity 83.3%, specificity 93.3%, AUC: 0.912, 95% CI: 0.849-0.975) for day 4, and ≥3.25 (sensitivity 78.3%, specificity 83.3%, AUC: 0.827, 95% CI: 0.74-0.913) for day 7. The distribution of the newly identified cut-offs when compared between the three groups is displayed in Table 2. The number of patients with higher ONSD, in terms of either cut-off value, was greater in the severe and non-severe preeclampsia groups compared to controls (Figure 2).

Association of ONSD measurements with maternal and neonatal outcome

The newly derived ONSD cut-off was compared with two previous cut-offs, one developed internationally and another specific to India (12,17) in terms of the relationship with maternal and neonatal outcome.

Neurological symptoms were present in 22.2% (20/90) patients, all among patients with severe preeclampsia. Emergency caesarean section was needed in 47.7% (43/90), mostly among patients with severe preeclampsia (20/30 vs. 10/30 among controls, p=0.03). Neonatal SNCU admission was needed in 27.8% (25/90) and neonatal mortality was 8.9% (8/90), the

Table 1. Baseline demographics, clinical and laboratory features of the entire cohort

Characteristics	Preeclampsia group, (n=60)	Severe preeclampsia group, (n=30)	Non-severe preeclampsia group, (n=30)	Non-preeclampsia group, (n=30)	p[a]	p[b]
Age in years	26 (20.25-29.75)	26 (22-30.25)	22.5 (20-28.25)	23 (20-27.25)	0.13	0.263
POG	38 (36.45-39)	37.2 (36.15-38.2)	39 (37.275-39.7)	38.45 (38-39.4)	<0.001	0.005
BMI	28 (26-30)	28 (26-31.25)	26 (23.75-30)	24 (22-26)	<0.001	0.001
SBP	160 (150-170)	170 (167-180)	150 (141.5-154.5)	118 (109.5-126)	<0.001	<0.001
DBP	106 (92.5-117)	116 (110-118.5)	93 (90-100)	74 (68-80)	<0.001	<0.001
24-hour urine protein excretion (mg/day)	320 (300-350)	340 (317.25-385)	308 (287.5-326)	276 (264.25-286)	<0.001	<0.001
Platelet (/μL)	150000 (112000-207500)	140000 (107000-180000)	167500 (120000-275000)	276000 (228750-354250)	<0.001	<0.001
Urea (mg/dL)	27.5 (22-30)	30 (23.5-37)	25 (21.5-28.5)	26 (23.5-28)	0.07	0.56
Creatinine (mg/dL)	0.8 (0.7-0.9)	0.9 (0.775-0.9)	0.8 (0.7-0.9)	0.9 (0.775-0.9)	0.15	0.43
AST (IU/L)	49 (36-141.5)	69 (37-180)	47.5 (35.5-110.5)	39 (35.75-45)	0.036	0.023
ALT (IU/L)	48 (36-119.5)	59 (36-137.25)	48 (33.5-84.5)	36.5 (34-40)	0.013	0.010
FHR	136 (118-140)	136 (114.5-140)	137 (124.5-140)	137 (132-142)	0.085	0.073
Birth weight	2.9 (2.425-3.2)	2.7 (2.2-3.1)	3 (2.6375-3.65)	2.85 (2.6-3.2)	0.086	0.67
Past history PIH	9 (15)	5 (16.7)	4 (13.3)	0	0.071	0.025
Headache	18 (30)	18 (60)	0	0	<0.001	0.001
Visual disturbance	9 (15)	9 (30)	0	0	<0.001	0.025
Epigastric pain	11 (18.3)	11 (36.7)	0	0	<0.001	0.012
FGR	11 (18.3)	7 (23.3)	4 (13.3)	0	0.013	0.012
Pulmonary oedema	1 (1.7)	1 (3.3)	0	0	0.99	0.99

Cells indicate median (25-75 percentile) or frequency (percentage). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BMI: Body mass index in kg/m^2, DBP: Diastolic blood pressure (mmHg), FGR: Fetal growth restriction, FHR: Fetal heart rate, PIH: Pregnancy induced hypertension, POG: Period of gestation (weeks), SBP: Systolic blood pressure (mmHg). [a]P-value derived from Kruskal-Wallis test or 2x3 chi-squared test (omnibus test result comparing severe preeclampsia, non-severe preeclampsia and non-preeclampsia). In bivariate tests between severe group versus control group, additional significant difference was in FHR (p=0.025); in comparisons between non-severe group and control group no additional significant differences were found, but differences in terms of POG (p=0.73), BMI (p=0.08), AST (p=0.15) and ALT (p=0.118) lost significance, [b]Comparison between pooled patients of preeclampsia patients versus non-preeclampsia subjects

Table 2. Comparison of different optic nerve sheath measurement cut-offs

ONSD measurements	Preeclampsia group, (n=30)	Severe preeclampsia group, (n=30)	Non-severe preeclampsia group, (n=30)	Non-preeclampsia group, (n=30)	p[a]	p[b]
ONSD day P	5.2 (4.6-5.7)	5.65 (4.875-5.9)	4.88 (3.975-5.4)	3.7 (3.175-4.2)	<0.001	<0.001
ONSD day P >5.8 mm	14 (23.3)	13 (43.3)	1 (3.3)	0	<0.001	0.004
ONSD day P >4.6 mm	48 (80)	27 (90)	21 (70)	5 (16.7)	<0.001	<0.001
ONSD day P ≥4.25 mm	49 (81.7)	28 (93.3)	21 (70)	5 (16.7)	<0.001	<0.001
ONSD day 4	4.6 (4.2-5)	4.8 (4.575-5.4)	4.25 (3.95-4.8)	3.2 (2.8-3.6)	<0.001	<0.001
ONSD day 4 >5.8 mm	2 (3.3)	2 (6.7)	0	0	0.33	0.55
ONSD day 4 >4.6 mm	37 (61.7)	23 (76.7)	14 (46.7)	1 (3.3)	<0.001	<0.001
ONSD day 4 ≥4.05 mm	50 (83.3%)	30 (100)	20 (66.7)	2 (6.7)	<0.001	<0.001
ONSD day 7	3.7 (3.4-4.075)	3.9 (3.6-4.325)	3.6 (2.975-3.825)	2.95 (2.6-3.2)	<0.001	<0.001
ONSD day 7 >5.8 mm	0	0	0	0	-	-
ONSD day 7 >4.6 mm	8 (13.3)	6 (20)	2 (6.7)	0	0.025	0.036
ONSD day 7 ≥3.25 mm	47 (78.3)	26 (86.7)	21 (70)	5 (16.7)	<0.001	<0.001

Cells indicate median (25-75 percentile) or frequency (percentage). ONSD: Optic nerve sheath diameter. [a]P-value derived from Kruskal-Wallis test or 2x3 chi-squared test. [b]Comparison between preeclampsia patients and non-preeclampsia subjects, day P: Day prenatal (first day of ONSD measurement before delivery after admission in hospital), day 4: Day 4 postpartum, day 7: Day 7 postpartum

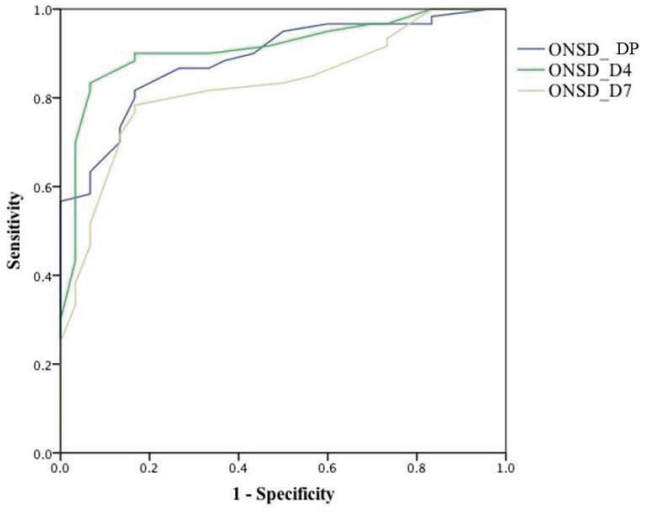

Figure 1. ROC curve analysis was performed to identify optimal cut-off values for ONSD to preeclampsia
ROC: Receiver operating characteristic, ONSD: Optic nerve sheath diameter

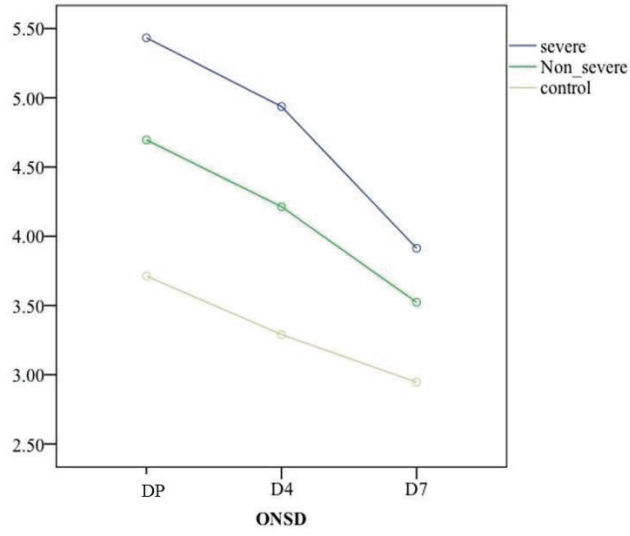

Figure 2. The number of patients with higher ONSD, in terms of either cut-off value in the severe and non-severe preeclampsia groups compared
ONSD: Optic nerve sheath diameter

latter exclusively among patients with severe preeclampsia. Association of these outcome measures with the optic nerve sheath measurements are given in Table 3. Higher ONSD were seen in patients with neurological symptoms and associated with physician's decision to perform emergency caesarean section. Among patients with severe preeclampsia, patients with neurological symptoms (n=20) had higher ONSD compared to patients without neurological symptoms (n=10) on day P [respectively 5.8 (5-5.9) vs. 5.4 (4.5-5.6), p=0.028], on day 4 [respectively 5.1 (4.7-5.5) vs. 4.6 (4.2-5), p=0.017] and on day 7 [respectively 4.05 (3.7-4.6) vs. 3.6 (3.1-3.7), p=0.008]. In this group, no significant association with other outcome measures were noted, except the need for emergency caesarean section with ONSD on day 7 [4.1 (3.7-4.6) vs. 3.6 (3.1-3.7), p=0.005]. Among patients with preeclampsia, different cut-offs for ONSD were associated with development of neurological symptoms. These are detailed in Table 4.

Discussion

In severe preeclampsia and eclampsia neurological complications may arise from co-existence of reversible

Table 3. Association of optic nerve sheath measurements with pregnancy and neonatal outcomes

	Neurological symptoms			Neonatal death			SNCU admission			Emergency caesarean section		
	Absent, (n=70) Present, (n=20)			Absent, (n=82) Present, (n=8)			Absent, (n=65) Present, (n=25)			Absent, (n=47) Present, (n=43)		
ONSD day P	4.2 (3.6-5.1)	5.8 (5-5.9)	<0.001	4.7 (3.8-5.4)	5.3 (4.7-6.1)	0.14	4.6 (3.8-5.3)	5.2 (4.6-5.8)	0.016	4.2 (3.6-5.1)	5.1 (4.2-5.8)	0.001
ONSD day 4	4 (3.2-4.6)	5.1 (4.7-5.6)	<0.001	4.2 (3.4-4.8)	4.6 (4.1-5.2)	0.23	4 (3.2-4.8)	4.6 (4.25-4.9)	0.02	3.8 (3.2-4.6)	4.6 (4-5)	<0.001
ONSD day 7	3.2 (2.8-3.6)	4.05 (3.7-4.6)	<0.001	3.45 (2.8-3.8)	3.95 (3.1-4.7)	0.09	3.4 (2.8-3.8)	3.7 (3.2-4)	0.09	3.2 (2.7-3.6)	3.8 (3.2-4.2)	<0.001

Cells indicate median (25-75 percentile). ONSD: Optic nerve sheath diameter, SNCU: Sick new-born care unit. P-values were calculated using the Mann-Whitney U test

Table 4. Comparison of different optic nerve cut offs on day P in terms of their association with pregnancy and neonatal outcomes

	Neurological symptoms			Emergency caesarean section			Neonatal death		
	Present, (n=20)	Absent, (n=40)	p	Present, (n=33)	Absent, (n=27)	p	Present, (n=7)	Absent, (n=53)	p
ONSD day P >5.8 mm	11 (55)	3 (7.5)	<0.001	12 (36.4)	2 (7.4)	0.008	2 (28.6)	12 (22.6)	0.73
ONSD day P >4.6 mm	19 (95)	29 (72.5)	0.04	29 (79.4)	19 (70.4)	0.42	6 (85.7)	42 (79.2)	0.68
ONSD day P ≥4.25 mm	20 (100)	29 (72.5)	0.009	30 (90.9)	19 (70.4)	0.041	6 (85.7)	43 (81.1)	0.77

Cells indicate frequency (percentage). ONSD: Optic nerve sheath diameter

cerebral vasoconstriction syndrome and posterior leuco-encephalopathy syndrome, giving rise to clinical features like headache, reversible blindness, confusion, and convulsions (18-21). Reversible cerebral vasoconstriction syndrome is associated with subarachnoid and intracerebral haemorrhage and posterior leukoencephalopathy syndrome results in diffuse vasogenic oedema (22,23). Such vasogenic oedema happens due to failure of cerebral autoregulation, disruption of blood brain barrier and endothelial dysfunction (23,24). The resultant cerebral oedema arising from both vasogenic oedema and vasoconstriction-induced cytotoxic oedema contributes to raised ICP (24). Invasive monitoring of ICP is expensive and associated with complications, such as bleeding and infection (17). Performing MRI scans for regular assessments and comparisons may not be cost effective in resource poor countries and transportation of critically ill pregnant mothers to radiology departments may be hazardous (25). USG is a less expensive, quick, real time, and dynamic imaging modality with an objective endpoint in this situation. It has been observed that about 25 scans are enough for an inexperienced sonographer to become proficient in its use (26). In contrast, fundoscopy for papilledema to detect raised ICP has limitations, as papilledema takes time to become evident (27). Moreover, the findings are subjective with inter-observer variability and detection of early papilledema can be a diagnostic challenge (28).

Optic nerve, being a direct extension of the CNS, and unlike other cranial nerves, is surrounded by the meninges and is subjected to the same pressure changes as occur in the intracranial compartment when CSF pressure increases. The increased CSF pressure is transmitted directly to the subarachnoid space between the nerve and its sheath, leading to distension of the intra orbital part of the sheath, particularly the retrobulbar segment (17). The resultant increase in ONSD, can be measured by USG B scan and provides strong evidence of intracranial hypertension (29). When compared with invasive monitoring of ICP, an ONSD value >5.8 is associated with 95% risk of raised ICP (>20 mmHg) (12). Average ONSD among Indian women, aged between 18 and 40 years, was 4.6 mm (17). In this study we compared ONSD measurements with the maternal and neonatal outcomes at both these cut-offs values of 5.8 mm and 4.6 mm. The two prominent studies on changes in ONSD measurements in preeclampsia by Dubost et al. (9) and Brzan Simenc et al. (30) did not a significant correlation between severity of the disease and increased ONSD values, probably due to small sample size. Incidence of raised ICP (ONSD >5.8 mm) in severe preeclampsia was found to be 19% by Dubost et al. (9) and 43% by Brzan Simenc et al. (30). In our study, it was 43.3%, similar to the study by Brzan Simenc et al. (30). We found a significant association between increased ONSD and neurological manifestations in our study at different cut off values including the newly derived optimal cut-off, particularly in the pre delivery state. A study with transcranial Doppler has shown a gradual reduction of cerebral oedema in preeclampsia and eclampsia over 5 to 6 days after delivery (31). A similar

reduction in ONSD in severe preeclampsia was found following delivery over one week in these three studies. Both Dubost et al. (9) and Brzan Simenc et al. (30) found higher ONSD values after delivery and 5 days postpartum when compared with uncomplicated normotensive pregnant controls. We too found significantly higher ONSD measurements when compared to that of uncomplicated pregnancy at the 4.6 mm cut-off and at the newly derived cut-off values over 7 days postpartum. However, the data regarding time course of persistence of enlarged ONSD once dilated is lacking. Rajajee et al. (32) postulated a delayed reversibility of ONSD in long standing increased ICP. Bala et al. (33) commented that increase in OSND occurred before manifestation of neurological features when ICP starts to increase and the reverse occurs during resolution of intracranial hypertension, with ONSD reversal lagging behind resolution of CNS manifestations. Some studies have shown persistent excess fluid accumulation in extravascular lung spaces for several days after delivery in severe preeclampsia (34,35). This information, along with the findings of increased ONSD over seven days post-delivery highlights the importance of intensive monitoring of such critical patients for several days after delivery.

Study Limitations

The limitations of our study were that we did not compare our findings with invasive ICP monitoring and we did not have MRI brain scans for correlation.

Conclusion

We were able to identify much lower new cut off values for ONSD, at which neurological manifestations became evident. Further studies involving larger sample sizes and longer duration of follow-up are needed to be undertaken. Furthermore, ONSD measurements can be compared with invasive ICP values, MRI brain studies and treatment outcomes using diuretics and/or fluid restrictions.

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Ethical Committee Approval: *The study was approved by the Calcutta National Medical College Institutional Ethics Committee (approval number: CNMC-GYN-228, date: 07.10.2019).*

Informed Consent: *Informed consent was obtained from study subjects.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: A.M.; Concept: J.B.; Design: N.B.; Data Collection or Processing: Sw.M.; Analysis or Interpretation: S.M.; Literature Search: N.K., R.B.; Writing: J.B.*

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Obstetric and neonatal complications in large for gestational age pregnancy with late gestational diabetes

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Abstract

Objective: Gestational diabetes (GDM) is increasing in prevalence with effects starting in-utero, leading to excessive fetal growth. It is the leading cause of many perinatal complications. The aim was to determine the rate of obstetric and neonatal complications in pregnant women with high fetal weight and a recent diagnosis of GDM during the third trimester, despite normal earlier glycemic control.

Material and Methods: Prospective cohort study over four years involving pregnant women regularly visiting a single center who had normal glycemic index at 24-28 gestational weeks and ultrasonography (US) suggested high fetal weight during the third trimester. Oral glucose tolerance test was given, dividing the sample into the late GDM (LGDM) and the non-LGDM group.

Results: Of 176 women, 24 (13.64%) had LGDM, and 152 (86.36%) had non-LGDM. After exclusions these groups' sizes were (n=21) in LGDM and (n=132) in non-LGDM. Hemoglobin A1c level was significantly higher in LGDM than non-LGDM (5.9% versus 5.1%). However, obstetric and neonatal complications were largely comparable ($p \geq 0.05$) but higher in LGDM than non-LGDM women. Exceptions to this were birth weight (3219 g versus 3326 g), large for gestational age at delivery (85.72% versus 88.64%), and gestational age at delivery (37.9 versus 38.2 weeks) in the LGDM vs. non-LGDM groups, respectively. There was a significantly higher cesarean section (CS) rate (76.19% versus 51.52%; $p<0.05$) in the LGDM group.

Conclusion: The rate of newly diagnosed LGDM in pregnant women with high fetal weight during the third trimester by US was 13.64%. They had comparable obstetric and neonatal complications with non-GDM women, except for the rate of CS that was significantly higher in LGDM women. (J Turk Ger Gynecol Assoc 2023; 24: 12-7)

Keywords: Hyperglycemia, macrosomia, third-trimester gestational diabetes

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Introduction

Gestational diabetes mellitus (GDM) is a state of glucose intolerance during pregnancy and affects 3.2% of pregnancies in Iraq and 3-10% globally, varying by sample populations and diagnostic criteria (1,2). GDM exaggerates fetal growth and directly influences adverse obstetric and neonatal events (1,3). The diagnosis of GDM is usually confirmed by an oral glucose tolerance test (OGTT), which is undertaken between 24-28 gestational weeks, while fetal growth has the fastest increase between 20-28 gestational weeks, that is prior to or concurrent with the OGTT. Correct diagnosis and effective management of GDM as early as possible may improve fetal weight, obstetric complications, which include, high cesarean section (CS) rate,

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postpartum hemorrhage, preterm delivery (PD), pregnancy-induced hypertension, and polyhydramnios, and neonatal adverse events, such as shoulder dystocia, hypoglycemia, and high admission rate to neonatal intensive care (4,5).

A high glycemic index in pregnant women is a major cause of excessive fetal growth, constituting up to 45% of elevated fetal weight cases. However, many pregnancies are complicated with high fetal weight after 28 weeks' gestation with normal earlier glucose measurements. There is limited published evidence concerning diagnosis and management in this situation (2,6,7).

The aim of this study was to investigate obstetric and neonatal complications in pregnant women during the third trimester, who had high fetal weight suggested by ultrasonography (US) after 28 weeks of gestation and were recently diagnosed with GDM, despite an earlier normal glycemic index.

Material and Methods

The study was carried out over a four-year period, from May 4, 2017 until May 3, 2021. The study design was a prospective cohort study and involved pregnant women visiting the Obstetrics and Gynecology Department of Medical City Hospital in Baghdad, Iraq. This is the main tertiary referral hospital in the country. This work was approved by the ethical and scientific committees of the College of Medicine and Al-Kindy College of Medicine/University of Baghdad (approval number: 547, date: 29.03.2017). Informed consent was taken from all involved women.

Inclusion criteria

Singleton pregnant women who had regular antenatal care (ANC) visits starting from the first trimester until delivery, with no diagnosis of GDM during the second trimester, either by screening OGTT or blood sugar measurements (fasting and postprandial) at 24-28 weeks' gestational age and who had the suspicion of large for gestational age (LGA) fetus by US examination at 32 weeks' gestational age during the third trimester.

Exclusion criteria

Multiple pregnancy, congenital anomalies diagnosed by US, irregular ANC, pre-pregnancy diabetes mellitus, diagnosis of GDM during the second trimester, and women who missed glucose measurements during the period 24-28 gestational weeks. Women who lost contact with the researchers during the study period or delivered outside the hospital were also excluded.

Carpenter-Coustan criteria were applied to diagnose GDM using OGTT (100 gram) with the following blood sugar reference readings: 95, 180, 155, and 140 mg/dL of fasting, one hour, two hours, and three hours postprandial, respectively. If the pregnant women had (or exceeded) at least two of these thresholds, the diagnosis of GDM was confirmed (8). In addition, hemoglobin A1c (HbA1c) was also measured in the involved women at this stage.

The weight of the fetus was estimated by US using the Hadlock formula, and LGA pregnancy was assumed when the estimated weight of the fetus exceeded 90th percentile of weight for gestational age (9).

When the included pregnant women had the suspicion of LGA during the third trimester, OGTT was done within 2-3 days. Subsequently, two study groups were created; the late GDM (LGDM) group, and non-LGDM group. In LGDM women, lifestyle management was advised with frequent blood glucose monitoring and management was undertaken by an expert obstetrician.

The following obstetric complications were investigated: PD, polyhydramnios, pregnancy induced hypertension, shoulder dystocia, and operative delivery by CS, while the observed neonatal complications were hypoglycemia and admission to the neonatal intensive care unit (NICU). These complications were documented for all included women and compared between the GDM and non-GDM groups. Labor was managed for all involved women by the attending obstetrician and neonatologist, and all perinatal events and delivery details were documented.

Statistical analysis

Mann-Whitney U test was used for continuous parameters, while Pearson's χ^2 test or Fisher's exact test was performed for proportions. Statistical Package for the Social Sciences statistics software, version 23.0 (IBM Corp., Armonk, NY, USA) was used to complete the calculations. A p-value <0.05 was considered significant.

Results

The total number of involved pregnant women was 176 were tested using OGTT after an excessive fetal weight had been suggested on US during the third trimester. Of them, 24 women (13.64%) had abnormal OGTT, indicating a diagnosis of LGDM (LGDM group), while the remainder, 152 women (86.36%), had normal OGTT (non-LGDM group). For LGDM and non-LGDM groups, 3 and 20 women, respectively, were excluded from the study because of missed data or delivery outside the research center. Thus, final total in the LGDM group was 21, while it was 132 for the non-LGDM group. In the LGDM group, three women were managed by diet while the remaining 18 were on medical treatment.

General characteristics of both groups were comparable without statistical significance ($p \geq 0.05$), with the exception of

HbA1c, which was significantly higher in the LGDM than in non-LGDM women (5.9% versus 5.1%) (Table 1).

Table 2 shows all neonatal and obstetric complications, compared between the two groups. Most variables were statistically comparable, although there was a tendency for babies in the LGDM group to be lighter weight at birth, to have an earlier gestational age at birth and to be less likely to be LGA.

Table 3 compares the characteristics of the women in the two groups at the time of OGTT. Gestational age (34.7 versus 35.2) and estimated fetal weight (2674 versus 2719) were comparable ($p \geq 0.05$) between both groups, while OGTT, as expected, was significantly different ($p < 0.05$).

Details of delivery mode are shown in Table 4. LGDM women had significantly ($p < 0.05$) higher rates of CS and lower rates of normal delivery than non-LGDM women (76.19% versus 51.52%, and 23.8% versus 48.48%, respectively). The rates of induction of labor and premature rupture of membranes did not differ between the groups. Frequencies of different indications of CS, including

Table 1. General features of involved women

Variable	LGDM (n=21)	Non-LGDM (n=132)	p
Age (years), median (range)	23.4 (18-43)	22.7 (19-46)	0.62
Nulliparous, n (%)	13 (61.90%)	79 (59.85%)	0.81
Family history of diabetes mellitus, n (%)	4 (19.05%)	22 (16.67%)	0.53
Pre-pregnancy body mass index (kg/m^2), median (range)	26.1 (17.4-38.3)	25.8 (17.2-39.8)	0.14
Hemoglobin A1c, median (range)	5.9 (4.8-7.3)	5.1 (4.4-6.2)	<0.05
LGDM: Late gestational diabetes mellitus			

Table 2. Obstetric and neonatal outcomes of involved groups

Variable	LGDM (n=21)	Non-LGDM (n=132)	p
Birth weight (g), median (range)	3219 (2472-4596)	3326 (2411-4480)	0.38
Polyhydramnios, n (%)*	1 (4.76%)	5 (3.79%)	0.79
Pregnancy induced hypertension, n (%)*	1 (4.76%)	3 (2.27%)	0.09
Large for gestational age at delivery, n (%)**	18 (85.72%)	117 (88.64%)	0.46
Gestational age at delivery (weeks), median (range)	37.9 (34.3-41.1)	38.2 (34.5-40.8)	0.18
Shoulder dystocia, n (%)*	1 (4.76%)	5 (3.79%)	0.42
Preterm delivery, n (%)*	2 (9.52%)	9 (6.82%)	0.39
Fetal sex (male), n (%)*	12 (57.14%)	65 (49.24%)	0.61
Neonatal hypoglycemia, n (%)*	1 (4.76%)	4 (3.03%)	0.34
Neonatal intensive care unit admission, n (%)*	2 (9.52%)	10 (7.58%)	0.22
*Data are presented as n (%) and were compared with Fisher's exact test, **Data were compared with Pearson's χ^2 test, LGDM: Late gestational diabetes mellitus			

Table 3. Data of involved women at time of oral glucose tolerance test during the third trimester

Variable	LGDM (n=21)	Non-LGDM (n=132)	p
Gestational age at presentation (weeks), median (range)	34.7 (29.5-39.2)	35.2 (30.3-40.6)	0.28
Estimated fetal weight at presentation (g), median (range)	2674 (1253-3587)	2719 (1304-3926)	0.31
Oral glucose tolerance test measurements (mg/dL), median (range)			
- Fasting	88 (64-128)	80 (61-105)	<0.05
- After 1-hour	191 (156-280)	149 (73-212)	<0.05
- After 2-hours	169 (101-274)	124 (82-165)	<0.05
- After 3-hours	136 (58-221)	104 (53-158)	<0.05
High oral glucose tolerance test, n (%)*			
- Fasting	4 (19.05%)	4 (3.03%)	<0.05
- After 1-hour	15 (71.43%)	6 (4.55%)	<0.05
- After 2-hours	17 (80.95%)	7 (5.30%)	<0.05
- After 3-hours	13 (61.90%)	8 (6.06%)	<0.05
*Data are presented as n (%) and compared with Fisher's exact test, LGDM: Late gestational diabetes mellitus			

Table 4. Details of delivery characteristics

Variable	LGDM (n=21)	Non-LGDM (n=132)	p
Delivery type, n (%)*			
- Normal vaginal delivery	5 (23.8%)	64 (48.48%)	<0.05
- Cesarean section	16 (76.19%)	68 (51.52%)	-
Labor induction, n (%)*	13 (61.90%)	69 (52.27%)	0.69
Premature rupture of membranes, n (%)*	3 (14.29%)	22 (16.67%)	0.75
Cesarean section indication, n (%)*			
- Previous cesarean section	10 (47.62%)	58 (43.94%)	0.56
- Placental abnormalities	1 (4.76%)	6 (4.55%)	0.91
- Fetal deceleration	2 (9.52%)	11 (8.33%)	0.64
- Failure to progress	4 (19.05%)	23 (17.42%)	0.72
- Malpresentation	1 (4.76%)	3 (2.27%)	0.09
*Data were compared with Fisher's exact test, LGDM: Late gestational diabetes mellitus			

previous CS, placental abnormalities, fetal deceleration, failure to progress, and malpresentation, were higher in the LGDM than non-LGDM group but failed to reach significance.

Discussion

Many previous studies have discussed obstetric and neonatal complications in GDM pregnancy, but few have addressed the issue of LGDM in the third trimester (1,6,10). In this study, we tried to select pregnant women with suggested high fetal weight during the third trimester who surprisingly appeared to have GDM despite their prior normal glucose readings during the second trimester.

The prevalence rate of LGDM women during the third trimester in our sample was 13.64%, which was comparable with one previous study (13.5%) (11) but twice that of another earlier study (6.7%) (12). The reason behind that might be due to the feature of excessive fetal weight in our sample of pregnant women who are likely to have high blood sugar as insulin resistance is a progressive phenomenon during pregnancy, and GDM is the primary etiology of increased fetal growth (13).

HbA1c was significantly elevated in LGDM women, indicating higher glucose readings for the previous 2-3 months. However, this interpretation may be unreliable during pregnancy due to physiological changes, including rapid erythrocyte turnover (14).

In our study, although LGDM women had higher suggested fetal weight during the third trimester, neonatal birth weight and LGA at delivery were lower than non-LGDM women. This may be explained by effective GDM management, which influenced perinatal outcomes (15). Other reported perinatal outcomes including polyhydramnios, pregnancy-induced hypertension, shoulder dystocia, PD, neonatal hypoglycemia, and admission to NICU, were slightly higher in the LGDM group, in agreement with local and international figures (13,16,17).

The LGDM women in this cohort had significantly higher OGTT measurements, including fasting and 1-, 2-, and 3-hour postprandial readings, confirming the diagnosis of GDM in these women (18).

Rates of CS were significantly higher in LGDM women, which might be due to increased but not significantly increased rates of CS indications, including previous CS, placental abnormalities, fetal deceleration, failure to progress, and malpresentation.

Higher chances of operative delivery in GDM patients were reported in several previous studies, while our non-GDM women had a rate of CS delivery similar to the local rate (17,19-21).

The significantly high rate of CS in LGDM women in our sample was higher than the local rates of CS in adult and adolescent pregnant women (22,23).

This study has the advantage of a prospective assessment of newly diagnosed GDM women during late pregnancy in the third trimester, with suspected excessive fetal growth and normal earlier glucose measurements. LGDM could be missed because of comparable obstetric and neonatal complication rates (11), unless identified using the approach described herein. Although obstetric and neonatal complications were slightly but insignificantly increased in LGDM compared to non-LGDM women, the benefit of identifying women with GDM may extend beyond the current pregnancy. This is because GDM in a previous pregnancy is considered a risk factor for early GDM in subsequent pregnancies with more severe perinatal complications that may be similar to pre-pregnancy diabetes. Moreover, GDM diagnosis may suggest increased monitoring for diabetes and it may predict overt diabetes within some subsequent timeframe (24).

Study Limitations

This study has some limitations. OGTT was not routinely applied to all pregnant women during mid-pregnancy because

of limited resources in our setting, and was replaced by blood glucose profile (fasting and postprandial) in some pregnant women, which is less sensitive for diagnosing GDM (25). In addition, the small sample size could be considered a limitation and may make the findings less reliable despite the long study period. In addition, the single-center design of the study may affect the generalizability of the results. However, this rare presentation of pregnant women with LGDM needs to be given more consideration, to prevent possible complications in the existing pregnancy and possible complications in future pregnancies.

Conclusion

The rate of newly diagnosed GDM during the third trimester in pregnant women high fetal weight suggested by US was 13.64%. LGDM and non-LGDM pregnant women had comparable non-significant obstetric and neonatal complication rates. Operative delivery by CS was significantly more likely in LGDM women, possibly due to increased concerns about CS indications, despite the rates of these being similar between the two groups.

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Does advanced paternal age affect global DNA methylation of human spermatozoa and intracytoplasmic sperm injection outcome?

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Abstract

Objective: This study was performed to (I) evaluate the potential effect of advanced paternal age on global DNA methylation in spermatozoa, and (II) to investigate the association between the outcome of intracytoplasmic sperm injection (ICSI), semen parameters, and advanced paternal age.

Material and Methods: This study comprised 230 semen samples collected from males with a mean age of 38.2±8.5 years. Medical records were used to gather clinical information related to the female partner. The participants were divided into three groups depending on age: age <30 years; age 30-40 years; and age >40 years. The DNA was extracted from purified spermatozoa. Then the sperm global DNA methylation, sperm DNA fragmentation, and chromatin decondensation were evaluated by an ELISA, TUNEL, and Chromomycin A3 staining, respectively.

Results: The sample counts were n=50 (21.8%), n=90 (39.1%) and n=90 (39.1%) for the <30, 30-40 and >40 year age-groups, respectively. A significant variation was found in the age of males included in this study ($p<0.001$). There was a significant reduction in sperm count, total motility, and non-progressive motility in the older group compared to the younger group ($p<0.001$). There was also a significant elevation in chromatin decondensation, DNA fragmentation, and global DNA methylation of spermatozoa in the older age group ($p<0.001$). Finally, there was a significant positive correlation between the percentage of non-motile sperm, sperm chromatin decondensation, DNA fragmentation, global DNA methylation status, and paternal age ($p<0.001$).

Conclusion: These results suggest that advanced paternal age increased the DNA fragmentation, chromatin decondensation, and global DNA methylation level in human spermatozoa, which negatively affects the ICSI outcomes in couples undergoing ICSI cycles. (J Turk Ger Gynecol Assoc 2023; 24: 18-27)

Keywords: Global methylation, ICSI outcomes, paternal age, spermatozoa

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Introduction

In assisted reproductive technologies (ART), maternal age plays a critical role in determining the success rate of the intracytoplasmic sperm injection (ICSI) process. Several studies have shown that paternal aging can directly damage sperm DNA, increase the level of sperm DNA methylation (1,2), and increase the rate of sperm damage through the production of excessive reactive oxygen species (3,4). Other studies reported that human spermatozoa have a very distinct pattern of age-associated alteration (5,6), whereas one study observed an increase in global spermatozoa DNA methylation and a strong bias toward regional loss of methylation at sites known to be impacted by aging (7). It has been reported that male age was associated with alterations in sperm DNA methylation levels at 1,698 CpGs and 1,146 regions, which were associated

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with more than 750 genes enriched in embryonic development, behavior, and neurodevelopment (6). Additionally, the DNA methylation profiles in sperm of young men (≤35 years) and older men (≥50 years) were compared and the authors identified around 49,792 differentially methylated CpG sites related to neurodevelopmental relevant diseases (8). Another study observed that there are more hypermethylated (62%) than hypomethylated (38%) CpG sites in sperm from older aged men and the distribution of age-related hyper- and hypo-methylated CpGs in sperm is not random. The CpG sites that were hypermethylated with advanced age were frequently located in the distal region to genes, whereas hypomethylated sites were near gene transcription start sites. Consequently, the effect on gene function is potentially related to diseases in offspring (9). Despite these recent advances, data about age-associated sperm DNA methylation is still limited.

An association was reported between the reduction in the rates of pregnancy and advancing paternal age (10). Furthermore, several studies have observed a negative correlation between sperm quality and paternal age, which negatively influences embryo cleavage and in vitro fertilization (IVF) clinical outcomes (11-13). In contrast, other studies have shown that paternal age has no influence on the fertilization rate, embryo quality (14), and the rate of pregnancy during conventional IVF techniques (15). Nevertheless, there is currently a lack of consensus concerning the contribution of paternal aging to sperm parameters, sperm DNA integrity, and clinical ICSI outcomes (16-18). Human and animal studies have reported that ART is associated with epigenetic changes in embryonic and extra-embryonic tissues (19,20). Additionally, epigenetic events may impair key steps during fertilization, implantation, embryo development, and sperm maturation (21,22). Epigenetics is defined as alterations in gene expression without changing the DNA sequence (23). Well-known epigenetic regulation mechanisms include DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) (24).

DNA methylation is defined as the addition of a methyl group (CH3) to the fifth (C-5) position of the cytosine ring in CpG dinucleotides by DNA methyltransferase to form 5-methylcytosine (5-mC), where the S-adenosyl-methionine is used as a donor for the methyl group (25,26). The CpG dinucleotides can be found in clusters that have been termed CpG islands and are characterized by less methylation than non-CpG islands (27). It is worth noting that CpG islands are found in about 60-70% of gene promoters (28). The methylated state of CpGs has a crucial impact on gene transcription during embryonic growth, genomic imprinting, X-chromosome inactivation, and tumor development (29,30). Several studies have illustrated that changes in DNA methylation of specific genes in germ cells are associated with oligozoospermia, reduced sperm progressive motility, and abnormal sperm morphology (31,32). A previous study noted that the alteration in the DNA methylation level of male spermatozoa may influence the developmental potential of embryos (33). Several studies have highlighted that increased paternal age influences testicular function (34), sperm parameters (35), sperm DNA integrity (1), and epigenetics (36). However, there is still no consensus around the influence of paternal age on global DNA methylation of human spermatozoa and the reproductive capacity of males during ICSI cycles. Therefore, this study was designed to (I) evaluate the potential effect of advanced paternal age on global DNA methylation, DNA fragmentation, DNA condensation in human spermatozoa, and ICSI outcomes, and (II) investigate the relationship between ICSI outcomes, semen parameters, and paternal age.

Material and Methods

Study population

This prospective study comprised two hundred and thirty couples with a mean age for males of 38.2±8.5 years and for females of 36.1±5.9 years. The study was conducted between May 2010 and September 2013. All cases underwent the first ICSI cycles at Al-Basma Fertility Center, Palestinian Territories. All participant women were selected according to the following inclusion criteria: first ICSI cycle; embryo transfer after three days from the injection; undergoing gonadotropin-releasing hormone (GnRH) antagonist stimulation protocols; normal body mass index; and women who have a regular menstrual cycle. Exclusion criteria for females included: tobacco smoking (cigarette or water pipe); alcohol drinkers; diabetes mellitus; women using an oral contraceptive; women suffering from endocrine abnormality; and endocrine disorders including polycystic ovarian syndrome, history of ovarian surgery, and endometrioma. Male partner exclusion criteria were: diabetes mellitus; alcohol drinkers; smokers; the presence of anti-sperm antibodies; varicocele; Y chromosome microdeletions; karyotype abnormalities; history of surgical operation in the reproductive system; abnormal hormonal parameters; and abnormal body mass index. Medical records were used to gather general and medical information that included age, body mass index, menstrual history, the number of retrieved oocytes, mature oocytes, immature oocytes, fertilized oocytes, the number of embryos transferred, and the value of Beta-human chorionic gonadotropin (β-hCG). The participants were divided into three groups depending on the male partner's age: <30 years; 30-40 years; and >40 years.

Ethics approval and consent to participate

This study was approved by the Health Research Council, Palestinian Territories (approval number: 03/10, date: 23.03.2010), and consent was provided in accordance with the Declaration of the Helsinki Committee. Samples were analyzed according to the guidelines and standard procedures of the Al Basma Fertility Center, Palestinian Territories. All participants gave written informed consent to participate in this study.

Ovarian stimulation and embryo transfer

All women included in the present study underwent ovarian stimulation using GnRH antagonist protocols with a recombinant follicle-stimulating hormone. Briefly, ultrasonography was conducted on the third day of the menstrual cycle to evaluate the anatomical characteristics of the female reproductive system and to determine the antral follicular count. The basal levels of estradiol (E2), FSH, luteinizing hormone, prolactin, and anti-Müllerian hormone were measured by immunoassay using a Tosoh AIA-360 instrument (Tokyo, Japan). A GnRH antagonist was administered once the dominant follicle was >14 mm and continued to the day of hCG administration. When at least three follicles were ≥18 mm, ovulation was triggered with hCG. The oocyte pickup was scheduled for 33-36 hours after the administration of 5,000 to 10,000 IU of hCG (Pregnyl), depending on the age of the women and the number of oocytes. The fertilization status of oocytes was checked after 16-18 hours from ICSI. The criteria for normal fertilization were the presence of two clearly visible pronuclei. Embryo cleavage and quality were evaluated 48 hours after ICSI. For each couple, a maximum of three embryos with high quality (grade I or II) were transferred into the uterine cavity after three days from ICSI. All women received luteal support with vaginal progesterone until a pregnancy test was performed. The women were described as pregnant women when the β-hCG hormone level reached >5 mIU/mL.

Semen collection and sperm purification

At the time of the ICSI cycle, semen samples were collected by masturbation after three days of abstinence from sexual intercourse. Semen samples were allowed to liquefy for 30 minutes at 37 °C. Then, the count of spermatozoa was evaluated immediately using a Makler counting chamber (Sefi-Medica, Haifa, Israel). Semen parameters were analyzed according to the World Health Organization guidelines (37). All samples underwent the somatic cell lysis buffer (SCLB) protocol to remove somatic cells and other debris from the sample before DNA extraction from spermatozoa. Briefly, the liquefied semen samples were loaded onto 45% over 90% discontinuous Puresperm gradients (Nidacon International AB, Sweden) and then centrifuged at 500x g for 20 minutes at 22 °C. Then the pure spermatozoa were incubated with SCLB on ice for half-hour and subsequently washed three times with phosphate-buffered saline (PBS), and centrifuged at 500+g for 10 minutes (38,39). Finally, microscopic examination was used to confirm purity of the semen samples from somatic cells and other debris.

DNA fragmentation of human spermatozoa (TUNEL assay)

The DNA fragmentation of spermatozoa (sperm apoptosis) was assessed using the terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling (TUNEL) assay. The TUNEL assay was performed using an in situ cell death detection kit following the guidelines of the manufacturer (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, smears were prepared using 10 μL of sperm suspension on microscope slides and allowed to air-dry and then fixed with 4% paraformaldehyde phosphate-buffered saline, pH 7.4, for two hours at room temperature, then rinsed with PBS. Smears were then permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate, pH 6.0, for 15 minutes at room temperature; 50 μL of the terminal deoxyribonucleotide transferase (TdT)-labelled nucleotide mixture (50 μL of enzyme solution and 450 μL of label solution) was added to each slide and incubated in a humidified chamber at 37 °C overnight in the dark. Negative controls without TdT were run in each replicate. Then, slides were rinsed twice in PBS and left to air dry, followed by adding 25 μL of 5 μg/mL DAPI stain solution to each slide as a counterstain and then coverslipped. For evaluation, a total of 500 spermatozoa were analyzed, by distinguishing spermatozoa stained bright green (TUNEL positive, fragmented DNA) from those stained dull green (TUNEL negative, with intact DNA). A Zeiss Photomicroscope III was used for the fluorochrome evaluation (Zeiss Photomicroscope III, Germany) (40).

Sperm chromatin decondensation (Chromomycin A3 staining)

Chromomycin A3 (CMA3) staining was used to evaluate chromatin non-condensation in human spermatozoa. Briefly, three semen smears were prepared from each sample and all smears were fixed using a fixative solution of methanol:glacial acetic acid, 3:1 respectively) at 4 °C for 20 minutes. Semen smears were air-dried at room temperature. After that, each smear was covered by 50 μL of staining solution (Sigma-Aldrich, St. Louis, MO, USA) and then incubated in a dark place at room temperature for 20 minutes. PBS was used to wash all slides, then the slides were mounted with 1:1 (v/v) glycerol/PBS incubated overnight at 4 °C. To estimate the results of CMA3 staining, the fluorescence microscope (Zeiss Photomicroscope III, Germany) was used to analyze 200 spermatozoa on each smear. Finally, the CMA3 staining was evaluated by differentiating the spermatozoa that stained with bright yellow (positive, bad spermatozoa) from spermatozoa that stained with dull yellow (negative, good spermatozoa) (40,41).

DNA extraction from human spermatozoa

The Isolate II DNA/RNA/Protein Kit was used to isolate DNA from human spermatozoa. At first, 600 μL of lysis buffer was added to 200 μL of pure spermatozoa. Then the mixture was vortexed for 15 seconds. After that, all the lysate was transferred to a DNA column and centrifuged for 1 minute at 14,000 g. All the procedures were carried out according to the guidelines of the manufacturer (Bioline, UK). The purity and the concentration of isolated nucleic acid were assessed using a Nanodrop spectrophotometer-2,000c (Thermo Scientific, USA), to ensure that the quantity and quality of isolated DNA were suitable and adequate for global sperm DNA methylation assay.

Evaluation of global DNA methylation in human spermatozoa

The status of global DNA methylation (5-methylcytosine) in all DNA samples was evaluated using the MethylFlash™ Methylated DNA Quantification ELISA Kit (colorimetric) according to the guidelines of the manufacturer (EpiGentek Group Inc, USA). Briefly, 100 ng of extracted DNA was incubated with the DNA binding buffer solution provided with the kit for 1.5 hours at 37 0C. During this assay a blank, a positive and a negative control were included in triplicate. After washing the microwell four times, methylated DNA capture solution was added to each well and incubated at 22 °C for one hour. After that, detection antibodies were added to each well and incubated for half an hour at room temperature. After washing three times, the developing solution was added to each well and incubated at room temperature in a dark place for six minutes, and at the end of this time, stop solution was added. A microplate ELISA reader was used to determine the absorbance at 450 nm. The global DNA methylation level was calculated using the equation: 5-mC(ng) = [(sample OD - blank OD)/100].

Statistical analysis

All the data were analyzed using IBM SPSS for Windows, version 24.0 (SPSS Inc., Chicago, IL, USA). The skewness test, kurtosis test, and Shapiro test were used to investigate the normality of data distribution. Kruskal-Wallis (H test) and Mann-Whitney (U test) were applied to compare quantitative variables between the study groups. The Spearman rank correlation coefficient was used to study the association between paternal age and other clinical parameters. A p-value of <0.05 was accepted as indicating statistical significance.

Results

Clinical parameters and ICSI outcome among different age groups

This study comprised 230 semen samples collected from males with a mean age of 38.2±8.5 years. On stratification by male partner age, the groups comprised n=50 (21.8%), n=90 (39.1%) and n=90 (39.1%) for the <30, 30-40 and >40 year age-groups, respectively. As expected there was a significant difference between the age of the males in the different groups ($p<0.001$) but there was no significant difference between the female partner's ages ($p=0.676$) (Table 1). A significant reduction was found in the sperm count, percentage of total sperm motility, progressive motility, and non-progressive motility between the different age groups ($p<0.001$). A significant increase was observed in semen volume and the percentage of non-motile sperm ($p=0.022$ and $p<0.001$, respectively) among the different age groups. Additionally, a significant variation was observed among the different age groups in the proportion of spermatazoa with normal and abnormal forms ($p=0.013$). There were also significant differences between the different age groups in terms of oocyte fertilization rate and the number of embryos transferred ($p<0.001$ and $p=0.041$, respectively). Furthermore, a significant decrease in the level of ß-hCG was noted with increasing age ($p=0.009$). There was a significant increase in the degree of sperm chromatin decondensation (Figure 1), sperm DNA fragmentation (Figure 2), and the global sperm DNA methylation level (Figure 3) in older males compared to younger ($p<0.001$).

Correlation between the paternal age and clinical parameters of the study population

There was a significant negative correlation between the sperm count ($r=-0.581$, $p<0.001$), percentage of total sperm motility ($r=-0.391$, $p<0.001$), progressive motility ($r=-0.359$, $p<0.001$), non-progressive motility ($r=-0.351$, $p<0.001$), the level of ß-hCG ($r=-0.166$, $p=0.01$), and paternal age (Table 2). In contrast, a significant positive association was found between semen sample volume ($r=0.220$, $p<0.001$), percentage of non-motile sperm ($r=0.391$, $p<0.001$), degree of sperm chromatin decondensation ($r=0.423$, $p<0.001$), sperm DNA fragmentation ($r=0.391$, $p<0.001$), degree of global DNA methylation ($r=0.321$, $p<0.001$), and increasing paternal age.

Clinical parameters and ICSI outcome in pregnant women compared to non-pregnant

There was a significant increase in the number of collected oocytes ($p=0.029$), mature oocytes ($p=0.044$), fertilized oocytes ($p=0.023$), embryo cleavage ($p<0.001$), number of embryos transferred ($p<0.001$), and the levels of ß-hCG ($p<0.001$) in pregnant compared to non-pregnant women (Table 3). Additionally, a significant decline was found in the level of sperm chromatin condensation (Figure 4), sperm DNA fragmentation (Figure 5), and degree of global DNA methylation (Figure 6) in the partners of pregnant women compared to the partners of non-pregnant women ($p<0.001$).

Table 1. Clinical parameters and ICSI outcome among different paternal age groups (n=230)

Clinical parameters	Study population		Age <30 years, (n=50)		Age 30-40 years, (n=90)		Age >40 years, (n=90)		p
	Median	SD	Median	SD	Median	SD	Median	SD	
Paternal age (years)	38.0	8.5	27.0	1.6	35.0	3.3	48.0	2.6	<0.001
Semen volume (mL)	3.50	2.38	3.50	1.66	3.50	2.33	3.90	2.67	0.022
Sperm count (milli/mL)	58.00	63.06	144.00	63.94	60.85	63.50	31.65	29.86	<0.001
Percentage of total sperm motility	46.00	23.81	55.00	22.20	50.00	17.98	22.50	24.55	<0.001
Percentage of progressive motility	26.00	20.36	38.50	18.62	27.00	16.21	9.50	22.34	<0.001
Percentage of non-progressive motility	15.00	10.42	18.50	10.17	20.00	10.16	9.50	9.35	<0.001
Percentage of non-motile sperm	54.50	23.94	45.00	22.27	50.00	18.53	77.50	24.55	<0.001
Percentage of sperm normal form	8.50	13.26	7.00	8.02	14.50	14.54	7.00	13.47	0.013
Percentage of sperm abnormal form	91.50	13.26	93.00	8.02	85.50	14.54	93.00	13.47	0.013
Maternal age (years)	37.0	5.9	36.0	5.5	37.0	6.6	37.5	5.4	0.676
Number of collected oocytes	8.00	7.13	8.00	6.27	10.00	8.17	7.00	6.19	0.060
Number of mature oocytes	6.00	5.38	6.00	5.30	6.50	5.86	5.00	4.89	0.407
Number of fertilized oocytes	5.00	4.31	4.50	4.89	5.00	4.24	4.00	4.08	0.865
Oocytes fertilization rate	61.11	24.07	58.33	24.25	50.00	22.41	66.67	23.12	<0.001
Number of embryo cleavage	4.00	4.21	4.00	6.19	3.00	3.38	4.00	3.51	0.271
Number of embryo transferred at day 3	3.00	1.18	3.00	0.77	2.00	1.33	3.00	1.19	0.041
ß-hCG level	4.50	40.15	68.00	43.24	4.20	39.69	3.80	36.43	0.009

SD: Standard deviation, ß-hCG: Beta human chorionic gonadotropin

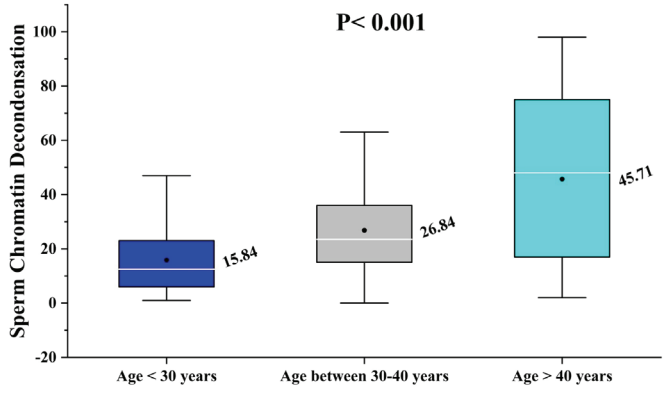

Figure 1. Sperm chromatin decondensation compared between the different paternal age groups

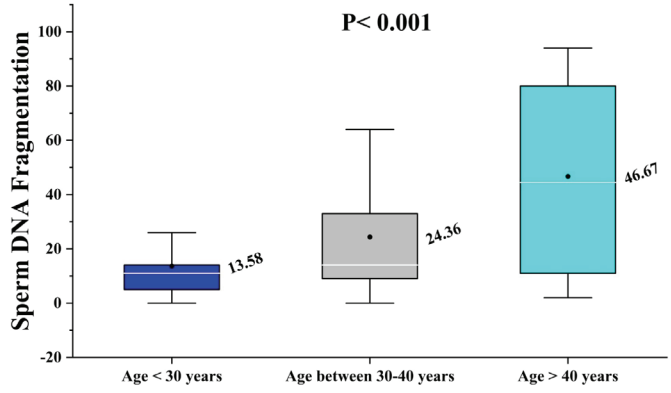

Figure 2. DNA fragmentation in human spermatozoa compared between the different paternal age groups

Correlation between paternal age and clinical parameters of women who became pregnant.

As illustrated in Table 4, there was a significant positive correlation between the degree of sperm chromatin decondensation (r=0.309, p<0.001), sperm DNA fragmentation (r=0.244, p=0.01), global sperm DNA methylation (r=0.269, p=0.01), and the male partner's age in the group of women who became pregnant. In contrast, no significant associations were identified between ICSI outcome and male partner's age in the pregnant women group.

Discussion

Currently, much attention has been paid to studying the impact of paternal age on ICSI outcome and fertilization rate. Several studies have reported that older paternal age contributes negatively to semen production, fertility, pregnancy outcome, and ICSI outcomes (42-44). Other studies have shown that increasing paternal age is linked to genetic and epigenetic abnormalities in spermatozoa (18,45,46). In this study, we assessed the potential effect of differences in paternal age on ICSI outcomes and global DNA methylation in human

spermatozoa. The present study identified a significant reduction in sperm count, sperm total motility, progressive motility, non-progressive motility, and other semen parameters between the different paternal age groups. These findings are in agreement with other studies that showed that sperm counts and other semen parameters decrease with increasing paternal age (47-50). Additionally, these findings are in agreement with other studies that found a significant decline in sperm motility and fecundity status in males aged older than 40 years compared to males aged 35 years old or less (50-52). However, some other studies have shown no significant differences in semen volume, sperm concentration, sperm motility, and morphology between different paternal age groups (35,53). Other earlier reported no drastic effects on semen parameters of healthy men or men with proven fertility with age (54,55). This inconsistency in the findings might result from the lack of control for some confounding factors, such as the duration of abstinence time and the method used for semen collection.

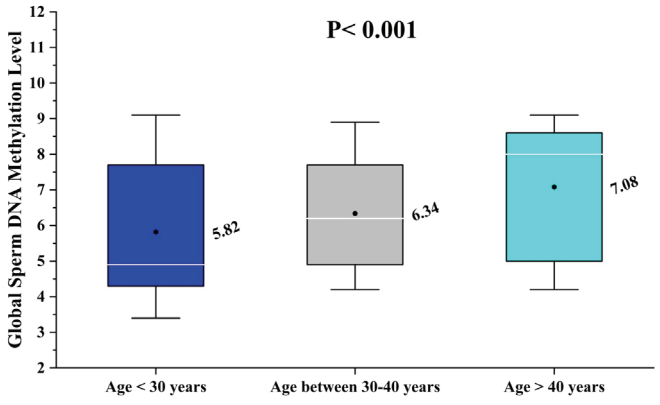

Figure 3. Global DNA methylation level in human spermatozoa compared between the different paternal age groups

A significant increase was found in the level of global DNA methylation, sperm chromatin decondensation, and sperm DNA fragmentation in the older male group compared to younger men. These results support the findings of previous studies that showed an increase in the level of global DNA methylation (56) and sperm DNA fragmentation in older males (57,58). Other previous studies have reported that paternal age is associated with hypermethylation globally (2,59). In addition, another study showed an increase in global 5-methylcytosine

Table 2. Correlation between the paternal age and clinical parameters of the study population (n=230)

Clinical parameters	r	p	Clinical parameters	r	p
Semen volume (mL)	0.220	<0.001	Sperm DNA fragmentation (TUNEL-positive)	0.391	<0.001
Sperm count (million/mL)	-0.581	<0.001	Global sperm DNA methylation level (ng/μL)	0.321	<0.001
Percentage of total sperm motility	-0.391	<0.001	Number of fertilized oocytes	0.03	0.65
Percentage of progressive motility	-0.359	<0.001	Oocytes fertilization rate	0.201	<0.001
Percentage of non-progressive motility	-0.351	<0.001	Number of embryo cleavage	0.04	0.56
Percentage of non-motile sperm	0.391	<0.001	Number of embryo transferred at day 3	-0.03	0.67
Percentage of sperm normal form	-0.04	0.58	ß-hCG level	-0.166	0.01
Sperm chromatin decondensation (CMA3-positive)	0.423	<0.001			

Spearman rank correlation coefficient, r: Correlation coefficient, ß-hCG: Beta human chorionic gonadotropin

Table 3. Clinical parameters and ICSI outcome in pregnant women compared to non-pregnant (n=230)

Clinical parameters and ICSI outcome	Pregnant women, (n=106)			Non-pregnant women, (n=124)			p
	Mean	SD	Median	Mean	SD	Median	
Maternal age (years)	35.5	5.8	37.0	36.6	6.0	38.0	0.113
Number of collected oocytes	11.32	6.99	10.00	9.67	7.19	8.00	0.029
Number of mature oocytes	8.22	5.45	6.00	7.03	5.29	5.50	0.044
Number of fertilized oocytes	6.51	4.71	5.00	5.24	3.86	4.00	0.023
Oocyte fertilization rate	60.64	24.04	60.00	60.31	24.18	61.33	0.990
Number of embryo cleavage	6.05	5.01	4.50	4.10	3.13	3.00	<0.001
Number of embryo transferred at day 3	3.05	1.17	3.00	2.39	1.11	2.00	<0.001
ß-hCG level	78.41	20.18	84.25	2.86	1.05	2.60	<0.001

SD: Standard deviation, ß-hCG: Beta human chorionic gonadotropin, ICSI: Intracytoplasmic sperm injection

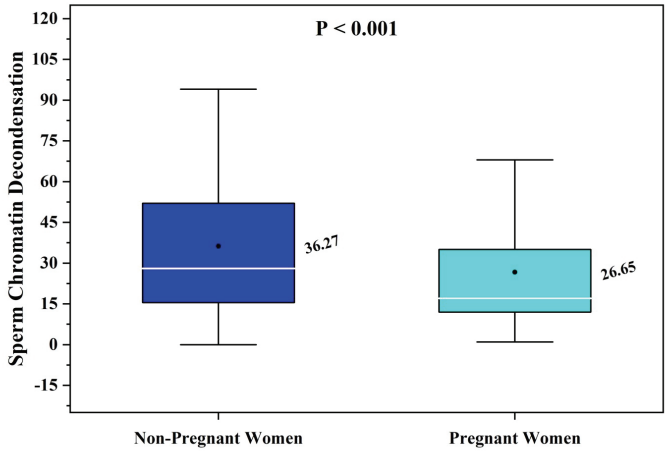

Figure 4. Sperm chromatin decondensation in male partners of pregnant women compared to the partners of non-pregnant women

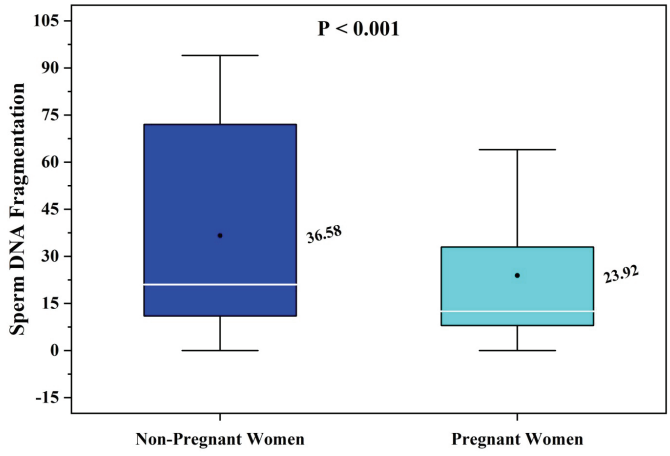

Figure 5. Sperm DNA fragmentation in male partners of pregnant women compared to the partners of non-pregnant women

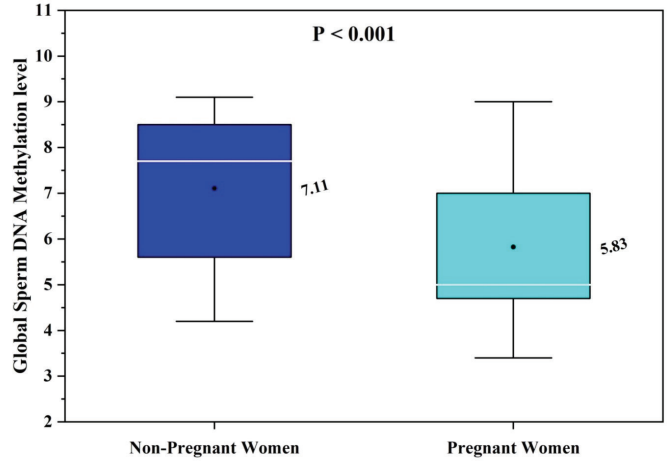

Figure 6. Global spermatozoa DNA methylation level in male partners of pregnant women compared to the partners of non-pregnant women

Table 4. Correlation between the paternal age and clinical parameters of the pregnant women (n=106)

Clinical parameters	r	p
Number of collected oocytes	-0.035	0.724
Number of mature oocytes	0.069	0.484
Number of fertilize oocytes	0.066	0.503
Oocyte fertilization rate	0.179	0.066
Number of embryo cleavage	0.041	0.679
Number of embryos transferred at day-3	-0.109	0.266
ß-hCG level	-0.176	0.071
Sperm chromatin decondensation (CMA3-positive)	0.309	<0.001
Sperm DNA fragmentation (TUNEL-positive)	0.244	0.01
Global sperm DNA methylation level (ng/μL)	0.269	0.01

Spearman rank correlation coefficient, r: Correlation coefficient, ß-hCG: Beta human chorionic gonadotropin

levels in spermatozoa obtained from men after 9-21 years from the first samples and an association between age and 5-methylcytosine in sperm (2). It has been suggested that increased methylation in the sperm of old males decreases the developmental potential of the resulting embryos, contributing to age-related fertility problems (60).

Recently, Jenkins et al. investigated DNA methylation in the sperm of 17 men collected 9-19 years apart and found 139 regions that became significantly hypomethylated and 8 others which became hypermethylated with increasing paternal age. Twenty-one of these sperm differentially methylated regions (DMRs) were confirmed by bisulfite sequencing (61). Another study used a genome-wide DNA methylation screen to compare sperm from young and old and revealed a significant loss of methylation in samples from older men of regions associated with transcriptional regulation (62).

The results of sperm DNA fragmentation and chromatin decondensation are in line with previous studies that found an increase in sperm DNA fragmentation in the age group ≥45 years compared to men <30 years old (1,63,64). However, other studies did not find the same variation with age (65,66). Based on the results of this study, the fragmentation of sperm DNA started to accelerate in men at age 41 years and older and this finding is in agreement with a previous study that found the acceleration point of sperm DNA fragmentation occurs at age 41.6 years (58). On the other hand, the results of DNA methylation disagree with previous studies that reported that male age is associated with a loss of sperm methylation at loci of key development genes (6,67). The difference in the findings of these studies might be due to the different techniques that were used during the evaluation of sperm DNA fragmentation or global DNA methylation, differences in the study population, inclusion criteria for participants, and sample processing.

This study showed a significant variation among the different age groups in the oocyte fertilization rate, the number of embryos transferred, and the level of β-hCG. Similar studies support these findings and suggest that paternal age impacts birth success rates (68), and leads to a reduction in the pregnancy rate from 12.3% in males aged <30 years to 9.3% in males ≥45 years old (13,69,70). A significant negative association has been found between most semen parameters, the level of ß-hCG, and paternal age. Such findings are in keeping with previous studies that showed that the reduction in sperm morphology, sperm motility, and sperm count are associated with increasing paternal age (49,52,71). Previous studies reported a negative association between sperm progressive motility and sperm normal morphology (72-74), assisted pregnancy rate (68), and paternal advancing age. In contrast, a significant positive association was observed between sperm chromatin decondensation, sperm DNA fragmentation, global DNA methylation level and paternal age. These positive correlations are in keeping with the results of earlier studies (64,75). The results of the present study support the findings of a previous study that found a strong correlation between an increase in sperm DNA methylation and advancing paternal age (76). However, the results of sperm DNA fragmentation do not match with the previous studies that found a significant negative correlation between the proportion of CMA positivity, and paternal age (76,77).

Conclusion

This study found that advancing paternal age increased the level of global DNA methylation, DNA fragmentation, and chromatin decondensation in human spermatozoa. Additionally, negative associations were identified between advancing paternal age and basic semen parameters. All of these findings may negatively affect ICSI outcomes and success rates in couples undergoing ICSI cycles.

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Ethics Committee Approval: *This study was approved by the Health Research Council, Palestinian Territories (approval number: 03/10, date: 23.03.2010), and consent was provided in accordance with the Declaration of the Helsinki Committee.*

Informed Consent: *All participants gave written informed consent to participate in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Data Collection or Processing: L.L.M., Y.M.M.; Analysis or Interpretation: L.M.M., Y.M.M.; Literature Search: Writing: L.L.M., Y.M.M.*

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Long-term outcomes of fetal posterior fossa abnormalities diagnosed with fetal magnetic resonance imaging

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Abstract

Objective: The diagnosis of posterior fossa abnormalities (PFA) in the intrauterine period and association with pregnancy outcomes are still controversial. PFA is generally referred to maternal-fetal medicine specialists. The primary purpose of PFA diagnosis is to screen for other accompanying abnormalities, provide prognostic information to families, and discuss the termination option.

Material and Methods: This retrospective study was conducted in patients diagnosed with PFA between January 2013 and September 2020 in a tertiary perinatology clinic. All patients underwent routine second-trimester ultrasound screening and definitive diagnosis was made by fetal magnetic resonance imaging (MRI) in the presence of a suspected anomaly.

Results: There were 164 fetal MRIs for fetal abnormalities during the study period and 22 (13.4%) were diagnosed with a PFA on fetal MRI. Indications for fetal MRI included four (18%) with Mega Cisterna Magna, two (9.1%) with rhomboencephalosynapsis, and thirteen (59.1%) with Vermian Hypoplasia-Dandy-Walker variant. Two patients, with neural tube defects and lumbosacral neural-tube defect are still alive. However, iniencephaly was detected in last patient who died in the postnatal period.

Conclusion: Diagnosis of PFA abnormalities is complex, and the prognosis in PFA is often unclear. The prognosis is not affected by maternal and fetal factors and allows the recognition of additional accompanying abnormalities. Fetal MRI is an imaging method that can provide retrospective examination and research, especially in pregnancies with poor prognoses. (J Turk Ger Gynecol Assoc 2023; 24: 28-32)

Keywords: Antenatal ultrasound, Dandy-Walker, fetal anomaly, fetal MRI, obstetric ultrasound, posterior fossa abnormalities

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Introduction

The cerebellum is the center for motor coordination, execution, and higher cognitive functions (1,2). In addition, the cerebellum may be associated with impaired spatial navigation and musical learning ability, as well as acting as a modulator for the motor, sensory, cognitive, emotional, and autonomic areas (1,3). Therefore, the effect of cerebellar anomaly on child development should be investigated.

The diagnosis of posterior fossa abnormalities (PFA) in the intrauterine period and the association with pregnancy outcomes are still controversial. PFA is usually referred to a maternal-fetal medicine specialist. The incidence of PFA is approximately 1:5,000 in live births (4). PFA is generally divided into two groups - primary and secondary. Primary PFA is further subdivided into Dandy-Walker Continuum (DWC) and other abnormalities of the cerebellar hemispheres (5).

The cerebellum has specific stages of development, so its development is different from other brain regions (6). Basic ultra-sonographic (USG) evaluation of PFA is usually performed in the second trimester transabdominally using a transverse

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view of the fetal head at the level of the trans cerebellar axial plane. Typically, measurements of the anteroposterior diameters of the cisterna magna are used for the evaluation of the posterior fossa (7). Recently, the mid-sagittal cranial plane has been highlighted as an essential diagnostic step in antenatal imaging of PFA, emphasizing that the best assessment of PFA can be achieved by analysis of a combination of mid-sagittal and axial sections (7).

Abnormal PF size is an essential sign in the diagnostic triage of fetuses with central nervous system malformations. It is enlarged in DWC and narrowed in Chiari II malformation. The sagittal expansion of the PF is usually associated with an elevated position of the tentorium and its occipital insertion into the torcular herophili region. In PFA, fetal magnetic resonance imaging (MRI) may be used when the diagnosis cannot be made by USG examination, or to confirm the diagnosis. It has been reported that potential USG diagnoses can change by 70% after fetal MRI (8).

There are limited publications in the literature on long-term postnatal outcomes of patients diagnosed with PFA by fetal MRI. The primary purpose of this article was to analyze the neurological outcomes of children diagnosed with PFA prenatally using fetal MRI.

Material and Methods

This retrospective study was conducted with patients diagnosed with PFA between January 2013 and September 2020 at the Perinatology Clinic of Ankara University Faculty of Medicine, Department of Obstetrics and Gynecology. All patients underwent routine second-trimester USG screening, and the definitive diagnosis was made by fetal MRI (Philips Ingenia 1.5 Tesla), in the presence of a suspected anomaly. In addition, patients diagnosed with any anomaly in another center and referred were also included in the study. USG examination of all patients was performed with Voluson 8 (GE Healthcare Ultrasound, Milwaukee, WI, USA), using a 4-8 Mhz convex transabdominal transducer, and a 5-9 Mhz transvaginal transducer. The records of patients who underwent postnatal MRI examination were also reviewed.

Demographic data of patients diagnosed with PFA were analyzed from hospital records, and an experienced perinatologist reviewed USG images. The images were compared with fetal MRI recordings. Amniocentesis was performed in mothers who accepted amniocentesis. When an abnormality was detected, information about the prognosis was given by the perinatology specialist. Termination of pregnancy (TOP) was performed according to parental choice. In Turkey, there is no upper limit for gestational week for TOP in the presence of severe abnormalities. Follow-up examinations were performed biweekly for up to 38 weeks. Some patients who did not undergo termination underwent MRI or computed tomography after birth to confirm the diagnosis. US results obtained at the time of diagnosis and accompanying abnormalities encountered during follow-up were recorded. Delivery type and week, postnatal neurological developmental results of the fetuses were recorded. Detailed neurological and developmental examination could not be performed because surviving babies were often resident far from the study center. Information about vision, speech and motor function of the patients was obtained from the parents by telephone. Mechanical ventilator dependence was investigated.

This study was conducted following approval by Ankara University Cebeci Hospital Clinical Research Ethics Committee (approval number: İ06-355-22) and written informed consent was obtained from all participants.

Statistical analysis

IBM SPSS, version 26.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation of research data. A descriptive analysis of the records of the patients was made and a table was created. Categorical variables were calculated as frequency and percentage. Continuous perinatal data were summarized using the median, mean, and standard deviation, and categorical factors were summarized using proportions.

Results

Between 2013 and 2020, 164 fetal MRIs were performed for fetal abnormalities, and 22 (13.4%) fetuses were diagnosed with a PFA on fetal MRI. The mean age of the patients was 23.68 (21-43) weeks. The mean gestational age was 26.34 (19-36) weeks during MR imaging. In the termination group, the mean gestational week was 26.27 (23-27). In the non-termination group, the mean gestational age at birth was 36.97 (35-40) weeks, and the mean birth weight was 2911.76 (425-4,100) grams. The ages of the surviving participants ranged from 1 to 8 years. The demographics of the patients are given in Table 1.

In terms of indications for fetal MRI, four (18%) were diagnosed with Mega Cisterna Magna (MSM), and the neurological examination of all patients was within normal limits. Two (9.1%) patients were diagnosed with rhomboencephalosynapsis; the first patient had preterm labor, and subsequently, the fetus died at 24 weeks, and the second patient had additional esophageal atresia, and her/his neurological examination is now within normal limits. Fourteen (63.6%) were diagnosed with DWC, and four of them were terminated. One pregnant woman presented with abruptio placentae and USG revealed that the fetus had no cardiac activity. Two (9.1%) died in the postnatal period, and both had additional abnormalities. One had a double out right ventricle, and the other had interrupted aorta and diastematomyelia. There were six

(27.3%) surviving infants and two of them had additional abnormalities besides neurological abnormalities. One had cerebellar hypoplasia, and the baby had speech delay and balance problems. The other had ventriculomegaly (31 mm) detected in the intrauterine period and currently has cerebral palsy. Neurological examination of four patients with isolated PFA was within normal limits. Two patients with neural tube defects and lumbosacral neural-tube defect are still alive. However, iniencephaly was detected in the last patient who died in the postnatal period. Abnormalities of fetus and long-term results of survivors are shown in Table 2.

Table 1. Demographic characteristics of patients

Demographic data	
Maternal age (years) [mean (minimum-maximum)]	23.68 (21-43)
Pregnancy age at MRI (years) [mean (minimum-maximum)]	26.34 (19-36)
Maternal additional disease	2 hypothyroidism, 1 GDM
Multiple gestations (n)	3
ART (n)	2
Birth age (weeks) [mean (minimum-maximum)]	26.27 (23-27)
Birth age (weeks, except terminations) [mean (minimum-maximum)]	36.97 (35-40)
Birth weight (grams, except terminations) [mean (minimum-maximum)]	2911.76 (425-4100)
The age range of children (years)	1-8

Mean (minimum-maximum). ART: Assisted reproduction technology, GDM: Gestational diabetes mellitus, n: Number, MRI: Magnetic resonance imaging

Discussion

Fetal MRI is generally preferred in patients whose definitive diagnosis cannot be made after second-trimester USG, and it reveals additional undetected findings when compared with USG at a rate of 60% (8). In this study, fetal MRI was performed between 26 and 34 weeks of gestation, in line with the literature. One of the main results is that if fetal PFA is isolated, the neurologic examination is usually within normal limits. If there is a fetal PFA, additional abnormalities should be investigated with detailed USG and, if necessary, MRI. Four patients had isolated DWC, and neurological examination findings were within normal limits in all of them. The image of the patient diagnosed with the DWC is shown in Figure 1. The mortality rate was 100% in all with additional findings. In the study of Scarlet et al. (8), the live birth rate was 87% in the group with isolated PFA, while the live birth rate was only 52% in the non-isolated group. Four of our eight patients with DWC had postnatal results available for follow up while in the other four, all had died with most being terminated without autopsy while one was intra-uterine exitus with an extra anomaly. Has et al. (9) detected DWC in 14 patients in a series of 78 patients and three of them had additional cardiac abnormalities. In our series, two patients had congenital heart abnormalities, and both patients died after cardiovascular surgery in the postnatal period. Therefore, it appears that cardiac abnormalities have a significant determinant role in neonatal prognosis.

Tablo 2. Clinical outcome of posterior fossa abnormalities

	Diagnosis	**Number of patients**	**Pregnancy outcome**	**Current status**	**Neurological outcome**	**Additional anomaly**
Primer posterior fossa abnormalities	MCM	4	Live birth	Living	Normal	None
	Rhomboencephalosynapsis	2	Live birth	Living	Normal	Esophageal atresia
			Preterm live birth	Postpartum exitus		Hydrocephalus
	DWC	14	9: Live birth	7 Living	3 Abnormal	1- Hydrocephalus 2- Cerebellar hypoplasia
					4 Normal	
				2 Postpartum exitus		1- DORV 2- Diastometamyelia + interrupted aorta
			4: TOP 1: IUEX			
Seconder posterior fossa abnormalities	NTD	2	Live birth	Arnold-Chiari malformation	Normal	
			Iniencephaly postpartum exitus			

DWM: Dandy-Walker Continuum, MCM: Mega cisterna magna, NTD: Neural-tube defect, TOP: Termination of pregnancy, IUEX: Intrauterine exitus, DORV: Double outlet right ventricule

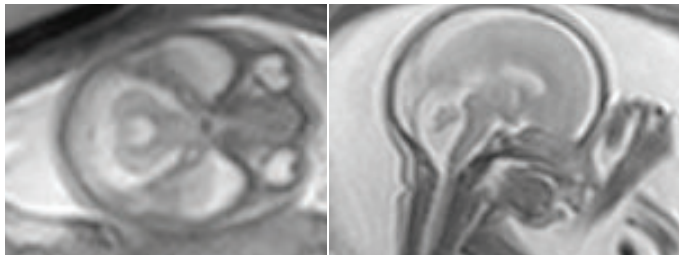

Figure 1. Dandy Walker continuum

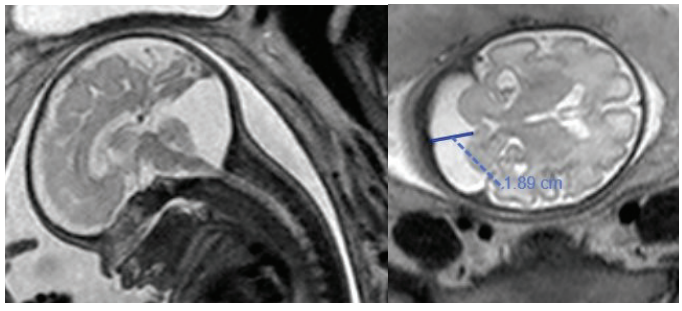

Figure 2. Mega cisterna magna

There are contradictory results in the literature regarding the spectrum of isolated DWC. In the series of six patients by Guibad et al. (10), three patients had normal neurological findings, but one patient also had partial trisomy seven and moderate-to-severe neurological impairment. In our study, four patients had isolated DWC, and all had average neurological results. If the genetic examination results are average in the isolated group, the prognosis is mostly good, and the family should be so informed.

Dror et al. (10) reported that the prognosis was good in patients with isolated MSM in their series of 29 patients, and they found that only the first step period was delayed. Four of our patients were diagnosed with isolated MSM, and all of them had average neurological examination findings. An MRI of a patient diagnosed with isolated MSM is shown in Figure 2. We did not detect the first-step delay in any of our patients with isolated MSM pathology.

Prenatal diagnosis of rhomboencephalosynapsis is rare and generally results in termination, while various degrees of neurological abnormalities were found in surviving infants (11,12). Poretti et al. (13) in a case series of five patients from 2009 reported that two patients had normal neurological development. One of our patients was diagnosed with isolated partial rhomboencephalosynapsis, and no additional neurological anomaly was detected. Prenatal and postnatal MRIs of this patient diagnosed with rhomboencephalosynapsis are shown in Figure 3.

One patient was diagnosed with unilateral cerebellar hypoplasia (UCH) and had a balance problem in the postnatal period. The prenatal and postnatal view of the patient with UCH is shown in Figure 4. Massoud et al. (14) reported that neurological prognosis

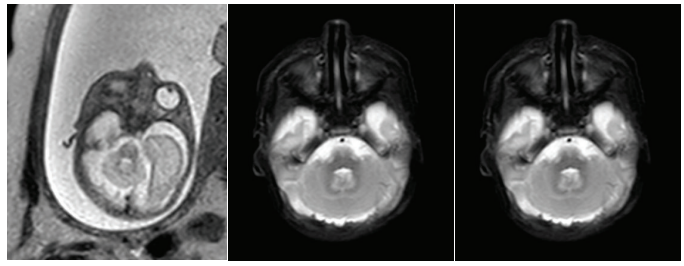

Figure 3. Rhomboencephalosynapsis

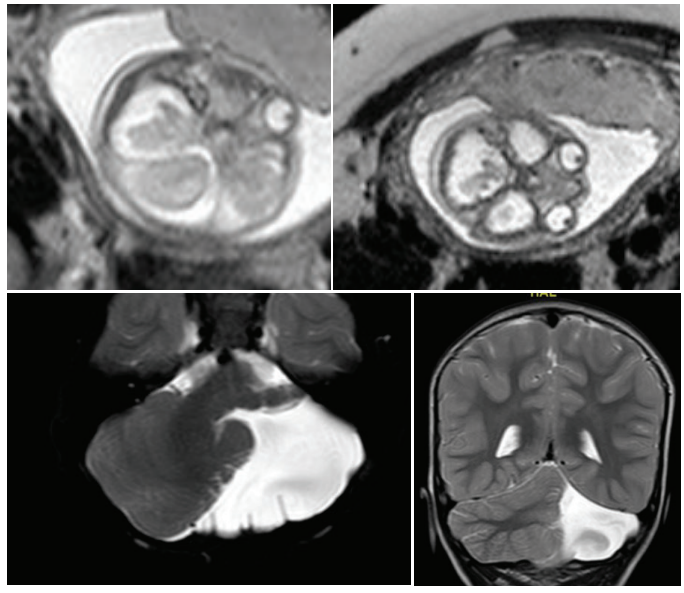

Figure 4. Unilateral cerebellar hypoplasia

is good if UCH is not associated with PFA, hemangioma, arterial abnormalities, cardiac abnormalities/aortic coarctation, eye abnormalities syndrome (PHACE syndrome), or an infection. The accompanying balance problem in our patient may be due to Vermian hypoplasia.

Diagnosis of PFA abnormalities is complex, and patient prognosis is unclear. The prognosis is not affected by maternal and fetal factors. Fetal MRI allows the recognition of additional accompanying abnormalities. Except for one case, fetal MRI findings were similar to fetal USG findings, in which one patient was diagnosed with DWC anomaly on USG, but fetal MR indicated UCH. Fetal MRI is an imaging method that can provide retrospective examination and research, especially in pregnancies with poor prognoses.

Conclusion

This study reports the long-term results of fetal PFA and we hope that these will be of interest to experts working in this field, given the limited data available. Our findings suggest that the prognosis of the child will be good if there is isolated MCM on fetal MRI and there is no extra anomaly. However, there may be extra anomalies that can only be detected in the postpartum

period. Therefore, we believe that USG and fetal MRI should be performed together for long-term outcome prediction in fetuses diagnosed with fetal PFA.

Ethics Committee Approval: *This study was conducted following approval by Ankara University Cebeci Hospital Clinical Research Ethics Committee (approval number: İ06-355-22).*

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

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The course of infection with the Delta variant of COVID-19 in pregnancy: analysis of clinical, laboratory, and neonatal outcomes

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Abstract

Objective: This study aimed to examine the effects of infection with the Delta variant of coronavirus disease-2019 (COVID-19) on the clinical course, laboratory parameters, and neonatal outcome in pregnant women.

Material and Methods: A total of 96 pregnant women who tested positive for the Delta variant of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) causing COVID-19 were retrospectively examined. The pregnant women were divided into three groups: Asymptomatic; non-severe; and severe. Age, obstetric history, symptoms and findings, blood tests, medication and vaccination history, clinical course, and perinatal outcome of pregnant women were analyzed.

Results: Pregnant women who tested positive for the Delta variant of SARS-CoV-2 had an intensive care unit (ICU) admission rate of 9.4% and a mortality rate of 5.2%. Pregnant women in the severe disease group had significantly higher rates of preterm birth and cesarean section compared with the non-severe and asymptomatic group. Pregnant women in the severe group had high C-reactive protein (CRP) levels at the time of admission. White blood cell count (WBC) and procalcitonin levels were increased in clinical follow-up in women in the severe group.

Conclusion: The Delta variant of SARS-CoV-2 was found to increase mortality rates in pregnant women compared to pre-Delta variants of COVID-19. In pregnant women infected with the Delta variant, advanced gestational age at diagnosis, high CRP, WBC, and procalcitonin levels were significantly correlated with poor prognosis. Pregnant women infected with the Delta variant and with severe COVID-19 had an increased risk for preterm delivery and cesarean section. Although newborns of women with severe disease were found to have significantly higher rates of ICU admission, there was no significant difference in neonatal mortality rates. We recommend close monitoring of CRP, WBC, and procalcitonin levels, in addition to symptoms, in pregnant women infected with the Delta variant of SARS-CoV-2 and diagnosed in the third trimester.

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Keywords: COVID-19, pregnancy, delta variant

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Introduction

Coronavirus disease-2019 (COVID-19) is a highly contagious infection caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and has so far resulted in nearly 300 million cases of COVID-19 and more than 5 million deaths worldwide (1,2). First detected in Wuhan, the capital of China's Hubei province in December 2019, COVID-19 has since spread rapidly worldwide and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (2). SARS-CoV-2 is an mRNA virus that acts mainly by binding to the

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angiotensin-converting enzyme 2 receptor, abundantly found in the respiratory and digestive tract and widely expressed during pregnancy (3,4). Similar to other RNA viruses, SARS-CoV-2 has genetically mutated over time, resulting in variants with different characteristics (5). WHO has classified SARS-CoV-2 variants into three groups based on the impact on public health, variation in transmissibility and virulence, and resistance to therapeutics and vaccines: variants of concern (VOCs), variants of interest (VOIs), and variants under monitoring. VOCs are the dominant group in the outbreak and comprise Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1,617.2) variants and Omicron (B.1.1.529) variant, which was added to the list on December 6, 2021. The Delta variant, first isolated in India in October 2020, was classified as a VOI on April 4, 2021, and as a VOC on May 11, 2021, due to its rapid spread worldwide (6). Data for November 2021 shows that the Delta variant has become the most common VOC, accounting for 99.7% of the variants isolated worldwide over the last 60 days (7).

Pregnancy comprises a unique immunological condition that is regulated by immune system mediating signals originating from the placenta (8-12). Pregnant women are at greater risk for viral infections than the normal population owing to the respiratory, circulatory, endocrine, immunological, and anatomical changes that occur during pregnancy (3,13,14). Pregnant women reportedly have higher rates of hospital admissions, pneumonia, requirement for ventilator support, and intensive care unit (ICU) admissions associated with COVID-19 than the non-pregnant population (15). Studies conducted with different subsets of population to compare the wild type (Wuhan) and the Alpha variant reported that the Delta variant had a higher virulence and was less affected by neutralizing antibodies induced by vaccination than other variants (16-18). However, few studies in the literature have examined the effect of this variant on the pregnant population. Furthermore, it is crucial to investigate the effects of the Delta variant on pregnant women, given that they are more severely affected by COVID-19 compared to the general population and have low vaccination rates worldwide (19). Therefore, the aim of the present study was to investigate the effect of the Delta variant of SARS-Cov-2 on the clinical course, laboratory parameters and neonatal outcome in pregnant women.

Material and Methods

The present study retrospectively assessed 96 pregnant women admitted to the University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital, Clinic of Obstetrics and Gynecology after testing positive for the Delta variant of SARS-CoV-2 following a polymerase chain reaction (PCR) examination between April 2021 and September 2021. The study was approved by the Ethics Committee of the University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital and conducted in accordance with the Helsinki Declaration of Ethical Principles (approval number: 917, date: 05.11.2021). Data of the patients included in the study were retrieved from the hospital archiving system and medical records. In PCR tests, the SL452R mutation, specific to the Delta variant, was detected using SARS-CoV-2 Emerging Plus kits (Bio-Speedy, İstanbul, Turkey). COVID-19 diagnosis was established through physical examinations, PCR tests from nasopharyngeal swabs, and X-ray and/or computed tomography (CT) to assess lung involvement in the presence of pneumonia. It was explained to pregnant women that chest CT can be performed safely in pregnancy, and after providing signed consent, symptomatic pregnant women underwent CT examinations (20). During the CT scan, the lower abdomen and pelvis of the pregnant woman were wrapped in protective aprons.

Among the 96 pregnant women, 49 were asymptomatic and 47 were symptomatic. Women were classified as asymptomatic or symptomatic. Asymptomatic pregnant women were admitted with obstetric indications. Symptomatic pregnant women were further divided into non-severe, and severe disease groups (21,22). Pregnant women with mild symptoms (fever, cough, and other upper respiratory symptoms), without abnormalities or with mild changes on chest CT (multiple areas of patch-like involvement and interstitial changes usually found in the outer zone of the lung and beneath the pleura) were included in the non-severe group. Patients were included in the severe group if they had at least one of the following: tachypnea (respiratory rate ≥30/minimum); hypoxia (SpO_2 <93%); the partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) ≤300 mmHg on blood gas analysis; respiratory or organ failure that required admission to the ICU; or the presence of shock.

There is no agreed and definitive treatment protocol for pregnant women with COVID-19. Low-molecular-weight heparin (LMWH) was used for thromboembolism prophylaxis. Steroids were used for a limited time (3-5 days) in pregnant women who demonstrated progressive deterioration of oxygen saturation, increased activation of a pro-inflammatory response, and rapid worsening of findings on chest imaging (23). Betamethasone was given to promote fetal lung maturation at or beyond 24 weeks of gestation in those who were at risk of preterm birth within 7 days. Antitussive agents and inhaled bronchodilators were used for supportive therapy. Also, patients were started on tocilizumab in the case of cytokine storm syndrome and broad-spectrum antibiotic therapy in the presence of concomitant suspected bacterial pneumonia (21,24).

Data items analyzed included age, obstetric history, symptoms and findings, blood tests, medication and vaccination history, clinical course, and perinatal outcomes. Postpartum hemorrhage was defined as blood loss of more than 1000

mL following cesarean delivery (25). IUGR was defined as estimated fetal weight <3rd centile based on sonographic measurements of the fetus along with end-diastolic flow loss on Doppler examination (26). Blood tests were reported at three different time points to better predict the course of the disease; at the time of admission, during the hospital stay, and at discharge. The tests administered during the hospital stay in symptomatic pregnant patients, that is, at the second time point, (excluding 5 pregnant women who died) were taken at the time of clinical worsening (such as the onset of oxygen therapy or the introduction of steroid therapy for pregnant women who were already on oxygen therapy). In asymptomatic pregnant women, tests administered during the hospital stay were used. In the newborns, birth weight, Apgar scores, umbilical cord blood gas analysis, neonatal intensive care unit (NICU) admission, and mortality rates were analyzed.

Statistical analysis

SPSS, version 20.0, was used to evaluate the data collected during the study (IBM SPSS, Armonk, NY, USA). Statistical significance was set at p-value <0.05. Based on the Shapiro-Wilk normality test results, multiple groups of continuous variables were compared with the non-parametric Kruskal-Wallis analysis test, and in case of significant differences, Dunn's multiple comparisons test was used to identify the groups that created the difference. As for descriptive statistics, letter indices were placed on grouped data for the results tabled in median values (minimum-maximum), and the differences between the groups were displayed. The chi-square and Fisher's exact test results were used to evaluate the distribution of categorical variables based on groups, and the results were expressed in frequency distributions and percentages. The repeated measures ANOVA model was used to explain the measurements taken at three different time points for the three independent groups, investigating also the main effect groups, and time as well as interaction terms.

Results

A total of 48.9% patients (n=47) were symptomatic pregnant women, of whom 72% (n=34) were categorized as non-severe and 28% (n=13) severe patients. There was no significant difference between the three groups in terms of mean age, gestational age, gravidity, parity, and abortus history. A significant majority of the symptomatic group consisted of pregnant women in the third trimester. There was no significant difference between symptomatic pregnant women in the non-severe and severe groups in terms of frequency of fever, cough, shortness of breath, diarrhea, and myalgia. The severe disease group had a significantly higher average heart rate and significantly lower average oxygen saturation than the non-severe disease group. A diagnosis of pneumonia based on physical examination findings was made in 91.1% of the pregnant women in the non-severe group and 100% of those in the severe group, and all of the symptomatic pregnant women underwent a CT scan of the chest. Radiological findings of pneumonia were detected in 50% of those in the non-severe group and 100% of those in the severe group. Steroid and LMWH use were significantly higher in the symptomatic group compared to the asymptomatic group. Furthermore, 19% of the symptomatic pregnant women (n=9) required ICU admission. Although all non-severe pregnant women were monitored in the clinic, 69% (n=9) of the pregnant women in the severe group were admitted to the ICU. Of the pregnant women admitted to the ICU, 55.5% (n=5) developed acute respiratory distress syndrome, and 33.3% (n=3) developed acute renal failure. A total of 55.5% (n=5) of the pregnant women in the ICU were intubated and all of the intubated patients died. The mortality rate in pregnant women was 5.2%. Of all the pregnant women who participated in the study, 46% (n=44) delivered their babies. The delivery rate in the severe disease group was 53.8%, and all deliveries were cesarean preterm deliveries owing to maternal conditions. Preterm delivery and cesarean section rates were significantly higher in the severe group compared to the non-severe and asymptomatic group (Table 1).

The average gestational age and weight at birth, and APGAR scores at 1 and 5 minutes were significantly lower in the newborns of the severe group than those of the non-severe and asymptomatic groups. The NICU admission rate was calculated at 20.5% for all the groups combined. There was no significant difference in NICU admission rates between the non-severe and asymptomatic groups but this rate was significantly higher in the severe group. There was no significant difference in umbilical cord blood gas pH values of newborns across the three groups. There were no neonatal deaths in this study (Table 2).

Blood samples collected from each patient at three different time points were evaluated to better predict the clinical course of the disease. There was no significant intergroup difference in the mean counts of white blood cell (WBC), neutrophils, platelets or lymphocytes, or in levels of hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, ferritin, and procalcitonin. WBC counts increased over time (p^2=0.002) in the severe group, and the rate of increase in this group was significantly higher than that in the non-severe and asymptomatic groups (p^3=0.001) (Graphic 1). Although the mean C-reactive protein (CRP) level was significantly higher in the severe group than in the non-severe and asymptomatic group, there was no significant increase in CRP levels in any of the three groups during clinical

Table 1. Age, obstetric history, symptoms and clinical findings, medication, clinical course, and perinatal outcomes

		Asymptomatic group, (n=49)	Non-severe group, (n=34)	Severe group, (n=13)	p-value
		n (%) or median (minimum-maximum)	n (%) or median (minimum-maximum)	n (%) or median (minimum-maximum)	
Age, years		29.0 (18.0-43.0)	29.5 (17.0-40.0)	29.0 (22.0-41.0)	0.708
Trimester	1st trimester	14 (28.5)[a]	2 (5.8)[a]	0 (0.0)[a]	0.025
	2nd trimester	5 (10.20)[a]	7 (20.5)[b]	2 (15.3)[b]	
	3rd trimester	30 (61.2)	25 (73.5)[b]	11 (84.6)[b]	
Gestational age, weeks		34.0 (8.0-40.0)	35.0 (7.0-39.0)	31.0 (27.0-36.0)	0.458
Obstetric history	Gravidity	2.0 (1.0-7.0)	3.0 (1.0-6.0)	3.0 (1.0-12.0)	0.145
	Parity	1.0 (0.0-5.0)	1.05 (0.0-5.0)	2.0 (0.0-9.0)	0.170
	Previous abortion	0.0 (0.0-3.0)	0.0 (0.0-2.0)	0.0 (0.0-3.0)	0.888
Symptoms and clinical findings					
Body mass index, kg/m^2		25.0 (20.4-29.8)	26.4 (21.2-29.9)	27.2 (22.5-30.4)	0.074
Smoking		7 (14.2)	3 (8.8)	1 (7.6)	0.748*
Systolic blood pressure, mmHg		100.0 (90.0-50.0)	110.0 (90.0-140.0)	100.0 (90.0-160.0)	0.865
Diastolic blood pressure, mmHg		60.0 (60.0-90.0)	60.0 (60.0-90.0)	60.0 (50.0-90.0)	0.812
Pulse, beats per minute		89.0 (70.0-121.0)[a]	96.0 (75.0-124.0)[b]	108.0 (93.0-125.0)[c]	<0.001
Saturation, (%)		98.0 (90.0-99.0)[a]	97.0 (88.0-99.0)[b]	93.0 (85.0-98.0)[c]	0.003
Fever		36.4 (36.1-37.0)	36.4 (36.2-37.1)	36.4 (36.1-36.9)	0.706
Cough		0 (0.0)[a]	32 (94.1)[b]	12 (92.3)[b]	<0.001
Shortness of breath		0 (0.0)[a]	28 (82.3)[b]	13 (100.0)[b]	<0.001
Diarrhea		0 (0.0)	0 (0.0)	0 (0.0)	N/A
Muscle pain		2 (4.9)[a]	26 (63.4)[b]	13 (31.7)[b]	<0.001
Exposed to someone with COVID-19		24 (48.9)[a]	34 (100.0)[b]	13 (100.0)[b]	<0.001
COVID-19 findings in chest CT	Positive	0 (0.0)	17 (50.0)	13 (100.0)	<0.001
	Negative	0 (0.0)	17 (50.0)	0 (0.0)	
	N/A	49 (100.0)	0 (0.0)	0 (0.0)	
Medication and vaccination					
Vaccination		0 (0.0)	0 (0.0)	0 (0.0)	N/A
Antibiotic		26 (53.0)[a]	19 (55.8)[a]	13 (100.0)[b]	0.007
Glucocorticoid		2 (4.0)[a]	33 (97.6)[b]	13 (100.0)[b]	<0.001
Tocilizumab		0 (0.0)[a]	0 (0.0)[a]	2 (15.3)[b]	0.016*
Low molecular weight heparin		34 (69.3)[a]	34 (100.0)[b]	13 (100.0)[b]	<0.001
Inhaler β2 agonists + antitussive syrup		0 (0.0)[a]	32 (94.1)[b]	13 (100.0)[b]	<0.001
Clinical course					
Viral pneumonia		0 (0.0)[a]	31 (91.1)[b]	13 (100.0)[b]	<0.001
Acute respiratory distress syndrome		0 (0.0)[a]	0 (0.0)[a]	5 (38.4)[b]	<0.001*
Renal failure		0 (0.0)[a]	0 (0.0)[a]	3 (23.0)[b]	0.002*
Intensive care unit admission		0 (0.0)[a]	0 (0.0)[a]	9 (69.0)[b]	<0.001*
Intensive care unit length of stay, days		0.0 (0.0-0.0)[a]	0.0 (0.0-0.0)[a]	3.0 (0.0-15.0)[c]	<0.001
Length of hospital stay, days		2.0 (1.0-3.0)[a]	4.0 (2.0-7.0)[b]	7.0 (3.0-22.0)[c]	<0.001
Intubation		0 (0.0)[a]	0 (0.0)[a]	5 (38.4)[b]	<0.001*
Maternal death		0 (0.0)[a]	0 (0.0)[a]	5 (38.4)[b]	<0.001*

Obstetrical findings					
Spontaneous abortion		5 (10.2)[a]	0 (0.0)[b]	0 (0.0)[b]	<0.001*
Threatened abortion		2 (4.0)	0 (0.0)	0 (0.0)	0.375
Hyperemesis gravidarum		9 (18.3)[a]	1 (2.9)[b]	0 (0.0)[b]	0.035*
Premature rupture of membranes		2 (4.0)	1 (2.9)	0 (0.0)	1.000
Preterm delivery		6 (12.2)[a]	3 (8.8)[a]	7 (53.8)[b]	0.002
Fetal distress		4 (8.1)	3 (8.8)	1 (7.6)	1.000
Intrauterine fetal demise		2 (4.0)	0 (0.0)	1 (7.6)	0.247*
Fetal growth restriction		0 (0.0)	0 (0.0)	0 (0.0)	N/A
Postpartum hemorrhage		0 (0.0)	0 (0.0)	0 (0.0)	N/A
Mode of delivery	Vaginal	18 (36.7)[a]	4 (11.7)[a]	0 (0.0)[a]	0.005*
	Cesarean section	7 (14.2)[a]	8 (23.5)[a]	7 (53.8)[b]	
	Not delivered	24 (49.9)[b]	22 (64.7)[a]	6 (46.1)[a,b]	
Indication for cesarean section	Maternal	0 (0.0)	0 (0.0)	6 (85.7)	0.002*
	Fetal	7 (100.0)[a]	8 (100.0)[a]	1 (14.3)[b]	

[a, b, c]: For medians expressed with indices such as a, b, and c; different indices indicate statistical difference. *: Fisher's exact p-value. The table prepared for group comparisons above shows p-values found to be significant in bold. For medians expressed by indices such as a, b, and c; statistically different ones have been marked with different letters, and those without statistical difference with the same letters. Likewise, where more than 20% of cells have an expected frequency of less than 5, the symbol "*" has been used for cross-tabulation (contingency tables) chi-square analysis, and Fisher's exact test p-value has been given instead of the chi-square p-value. COVID-19: Coronavirus disease-2019, CT: Computed tomography

Table 2. Neonatal outcomes

		Asymptomatic group, (n=25)	**Non-severe group, (n=12)**	**Severe group, (n=7)**	**p-value**
		n (%) or median (minimum-maximum)	**n (%) or median (minimum-maximum)**	**n (%) or median (minimum-maximum)**	
Gestational age at birth, weeks		37.0 (25.0-40.0)[a]	38.0 (35.0-39.0)[a]	32.0 (29.0-36.0)[b]	**0.035**
Birth weight, g		3000 (690-4200)[a]	3075 (1900-3550)[a]	1900 (1550-2900)[b]	**0.013**
Fetal gender	Male	12 (48.0)	9 (75.0)	4 (57.1)	0.367*
	Female	13 (52.0)	3 (25.0)	3 (42.9)	
1-min Apgar score		8.0 (0.0-8.0)[a]	8.0 (4.0-8.0)[a]	7.0 (4.0-8.0)[b]	**0.008**
5-minute Apgar score		9.0 (0.0-9.0)[a]	9.0 (6.0-9.0)[a]	8.0 (7.0-9.0)[b]	**0.011**
Umbilical cord gas analysis	pH	7.31 (7.18-7.37)	7.30 (7.12-7.42)	7.31 (7.24-7.37)	0.935
	Base deficit	1.45 (14.3-0.4)	8.9 (12-1.0)	2.4 (4.5-1.0)	0.253
	Lactate, mmol/L	2.70 (2.40-875)	3.3 (1.7-8.0)	2.4 (2.02-4.08)	0.669
Intensive care unit admission		1 (4.0)[a]	3 (25.0)[a]	5 (71.4)[b]	**<0.001***
Intensive care unit length of stay, days		0.0 (0.0-4.0)[a]	0.0 (0.0-8.0)[a]	8.0 (0.0-12.0)[b]	**<0.001**
Neonatal death		0 (0.0)	0 (0.0)	0 (0.0)	NA

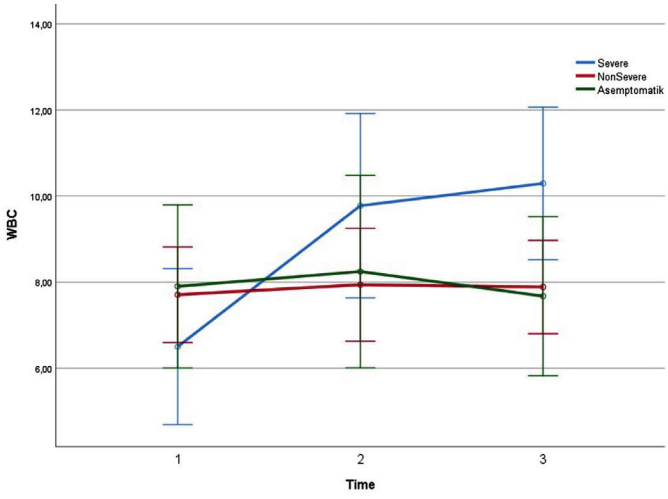
[a,b,c]: For medians expressed with indices such as a, b, and c; different indices indicate statistical difference. *: Fisher's exact p-value

follow-up (Graphic 2). Procalcitonin levels appeared to increase significantly initially in the severe group and remained high on clinical follow-up (Graphic 3) (Table 3).

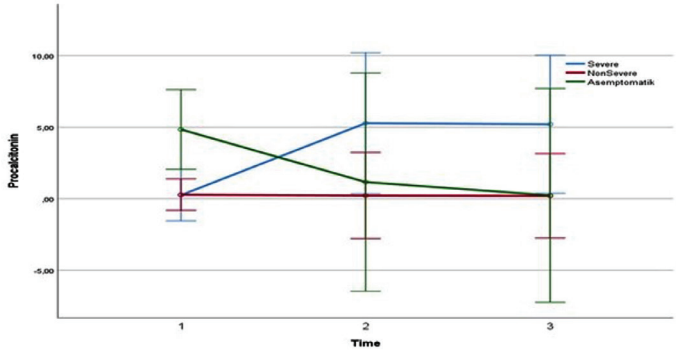
Discussion

This study revealed that a more advanced gestational age at initial diagnosis was associated with a poorer prognosis when pregnancy was complicated by infection with the Delta variant of SARS-CoV-2. A total of 19% of all admissions were ICU admissions, of which 55.5% were intubated, and all intubated patients died. Worsening of the maternal clinical picture was found to cause a significant increase in cesarean delivery, preterm delivery, low birth weight, and NICU admission rates. High CRP values in pregnant women were significantly correlated with severe disease. Increasing WBC and procalcitonin levels during follow-up of pregnant women were significantly associated with poor prognosis. Reportedly, symptomatic disease, severe disease, and ICU admission rates are significantly higher in pregnant women infected with the

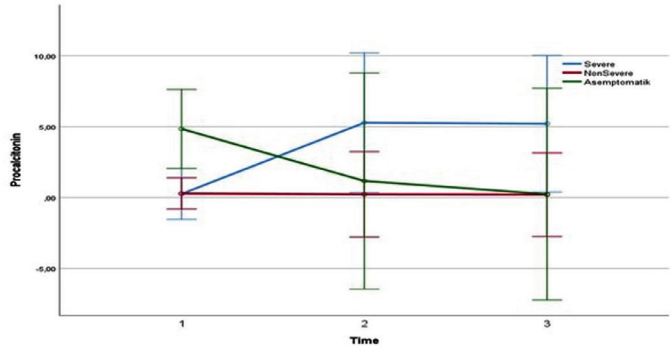
Delta variant of SARS-CoV-2 than in those with pre-Delta variants but there was no significant change in maternal mortality rates (27). Furthermore, 62.3-84% of pregnant women infected with the Delta variant were reportedly symptomatic. Also, 48-79% of symptomatic pregnant women are in the non-severe group and 21-36% were in the severe disease group. Several studies have reported that 4.9-29% of pregnant women infected with the Delta variant were admitted to the ICU, 33.3-80% of those in the ICU are intubated, and the mortality rate was 0-2%. Moreover, advanced gestational age at the time of initial diagnosis has been reported to be significantly associated with poor prognosis (27-29). Our study found that advanced gestational age at the time of initial diagnosis was significantly associated with poor prognosis. ICU admission and intubation rates were similar to those published in the literature. However, our study found a mortality rate of 5.2% in pregnant women infected with the Delta variant. This rate is remarkably high compared to both the literature on pregnant women infected with the Delta variant, and also higher than previous mortality rates found in our clinic in pregnant women infected with the Delta variant (2.99%, n=15/501). Our study found that COVID-19-related deaths in pregnant women increased with the Delta variant. A large number of pregnant women with COVID-19-related severe disease are referred to our tertiary center. This may be another reason why we found increased mortality rates.



Graphic 1. WBC x time relationship
WBC: White blood cell



Graphic 2. CRP x time relationship
CRP: C-reactive protein



Graphic 3. Procalcitonin x time relationship

There is a paucity of information in the literature on symptomatology in pregnant women infected with the Delta variant of SARS-CoV-2. It has been reported that 71% of symptomatic pregnant women infected with this Delta variant have a cough, 44.7% have a fever, 42% have dyspnea, 37% have myalgia-malaise, and 10.5% have diarrhea (28). In pregnant women infected with the Delta variant, lymphocytopenia has been reported at a rate of 27.8%, elevated AST at 11.5%, and elevated ALT at 9.8% (28). No significant difference has been reported among asymptomatic, non-severe, and severe groups of pregnant women infected with the Delta variant in terms of blood test parameters (lymphocyte, leukocyte, blood urea nitrogen, creatinine, AST, and ALT) at the time of admission (27). Our study found significantly higher CRP levels in pregnant women in the severe group than in the non-severe and asymptomatic group. Clinical follow-ups showed that increasing WBC and procalcitonin levels were significant predictors of poor prognosis.

The rate of chest CT findings with an appearance consistent with pneumonia has been reported at 88% in symptomatic pregnant women infected with the Delta variant of SARS-CoV-2 (28). In our study, 64% of the symptomatic pregnant women had findings consistent with pneumonia on their chest CT scans, but this varied from 100% in the severe group to 50% in the non-severe group.

While Wang et al. (28) reported no significant difference between pregnant women infected with the Delta variant and those with pre-Delta variants of SARS-CoV-2 in terms of preterm delivery rates, fetal weight, and adverse neonatal outcomes (NICU admission, hypoxic-ischemic encephalopathy, sepsis, ventilator support, meconium aspiration, birth trauma, stillbirth, etc.), Seasely et al. (27) reported an association between infection with the Delta variant and statistically

Table 3. Variation of blood test parameters over time

Variables	Asymptomatic group, (n=49)	Non-severe group, (n=34)	Severe group, (n=13)	p^1-value	p^2-value	p^3-value
WBC 1, $mm^3 x10^3$	9.03±3.25	7.77±3.6	6.52±1.86	-	-	-
WBC 2, $mm^3 x10^3$	8.25±2.02	7.94±3.49	9.78±5.16	0.584	**0.002**	**0.001**
WBC 3, $mm^3 x10^3$	8.97±3.41	7.82±2.87	10.21±3.98		-	-
Neutrophil 1, $x10^3$/uL	7±3.01	6.14±2.58	5.53±1.76	-	-	-
Neutrophil 2, $x10^3$/uL	6.56±1.81	5.91±3.06	7.93±3.18	0.347	**0.028**	**0.001**
Neutrophil 3, $x10^3$/uL	6.7±3.32	5.8±2.65	8.01±3.66	-	-	-
Lymphocyte 1, $x10^3$/uL	1.45±0.66	1.09±0.46	0.78±0.19	-	-	-
Lymphocyte 2, $x10^3$/uL	1.41±0.7	1.42±0.44	1.16±0.49	0.283	**<0.001**	0.263
Lymphocyte 3, $x10^3$/uL	1.73±0.73	1.61±0.59	1.52±0.69	-	-	-
Hemoglobin 1, g/dL	11.66±1.82	10.75±2.07	11.3±1.18	-	-	-
Hemoglobin 2, g/dL	10.78±1.91	10.43±0.9	10.29±1.16	0.453	**0.007**	0.534
Hemoglobin 3, g/dL	11.09±1.7	10.36±1.08	10.46±0.98	-	-	-
Platelet 1, $mm^3 x10^3$	210.33±70.83	197.38±59.16	178.62±46.69	-	-	-
Platelet 2, $mm^3 x10^3$	197.45±78.6	231.47±66.29	273.75±52.02	0.340	**<0.001**	**<0.001**
Platelet 3, $mm^3 x10^3$	210.8±72.04	253.15±80.7	284.54±65.72	-	-	-
AST 1, U/L	34.41±36.02	71.29±204.97	48.15±19.92	-	-	-
AST 2, U/L	43±15.58	52.52±67.84	38.83±31.96	0.849	0.411	0.849
AST 3, U/L	31.78±15.55	39.68±28.21	35.54±48.1	-	-	-
ALT 1, U/L	25.22±27.21	53.5±100.45	40.38±25.18	-	-	-
ALT 2, U/L	36.5±24.96	47±56.05	44.17±36.76	0.771	0.426	0.882
ALT 3, U/L	24±16.48	43±42.12	31.62±20.79	-	-	-
LDH 1, U/L	267.8±184.4	286.5±153.5	320.3±97.1	-	-	-
LDH 2, U/L	214.7±162.7	268.0±135.8	366.1±242.3	0.100	0.877	0.400
LDH 3, U/L	267.8±130.0	249.03±97.0	394.2±314.8	-	-	-
C-reactive protein 1, mg/dL	24.49±32.46	48.46±38.71	74.05±35.03	-	-	-
C-reactive protein 2, mg/dL	34.71±37.35	33.14±28.94	88.9±75.66	**0.033**	0.804	0.072
C-reactive protein 3, mg/dL	37.89±68.15	27.67±35.93	51.02±67.96	-	-	-
Ferritin 1, ng/mL	84.9±85.6	93.7±104.4	87.8±63.3	-	-	-
Ferritin 2, ng/mL	261.8±210.8	88.1±99.5	151.7±199.9	0.108	**0.001**	**0.007**
Ferritin 3, ng/mL	99.7±99.4	80.0±79.9	67.4±64.4	-	-	-
Procalcitonin 1, ng/mL	0.62±3.41	0.27±0.32	0.25±0.29	-	-	-
Procalcitonin 2, ng/mL	1.16±2.37	0.22±0.38	5.28±17.24	0.224	0.815	0.109
Procalcitonin 3, ng/mL	0.17±0.22	0.2±0.38	4.82±16.29	-	-	-

p^1: P-value for main effect group, p^2: P-value for main effect time, p^3: P-value for interaction term. The repeated measures ANOVA model was used in order to assess the measurements taken at three different time points for the variables of three independent groups. In this analysis, p^1-value shows the difference between groups defined as the main effect, p^2-value is used to evaluate the effect of time, p^3-value illustrates the effect called interaction term, WBC: White blood cell count, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase

increased rates of preterm delivery and NICU admission (27,28). The rate of preterm delivery has been reported at 73% and the rate of NICU admission at 74% in pregnant women infected with the Delta variant (27). Comparisons of neonatal outcomes among pregnant women infected with the Delta variant in asymptomatic, non-severe, and severe groups found no significant difference in terms of preterm delivery and poor neonatal outcomes (28). Our study found no increase in the incidence of preterm delivery complications such as spontaneous abortion, threatened abortion, and hyperemesis gravidarum in symptomatic pregnant women infected with the Delta variant (30-33). However, we found significantly higher cesarean and preterm delivery rates in the severe group compared to the asymptomatic and non-severe groups. We believe that maternal clinical features are the main determinant of preterm delivery and cesarean section rates in symptomatic

pregnant women. Our study found that the newborns of mothers in the severe group had significantly lower average gestational age at birth and birth weight, and significantly higher NICU admission rate compared to the newborns of mothers in the non-severe and asymptomatic group.

Study Limitations

The main limitation of this study is its retrospective design. Despite this, the present study is important due to the scarcity of data in the literature on the effects of the Delta variant of SARS-CoV-2 on pregnancy outcomes. We believe that the current study is strengthened by the inclusion and analysis of blood parameters at three different time points to better predict the course of the disease.

Conclusion

The Delta variant of SARS-CoV-2 leading to COVID-19 was found to result in increased mortality rates in pregnant women compared to pre-Delta variants. In pregnant women infected with the Delta variant, advanced gestational age at diagnosis, elevated CRP, WBC, and procalcitonin levels were found to be significantly correlated with poor prognosis. Pregnant women infected with the Delta variant of SARS-Cov-2 were also found to be at increased risk for preterm delivery and cesarean section in the presence of severe disease. Although intensive care admissions were found to be significantly higher in the newborns of pregnant women in the severe disease group, no significant difference was found in neonatal mortality rates. We recommend close monitoring of CRP, WBC, and procalcitonin levels in addition to symptoms, particularly in pregnant women infected with the Delta variant in the third trimester. We believe that swift decision-making for the delivery of the fetus can improve neonatal outcomes in case of impaired maternal oxygenation.

Ethics Committee Approval: *The study was approved by the Ethics Committee of the University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital and conducted in accordance with the Helsinki Declaration of Ethical Principles (approval number: 917, date: 05.11.2021).*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: M.R.G., S.C.O., Ş.T.; Concept: M.R.G., S.C.O., Z.G.Ö., Ş.T.; Design: M.R.G., S.C.O., Z.G.Ö.; Data Collection or Processing: M.R.G., Ş.T.; Analysis or Interpretation: S.C.O., Z.G.Ö.; Literature Search: S.C.O., Z.G.Ö., G.B.; Writing: M.R.G., S.C.O.; Critical review: S.C.O.*

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Postoperative care in the caesarean intensive care unit: experience from a tertiary maternity hospital

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Abstract

Objective: The aim was to determine whether follow-up in the intensive care unit (ICU) for the postoperative first eight hours was beneficial for early intervention in postpartum hemorrhage.

Material and Methods: In our hospital, all patients are admitted to the ICU for the first eight hours after cesarean section. Patients with postpartum hemorrhage after cesarean delivery who received medical and/or surgical treatment between 2016 and 2020 were reviewed in the presented study retrospectively.

Results: All cases (n=36,396) who underwent cesarean delivery were reviewed. Three hundred and fifty-nine patients with postpartum hemorrhage were identified and included. In the study group the time between cesarean section and diagnosis of postpartum hemorrhage was 10.1±19.1 hours, and the time between cesarean section and re-laparotomy was 9.26±23.1 hours. A total of three maternal deaths occurred after cesarean section in our hospital. In the last five years, the mortality rate in patients delivering by cesarean section was 3.9 per 100,000. The incidence of postpartum hemorrhage in cesarean deliveries at our hospital was calculated to be 1.0%, and the rate of obstetric near-miss events was calculated to be 0.6 per 1000 live births.

Conclusion: Follow-up of patients in the ICU in the first eight postoperative hours after cesarean section may result in a lower number of re-laparotomies due to postpartum hemorrhage, a shortened interval between cesarean section and re-laparotomy, and a lower maternal mortality rate. (J Turk Ger Gynecol Assoc 2023; 24: 42-7)

Keywords: Cesarean section, maternal mortality, postpartum hemorrhage, re-laparotomy

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Introduction

Maternal mortality rate is one of the most important health and development indicators of a country and postpartum hemorrhage (PPH) is the most common cause of maternal mortality (1). The maternal mortality rate in our country was reported to be 13.1 per 100,000 live births (2). Approximately 50% of maternal mortality is caused by PPH, which occurs within 24 hours after delivery. Therefore, close monitoring of the patient in the first eight hours after delivery is essential (3). In 2017, the American College of Obstetricians and Gynecologists changed its definition of PPH from the classic definition described above to a cumulative blood loss ≥1000 mL or hemorrhage associated with signs/symptoms of hypovolemia within 24 hours of delivery, regardless of route of delivery (4).

The rate of cesarean deliveries has increased worldwide. Considering that postpartum complications are more common in cesarean deliveries than in vaginal deliveries, it is inevitable that postpartum complications will also increase accordingly (5). Among these complications, uterine atony, the inability of the uterine muscles to contract after delivery, is the most

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critical complication leading to maternal mortality. About 80% of PPH is caused by uterine atony. Many organizations have published guidelines for the prevention or prompt diagnosis of PPH, especially uterine atony (4,6). The incidence of uterine atony after cesarean section was 6% in a large cohort study (7). In the present study, the aim was to investigate whether the follow-up of patients in the intensive care unit (ICU) after cesarean section (cesarean ICU) leads to early detection or prevention of postoperative complications. Our research question was, "do postpartum complications differ in frequency in patients followed up in ICU after cesarean section compared with the literature?"

Material and Methods

This study was a retrospective, single-center, hospital-based study. The University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Training and Research Hospital Institutional Review Board approved the study (approval number: 02, date: 10/02/2021). From January 1, 2016, to December 31, 2020, patients who underwent a medical or surgical procedure in the first 24 hours after cesarean delivery were identified. In these patients, the interval between the diagnosis of PPH and the time to re-laparotomy were determined. The amount of red blood cell suspensions or other blood products transfused to the patients was obtained from the medical records, and these results were compared with the literature. Patients who were anemic before cesarean section and had received preoperative or intraoperative blood transfusions were not included. Although previous studies have included cases of uterine atony detected intraoperatively, we excluded intraoperative atony in the present study. Therefore, only those patients who developed PPH during follow-up in the cesarean ICU were eligible for the study.

Since the establishment of our hospital 30 years ago, all postpartum patients were observed in the post-cesarean ICU during the first eight hours. Fever, pulse, and blood pressure were measured every 15 minutes for the first two hours, every 30 minutes for the second two hours, and every 60 minutes for the last four hours. In the ICU, each patient could be attended by four nurses and a senior obstetrician. Complete blood count was obtained at the second and sixth hour postoperatively in the ICU, and uterine tone examination and vaginal bleeding control were performed every 15 minutes (8). The diagnosis of PPH was made when the amount of bleeding exceeded 1000 mL in cesarean deliveries; the diagnosis was supported by a deterioration in vital signs and a drop in hemoglobin levels. Since risk factors, such as the patient's age, the presence of concomitant diseases, or bleeding disorders, increase PPH, the attending nurse cares more intensively for these patients.

All patients received 20 international unit (IU) of oxytocin (Synpitan fort®, Deva Ilac, İstanbul, Turkey). Ten IU was administered intraoperatively, with the remainder administered over the eight hours in the ICU following CS. After completion of the first eight hours, women with an uneventful postpartum course were transferred to the normal patient room. If patients have PPH, additional oxytocin [up to 40 IU, intravenous (IV)], methyl ergonovine (Metiler®, Adeka, Samsun, Turkey, 0.2 mg, intramuscular), misoprostol (Cytotec®, Pfizer, Germany, 600-800 μg, peroral, rectal or sublingual), tranexamic acid (Transamin®, Daiichi Sankyo, Japan, 100 mL, IV) were administered in the cesarean ICU. In patients with PPH who did not respond to drug treatment, bimanual uterine compression and/or uterine balloon tamponades were performed as a secondary line of treatment or as a bridge to a second surgical procedure in case of uterine atony. The following data were extracted from the hospital database and medical records: age; parity; number of abortions; concomitant diseases; and risk factors for PPH. Data used for analysis also included type and amount of supplemental uterotonics, amount of blood transfusion and type of blood products transfused, interval between cesarean section and re-laparotomy, and surgical technique used during re-laparotomy. Neonatal data included gestational age at birth, birth weight, and neonatal Apgar scores. Our hospital's protocols used previously published criteria to define Intrauterine Growth Restriction (9). Preeclampsia was defined as sustained blood pressure above 140/90 mmHg in association with proteinuria, maternal signs, symptoms, or laboratory findings (10). Premature preterm rupture of membranes (PPROM) was defined as fetal rupture of membranes before labor at less than 37 gestational weeks. Placental abruption was defined as the detachment of the placenta from the implantation site before delivery. The total number of births during the study period was also obtained from the hospital database.

Statistical analysis

Statistical Package for Social Sciences software for Windows, version 23.0, was used for statistical analysis (IBM Inc., Armonk, NY, USA). Descriptive statistics used in the present study included mean, standard deviation, median, minimum and maximum, frequency, and percentages.

Results

During the study period, a total of 77,157 births were recorded in our hospital. Of these, 769 were stillbirths, 36,196 deliveries were by cesarean section, and 6,554 were considered high-risk pregnancies. The remaining 29,642 deliveries by cesarean section were low-risk pregnancies. Over a five-year period, a

total of three maternal deaths occurred after cesarean section at our hospital. The mortality rate after cesarean delivery was 3.9 per 100,000 live births during the study period. The incidence of PPH was calculated to be 1.0% (359 cases in 36,196 cesarean deliveries). The causes of maternal death after cesarean delivery were massive PPH in two patients and disseminated intravascular coagulation (DIC) in one patient.

Amongst the 36,196 cesarean deliveries, 359 patients received additional medical and/or surgical treatment for PPH. The mean age of the patients was 28 years, the mean parity was 1, and 12 patients (3.4%) required transfusion because of prepartum anemia. Of these patients, 157 had a previous cesarean section. The other indications for cesarean delivery are listed in Table 1. The gestational age at delivery in these patients was 36.9±3.2 weeks, while 54 women (15%) delivered between 34-37 weeks, and 45 (12.5%) women delivered before 34 weeks. There were 104 pregnant women classified as high-risk pregnancies. There were 33 twin pregnancies (9.2%). PPROM was diagnosed in 14 pregnant women (3.9%), preeclampsia in 21 pregnant women (5.9%), and 15 pregnancies ended in stillbirth (4.1%) (Table 2). In this cohort 21 women (5.9%) had neonates >4000 g, and 45 women (12.3%) had neonates <2500 g. The median 1-minute and 5-minute Apgar scores were 9 and 10, respectively, and 59 infants (16.4%) were admitted to the neonatal ICU (Table 2).

A total of 37 patients (10.3%) received supplemental oxytocin alone to treat postpartum hemorrhage, and 322 patients (89.7%) received combined medical therapy (oxytocin +/- misoprostol +/- methylergonovine) to treat postpartum hemorrhage, while 150 patients (41.7%) received tranexamic acid. Bakri balloon tamponade was performed in 23 patients (6.4%), and 265 patients (73.8%) received transfusion of blood and blood products. Across the whole cohort requirement for blood and blood product transfusion after cesarean section was 0.7% (265/36196*100). The surgical approach for postpartum hemorrhage after re-laparotomy was uterine artery ligation in seven patients (1.9%) and B-Lynch compression suture in 11 patients (3%). Hysterectomy was required in seven patients (1.9%). Our hysterectomy rate after cesarean section was 0.02% (7/36196*100).

The diagnosis of PPH was made 10.1±19.1 hours after the first operation. In cases in which surgical intervention was performed, the mean interval between re-laparotomy and the first operation was 9.26±23.1 hours. Two patients (0.6%) developed DIC, three patients (0.8%) posterior reversible encephalopathy syndrome, and two patients (0.6%) developed eclampsia during follow-up. There was a single case of acute fatty liver (0.3%) and venous thromboembolism (0.3%). Near-miss was noted in 53 patients, and 11 patients (3.1%) required anesthesia ICU. The rate of near miss was 0.6 per 1000 live births (Table 3).

Table 1. Characteristics of patients

	Study population, (n=359)
Age*	28 (16-45)
Gravida*	2 (1-9)
Parity*	1 (0-7)
Abortion*	0 (0-5)
Pre-partum transfusion	12 (3.4%)
Indications for caesarean	
Placenta previa	6 (1.7%)
Fetal distress	61 (17%)
Labor arrest	61 (17%)
Ablatio placenta	17 (4.7%)
Severe preeclampsia	11 (3.1%)
Macrosomic fetus	16 (4.5%)
Previous caesarean	157 (43.7%)
Non-vertex presentation	24 (6.7%)
Umbilical cord prolapsus	6 (1.7%)

*Data are given as median (minimum-maximum) or n (%)

Table 2. Obstetric and neonatal outcomes

	Study population, (n=359)
Gestational age	
Mean (± standard deviation)	36.9±3.2
34-37 weeks, n (%)	54 (15%)
<34 weeks, n (%)	45 (12.5%)
>37 weeks, n (%)	260 (72.4%)
High-risk pregnancies	
Twin pregnancy	33 (9.2%)
Preeclampsia	21 (5.9%)
Placenta previa	6 (1.7%)
PPROM	14 (3.9%)
IUGR	15 (4.1%)
Stillbirth	15 (4.1%)
Neonatal birth weight, grams	
Mean (± standard deviation)	(3064±727)
>4000	21 (5.9%)
<2500	45 (12.3%)
Neonatal Apgar scores	
1 minute*	9 (0-9)
5 minute*	10 (0-10)
NICU admission	59 (16.4%)

*Data are given as median (minimum-maximum) or n, %. PPROM: Premature preterm rupture of membranes, IUGR: Intrauterine Growth Restriction, NICU: Neonatal intensive care unit

Table 3. Treatment for postpartum bleeding

	Study population, (n=359)
Uterotonics	
Additional oxytocin	37 (10.3%)
Combined (oxytocin ± misoprostol ± methylergonovine)	322 (89.7%)
Tranexamic acid use	150 (41.7%)
Bakri balloon tamponade	23 (6.4%)
Blood and blood product transfusion	265 (73.8%)
Re-laparatomy	11 (3.1%)
Surgical procedures performed during re-laparatomy	
Uterine artery ligation	7 (1.9%)
Hysterectomy	7 (1.9%)
B-Lynch	11 (3%)
The interval between diagnosis and primary operation time, hours*	10.1±19.1
The interval between re-laparotomy and primary operation time, hours*	9.26±23.1
Postpartum complications	
DIC	2 (0.6%)
Eclampsia	2 (0.6%)
Acute fatty liver	1 (0.3%)
Venous thromboembolism	1 (0.3%)
PRES	3 (0.8%)
Near miss events	53 (14%)
Need for anesthesia intensive care unit	11 (3.1%)

*Data are given as mean ± standard deviation or n (%). DIC: Disseminated intravascular coagulation, PRES: Posterior reversible encephalopathy syndrome

Discussion

The present study showed that follow-up in the cesarean ICU is beneficial in early intervention of postpartum complications after cesarean section. The incidence of PPH after cesarean delivery in our series was 1.0%, which was lower than the previously published incidence of between 4-6%. We believe this is due in part to the immediate care provided in the first eight hours. Although 73.8% of these patients required transfusion of blood and blood products, the need for re-laparotomy was low compared with the literature.

In terms of health economics, monitoring patients in the cesarean ICU is less costly than direct room care because four nurses and one physician are sufficient to provide adequate care. However, it is more costly than direct room transfer because of staffing, medical equipment, devices, ventilators, electricity costs, etc.

PPH is the leading and preventable cause of maternal morbidity and mortality worldwide (6,11,12). Early diagnosis, prompt intervention for hemorrhage, and appropriate treatment play a key role in preventing maternal mortality (13,14). In addition to publications reporting an incidence of 4-6% PPH (12), there are publications indicating an incidence of massive hemorrhage of approximately 5-15% (15). However, the 5-year incidence of postpartum hemorrhage after cesarean section in our hospital is lower than the previously reported world average. This low incidence may be due to the exclusion of intraoperative atony cases and vaginal deliveries with a lower threshold for PPH.

Our results show that intensive care after cesarean section appears to result in a lower incidence and earlier diagnosis of PPH. Kalisa et al. (16) reported a blood transfusion rate of 32.5% versus a re-laparotomy rate of 21.4% and a maternal mortality rate of 13.1% (13). In our study, the transfusion rate was 73.8%, but the re-laparotomy rate was 3.1%, and the mortality rate after cesarean section was 3.9%. Although the transfusion requirement was higher, the need for repeat laparotomy and the low maternal mortality rate suggest that close monitoring and early treatment are effective; it may also be suggested that the rate of surgical intervention can be reduced by early diagnosis of PPH. We hypothesize that early diagnosis will increase the response to medical treatment and thus reduce the number of surgical procedures.

Although many prenatal and intrapartum risk factors have been identified that increase the risk of PPH, there is no identifiable risk factor for most cases (16). Consistent with the literature, conditions that are considered risk factors for hemorrhage, such as multiple pregnancies, macrosomia, preterm labor, and placenta previa, were common in our series.

In the literature, the need for blood transfusion after cesarean section is reported to be 1-7% (17). Although in our setting the need for blood and blood product transfusion after cesarean section was 0.7%, this rate was lower than that reported in the literature. However, 73.8% of these patients with PPH had blood and blood product transfusion. These results suggest that transfusion after cesarean section was lower than reported in the literature because the ICU allows patients to be diagnosed early and receive early medical intervention. Nevertheless, we are more aggressive with blood transfusions in patients who are diagnosed with PPH.

A study by Holleboom et al. (18) found that after cesarean section, 7.2% of patients required supplemental oxytocin. In our study, although the need for additional oxytocin was 10.3%, 89.7% of patients with PPH were controlled with combined uterotonics without further intervention or treatment. We used a combination of these drugs by treating the patient individually during PPH. The need for additional medical treatment is higher than suggested in the literature, possibly because of early PPH diagnosis and rapid treatment in the cesarean ICU.

Uterine balloon tamponades can temporarily control the bleeding until appropriate management is achieved (16,19). According to a meta-analysis, the rate of use of the Bakri™ balloon was 0.20%, and two-thirds (67%) of insertions occurred after cesarean delivery (20). In our study, the rate of copper balloon use in patients with PPH after cesarean delivery was 6.4%, but lower than previously published data. Uterine artery ligation is a procedure that is now out of favor for the treatment of PPH (21). It was observed that the effect was not feasible when the cervicovaginal and uteroovarian branches were not ligated. In our study, the incidence of uterine artery ligation was 1.9%. Although it is easy to apply, it is less preferred in re-laparotomy because it is only an anesthetic.

Joseph et al. (21) found that the rate of hysterectomies associated with PPH was 73%, but it was not stated whether peripartum hysterectomies were included. In our study, the rate of hysterectomies related to PPH was 1.9%. According to another study, the rate of postpartum hysterectomy was 0.38%, excluding peripartum hysterectomies (22), and our rate of hysterectomy after cesarean section was 0.02%, which is far lower than the literature. The low rate of our hysterectomy is probably due to the early diagnosis of patients in the cesarean ICU and the fact that peripartum hysterectomies were not included in the statistics.

Levin et al. (23) and Akkurt et al. (24) found that the time between cesarean section and re-laparotomy was (25.2±35.6 hours and 15.7±3.2) hours, respectively. The time between cesarean section and relaparotomy was less in our series compared to the delay reported in the literature, but cesarean intensive care does not prevent the need for relaparotomy. In addition, the time in the ICU was prolonged in patients who may need re-laparotomy, and their follow-up was continued in this unit until they were taken to the operating room. The incidence of re-laparotomy after cesarean section has been reported in the literature to be 0.2%, 0.5%, and 0.7% (25-28). In our study, the incidence of re-laparotomy after cesarean section was 0.03% among 36,196 cesarean deliveries. This low rate may be due to the fact that patients were in the ICU for the first eight hours after cesarean section. The number of patients who required repeat laparotomy may be low because we intervened early and had a high blood transfusion rate. The fact that intraoperative bleeding was not considered in this study and the definition of postpartum hemorrhage was >1000 mL also resulted in these data being lower than those reported in the literature.

A systematic review reported that PPH rates and associated mortality are higher in low- and middle-income countries. The rate of postpartum near-miss was 2.1 per 1000 live births. Our near-miss rate of 0.6 per 1000 live births after cesarean section was significantly lower than the literature (29). We believe that this lower rate is due to being able to diagnose and treat early as a result of the close monitoring in the ICU after cesarean section.

Study Limitations

Our study has some limitations. First of all, we do not have a control group because all patients were admitted to the ICU after cesarean section according to the protocol of our hospital. Not including cases with intraoperative atony may have resulted in a falsely low incidence of PPH. Future prospective randomized studies, including a control group, are needed to support our findings. Retrospective design and incomplete data were the other limitations.

Conclusion

Compared with published data, the advantage of cesarean section intensive care may be earlier detection of PPH, resulting in less blood loss and lower maternal mortality rate after cesarean section. Cesarean section intensive care may also be associated with a decrease in near-miss events.

Ethics Committee Approval: *The University of Health Sciences Turkey, Etlik Zübeyde Hanım Women's Health Training and Research Hospital Institutional Review Board approved the study (approval number: 02, date: 10/02/2021).*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

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Barriers to the HPV vaccination program in the Eastern Mediterranean region: a narrative review

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Abstract

Objective: The human papillomavirus (HPV) vaccine is regarded as one of the most effective ways of preventing cervical cancer. Despite the massive burden of this disease, only two countries in the Eastern Mediterranean Region (EMR) have implemented a national HPV vaccination program. The aim of the present study was to assess the main barriers to the integration of HPV vaccination in the national vaccination programs of EMR countries.

Material and Methods: We performed a narrative review with no inclusion and exclusion criteria. The electronic databases we searched included Medline, Scopus, Embase, and Web of Science (last update; December 2021). The search was not subject to any limitation in terms of time or method. Studies that dealt with the obstacles or the needs of vaccination programs in EMR countries were included in the investigation.

Results: After a full-text screening, the report comprised of 31 studies from 15 EMR countries. All of the studies were descriptive. The most common barriers to HPV vaccination are the following: a) lack of knowledge and awareness, b) economic barriers in terms of the cost-effectiveness of the HPV vaccination program, c) social insecurity in conflict zones, d) cultural norms and religion.

Conclusion: EMR countries should focus on modifiable barriers to the vaccination program. Steps to improve HPV vaccination coverage in these countries should include enhancing social awareness and mobilization, ensuring the support of the Global Alliance for Vaccines and Immunization in eligible countries, using national resources in an optimal way, and addressing HPV vaccination in undergraduate medicine and paramedic curriculums. (J Turk Ger Gynecol Assoc 2023; 24: 48-56)

Keywords: Barriers to vaccination, cervical cancer, Eastern Mediterranean region countries, HPV vaccine

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Introduction

Cervical cancer is the fourth most common cancer among women worldwide (1). The prevalence of cervical cancer in the Sub-Saharan region is 11.4% and the consequent fatality rates are considerable (2). The human papillomavirus (HPV) is one of the most significant risk factors for cervical cancer. HPV can be transmitted through sexual contact, including vaginal or oral sex with an infected person (3). HPV has more than 100 strains, of which HPV16 and HPV18 account for about 70% of invasive cervical cancers (4,5). The HPV vaccine can prevent cervical cancer effectively. The World Health Organization (WHO) recommends routine HPV vaccination for girls aged 9-14 years (prior to sexual activity) through national immunization programs, "catch-up vaccinations" for unvaccinated adults older than 15 years with high-risk behavior, and for HIV-positive women (6). A minimum six-month interval must be ensured between the first and the second dose. The WHO currently does not recommend the HPV vaccination for boys (7). Since 2007 the HPV vaccine has been approved in the United States and

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the European Union (8). National HPV vaccination programs have been implemented in at least 107 countries worldwide (9). HPV vaccination is a part of the routine immunization program in many high-income countries (8).

The HPV vaccination rate for women in the USA and Australia is about 37.6% and 32%, respectively (10), but is lower in Asian countries (3). Implementation of an HPV vaccination program is probably the most effective way of reducing the burden of cervical cancer (11), especially when screening tests are not available, limited, or of poor quality (12). According to the WHO, no study has reported major adverse effects of the HPV vaccine (13).

However, the HPV vaccine is not affordable in those countries that need it the most. In fact, cervical cancer mirrors health inequity more than any other female cancer (8,14); approximately 90% of deaths due to cervical cancer occur in low-income countries (1).

The Eastern Mediterranean Region (EMR) of the WHO extends from Iran to Djibouti, covers 22 countries (Table 1) and a population of 680 million (15). Although some nations, including those of the Persian Gulf have common roots, the EMR countries differ significantly in terms of their geopolitical status, cultural backgrounds, income levels, and health expenditure rates. Moreover, some countries, such as Afghanistan, Yemen and Syria are experiencing prolonged social instabilities and conflict. A considerable part of the population of EMR countries has been displaced in recent years due to internal wars.

Despite the huge burden of cervical cancer, especially in low- and middle-income countries (16), only two nations in the EMR have implemented HPV vaccination in their national vaccination program (NVP) (17). The present study aimed to assess the barriers experienced in incorporating HPV vaccination in the NVP of EMR countries.

Material and Methods

Published articles which addressed HPV vaccination were searched for a narrative review.

Determining the research question

The review was based on the following research question: What are the main barriers to HPV vaccination in EMR countries?

Identifying relevant studies

We performed a systematic search in a range of electronic databases, including Medline, Scopus, Embase, and Web of Science (initial search in June 2021; last update in December 2021).

The following keywords were used: “HPV”, “vaccine” “vaccine strategy”, “barrier”, obstacle”, and “EMR countries”. The names of the 22 member states of the EMR were searched separately. The classification of EMR countries is summarized in Table 1.

Inclusion and exclusion criteria

All types of studies dealing with HPV vaccination in EMR countries, published exclusively in the English language, were included. Reports that were not in line with the purpose of the study or did not provide their full text were excluded.

Selection of studies

We reviewed all types of articles published about EMR countries. The search was not subject to any limitations in terms of time or methodology. Reference lists of all identified studies were screened manually. Vaccination-related guidelines published by the ministry of health in each EMR country and the WHO-EMRO were searched. The review process consisted of two screening steps: a) reviewing the title and summary of the articles, and b) reviewing the full texts.

Collating, summarizing, and reporting the results

We reviewed studies that included data concerning programs, strategies, barriers, and implementation of an HPV vaccination program in EMR countries. As we aimed to identify existing gaps and guide future research, we dispensed with quality assessment or an extensive data synthesis of the published reports.

Results

Search results

We reviewed investigations from 2012 to 2021 based on various methodologies. Of 85 publications found in the various databases, 10 were duplicate articles. After reviewing the titles and abstracts of the remaining reports, 45 were excluded.

Table 1. Classification of EMR countries

EMR subregions	Countries
Arab states	Bahrain, Iraq, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Syria, United Arab Emirates (UAE), Yemen, Occupied Palestinian Territory (OPT)
African countries	Djibouti, Egypt, Somalia, Sudan (North-East African nations)
	Libya, Morocco, Tunisia (North-West African nations)
Non-Arab countries	Iran, Afghanistan, Pakistan
EMR: Eastern Mediterranean Region	

Finally, 31 studies from 15 EMR countries [Arab states (n=23), African countries (n=3), and non-Arab countries (n=5)] were included in a narrative review. The characteristics of the studies are summarized in Table 2.

Cervical cancer and HPV prevalence

We lack precise statistics regarding the incidence and prevalence of cervical cancer for any EMR member state. Currently, in Afghanistan in each year about 862 women are identified with cervical cancer and 570 die from the disease (18). Cervical cancer ranks second after breast cancer in some countries, such as Yemen, Djibouti, Somalia and Afghanistan (19). An increase in life expectancy has been registered in all EMR countries. The risk of cancer is increasing with age. Both population growth and population aging have led to more numerous cases of cancer. Changes in lifestyle are regarded as one of the most important risk factors for cervical cancer. The HPV infection has increased markedly in recent times due to unsafe sexual activity (20). The overall prevalence of HPV in Egypt was estimated at 10-23% (21,22). Bansal et al. (23) estimated an HPV prevalence of 5.8% among women in Qatar with a normal cytology, and 18.4% in women with an abnormal cervical cytology. In Kuwait, the prevalence of HPV was reported to be about 2.5% and higher than 50% in women with a normal and abnormal cytology, respectively (24,25).

HPV vaccination status in EMR countries

According to the report of the WHO, at the annual workshop of the EMRO held in Lebanon in 2019 it was noted that only Libya and the United Arab Emirates) UAE (had integrated HPV vaccination in their national vaccination programs, while a third country (Morocco) plans to do so (26,27). In some countries, such as Iran, the private sector is responsible for providing vaccines. Therefore, the HPV vaccine is neither affordable nor available to all persons (28).

Barriers to HPV vaccination in EMR countries

Knowledge and awareness

Lack of knowledge is a fundamental obstacle to HPV vaccination. Adequate knowledge about the role of HPV as a risk factor for cervical cancer is crucial. In a study comprising 746 female students in Pakistan, Gul et al. (29) reported that nearly 85% had no information about the HPV vaccine and, surprisingly, more than 95% would like to receive it. Knowledge of the HPV vaccination was moderate or poor in Bahrain, Morocco, Oman, and Lebanon, expressed as a distrust of the vaccine (30-34).

Due to the absence or paucity of public education, the majority of the residents in the EMR countries are unaware of the availability of the HPV vaccine. Recommendations for HPV vaccination by physicians and the workforce in the health sector are very important, as these will influence parents' acceptance of the vaccine for their daughters (35). However, health care providers themselves do not have sufficient knowledge about the vaccine. Studies performed in Saudi Arabia and Iraq showed that physicians and medical students are poorly informed about the subject (36-39).

About 54% of student nurses in Sudan were aware of the HPV vaccine (40). The studies revealed that the main sources of information for medical and nursing students were mass media, including TV and web pages, as well as self-learning (36).

Published studies reveal that there is concern about the long- and short-term side effects of the HPV vaccine (30). Women are afraid of its potential side effects (20). In a study in Syria, Al Saad et al. (41,42) reported that mothers were unwilling to vaccinate their daughters against HPV due to concerns about the adverse effects and safety of the vaccine.

Economic barriers regarding the cost-effectiveness of HPV vaccination program

Given the population structure (large and growing young population) in EMR countries, the large number of persons eligible for vaccination, and the number of required doses (3 doses), the provision of a sufficient budget for public vaccination is a challenging issue for many countries in the region (34). The HPV vaccine is almost as expensive as other vaccines (35). The cost of each dose ranges from 13 to 100 US$ (43), making vaccination programs unaffordable for many low-income countries. The cost of one dose of HPV vaccine (Gardasil 4) is about 13 US$, as the cost is not covered completely or even partially by insurance companies (44). Therefore, the majority of eligible persons in Iran do not receive the vaccine (28). However, in a secondary analysis of the Iranian Ministry of Health data, Mohammadpour et al. (28) concluded that the use of Gardasil has increased very markedly in the last five years. Shaikh et al. (45) reported that the cost of vaccination is a barrier in Pakistan: the surveyed persons said they refrained from HPV vaccination because it was not affordable.

A correlation was observed between income levels and HPV vaccination (45). The Global Alliance for Vaccines and Immunization (GAVI) could negotiate with industry to provide a low-cost vaccine, or co-finance its provision. In 2011 Merck announced that a single dose of the HPV vaccine will be distributed on behalf of GAVI for 5 US$ (43). Similar programs could be devised to provide affordable HPV vaccines. It should be noted that the HPV vaccine is affordable for countries with a gross domestic product just below 1,000 US$ when each dose is supplied for 1-2 US$ (8). Some EMR nations, including Saudi

Table 2. Characteristics of studies

Author/year	Origin of the target population	Study type	Sample size	Study population	Results
Alsaad et al. (42), 2012	Syria	Descriptive/cross-sectional	345	Women	Less than a third of the participants were aware of HPV and the HPV vaccine.
Ortashi et al. (53), 2012	United Arab Emirates	Descriptive/cross-sectional	356	University students	46% of the participants said they accept the HPV vaccine. Fear of side effects (85%) and the absence of obvious benefits (38%) were among the most important factors hindering its acceptance.
Hwaid (38), 2013	Iraq	Descriptive/cross-sectional	198	Women	Knowledge about the availability of the HPV vaccine was inadequate. Only 23.2% of the participants were aware of it.
Grabiel et al. (63), 2013	Jordan	Descriptive/cross-sectional	129	Parents	The mean knowledge score about HPV and the HPV vaccine was 36 (range 0-80). 40% of parents mentioned the cost of the vaccine as a barrier to vaccination.
Aljuwaihel et al. (64), 2013	Kuwait	Descriptive/cross-sectional	206	Primary health care physicians	Information about the HPV vaccine was moderate. The percentage knowledge score was 63.4%.
Wigle et al. (65), 2013	Low- and middle-income countries	Review article	-	-	Social insecurity, internal wars and conflicts are barriers to HPV vaccination.
Ortashi et al. (66) 2014	United Arab Emirates	Descriptive/cross-sectional	640	Women	Knowledge about the HPV vaccine was low (37%) in the study population. However, 80% of women would be willing to receive the vaccination. 69% of participants were willing to be vaccinated. 17% of the women believed it might not be culturally acceptable. Vaccine safety was one of the foremost concerns of women.
Khatibi et al. (67), 2014	Iran	Descriptive modelling study	-	-	The cost of the vaccine may be viewed as a barrier in countries with limited resources.
Al-Darwish et al. (68), 2014	Saudi Arabia	Descriptive/cross-sectional	188	Medical students	67.7% of the participants were unaware of the availability of the HPV vaccine.
Gul et al. (29), 2015	Pakistan	Descriptive/cross-sectional	764	Female university students	The information level of the young population about the HPV vaccine was not high.
Selmouni et al. (32), 2015	Morocco	Descriptive/cross-sectional	653 women 659 men	Parents of girls aged 6-12 years	The acceptance rate of the HPV vaccine was 76.8 (95% CI: 73.3-79.9%) among women and 68.9% (95% CI: 65.2-72.5%) among men.
Zouheir et al. (33), 2015	Morocco	Descriptive/cross-sectional	1,044	Adolescents (12-17 years) and young persons (18-30 years)	The level of knowledge about the HPV vaccine was poor (20%). The acceptance rate of the HPV vaccine among the participants was 27%.
Abdallah et al. (40), 2016	Sudan	Descriptive/cross-sectional	246	Student nurses	A half of the participants (49.9%) lacked complete information about the HPV vaccine.
Saqer et al. (69), 2017	United Arab Emirates	Descriptive/cross-sectional	400	Women	Near a third (36.5%) of the population had heard about the HPV vaccine. 76.6% of parents were willing to vaccinate their daughters. Vaccine recommendation by health care providers and greater awareness of the vaccine on the part of husbands could influence the rate of vaccinated girls.

Jassim et al. (31), 2018	Bahrain	Descriptive/cross-sectional	300 women	Primary health care attendees	11.3 % of the participants had heard about the HPV vaccine.
Alwahaibi et al. (70), 2018	Oman	Descriptive/cross sectional	494	Patients attending an outpatient gynecology clinic, female staff of the clinic, university students	Patient awareness about the HPV vaccine was 5.9%. The awareness of staff members and students was 59.4% and 14.6%, respectively.
Abou El-Ola et al. (34), 2018	Lebanon	Descriptive/cross-sectional	1,193	Women	The main barrier to HPV vaccination is lack of knowledge and information about it. 34% of the participants were aware of the availability of the vaccine to prevent cervical cancer.
Al Saad and Jadoo (41), 2018	Syria	Descriptive/cross-sectional	400	Women	The cost of the HPV vaccine and lack of knowledge were major barriers hindering women's acceptance of the vaccine.
Hamdi (52), 2018	Middle East and North Africa	Review article	-	-	Traditional norms and religious issues could serve as barriers to HPV vaccination.
Shaikh et al. (45), 2019	Pakistan	Descriptive/cross-sectional	400	Young adults (18-26 years)	Approximately a half of the responders considered the vaccine time consuming and expensive. Poor knowledge of the vaccine and its cost were among the main barriers to HPV vaccination.
Husain et al. (30), 2019	Bahrain	Descriptive/cross-sectional	408 (268 women and 140 men)	Primary health care attendees	Awareness about HPV and the HPV vaccine is limited.
Abi Jaoude et al. (71), 2019	Lebanon	Descriptive/cross-sectional	228	Doctors, parents	The main barrier to HPV vaccination is its cost. Further barriers include concern about its safety and efficacy, and lack of knowledge about HPV.
Ibrahim et al. (39), 2019	Iraq	Descriptive/cross-sectional	240	Medical and nursing students	The responders had sufficient knowledge about the availability of the HPV vaccine; 81.9% of medical students and 50.5% of nursing students were aware of it.
Sallam et al. (72), 2019	Jordan	Descriptive/cross-sectional	376	Dentistry students (pre-clinical and clinical)	Less than a half of the participants (44% of pre-clinical and 36% of clinical students) were aware of the HPV vaccine.
Jradi and Bawazir (59), 2019	Saudi Arabia	Qualitative research	77	Women (general population, physicians, health care providers)	A small number of participants were aware of the HPV vaccine. Most of the population in Saudi Arabia did not know about the HPV vaccine and its efficacy. Lack of knowledge was among the most important barriers.
Anfinan (73), 2019	Saudi Arabia	Descriptive/cross-sectional	2,000	Physicians	Lack of knowledge about the HPV vaccine (21.1%) and being sexually inactive (14.7%) were the most common barriers.
AlMansoori et al. (74), 2019	United Arab Emirates	Descriptive/cross-sectional	110	Physicians	The percentage of physicians who recommended the HPV vaccine was high, but they lacked comprehensive knowledge about the HPV vaccine.
Anwari et al. (18), 2020	Afghanistan	Cohort model	-	-	In Afghanistan, targeting a single cohort with the HPV vaccine was potentially cost-effective (0.7 times the GDP per capita of 586 US$) from the perspectives of both, the government and society. Additional health benefits could be generated by a catch-up campaign, depending on the government's willingness to pay for the projected health outcomes.

Almazrou et al. (75), 2020	Saudi Arabia	Descriptive/cross-sectional		Physicians	More than a half of the participants were aware of HPV and the HPV vaccine. They believed that lack of knowledge among parents was one of the most important barriers to vaccination.
Mohammadpour et al. (28), 2020	Iran	Descriptive/cross-sectional	566	Vaccine recipients	The cost of the HPV vaccine could be regarded as a vaccination barrier
Rezqalla et al. (76), 2021	Kuwait	Descriptive/cross-sectional	1,341	Female school teachers	Lack of knowledge about the availability of the HPV vaccine.
HPV: Human papillomavirus, CI: Confidence interval					

Arabia, Oman and Qatar are not low-income countries, but their governments doubt the cost-effectiveness of HPV vaccination on a nationwide basis (28). It appears that the opinion of the government about the missing cost benefit of Gardasil is the main factor underlying the integration of the HPV vaccine in the NVP of each country (46). Health economy experts assessed the cost benefit, cost effectiveness, and affordability of the vaccine in Oman. They also evaluated the burden of HPV and stated that the integration of the HPV vaccine in the NVP of Oman is not cost effective (47).

The paucity of data about the burden of cervical cancer, its annual incidence, as well as the direct and indirect costs of treatment impairs the development of an up-to-date guideline and decisions about the HPV vaccine in EMR countries. The cost-effectiveness of mass vaccination against HPV depends on the incidence of cervical cancer and the cost of vaccine (48). To make an appropriate decision, experts would need to obtain valid data about the direct and indirect costs of treating precancerous lesions and invasive carcinoma. Vaccination is obviously a cost-effective measure in countries with a high incidence of cervical cancer (48).

Social insecurity in conflict zones

Timely delivery of the HPV vaccine in conflict zones is very challenging. It requires a reliable infrastructure, such as an uninterrupted cold chain. Schools are one of the appropriate places for the HPV vaccine. However, a very large number of girls in poor and conflict-ridden societies do not attend school or drop out after primary school (49). Of the 22 member states of the EMR, six suffer from internal wars and insecurity.

Cultural norms/religion

EMR countries have a relatively young population. Given social taboos, the age of sexual debut in this region is not clear. However, it is estimated that the usual age is 19-23 years (50). There is evidence of changing sexuality and gender relations in EMR countries. The age of marriage and the prevalence of premarital sexual activity are increasing (51). The HPV vaccine is known to prevent sexually transmitted diseases and this raises cultural as well as religious concerns in society. Given that all EMR member states are predominantly Muslim, a considerable proportion of the population in some countries are conservative in regard to sexual activity. Islam does determine the sexual behavior of individuals in EMR countries; sexual activity should occur within the context of marriage. In the same vein, religious activists may oppose the HPV vaccine because they believe it encourages premarital sexual activity (52). Cultural circumstances cause mothers to believe that their daughters are not at risk for HPV and do not need the vaccine (41). According to religious authorities, given that young people have no premarital sexual contact, they do not need the HPV vaccine (49). In a study comprising 600 women in the UAE, Ortashi et al. (53) noted that 17% of women believed the HPV vaccine may not be culturally acceptable.

Discussion

Cervical cancer is regarded as one of the leading causes of cancer deaths among women in EMR countries (19). This review summarizes the HPV vaccination program in the EMR. Barriers to vaccination were mainly lack of knowledge and awareness, doubts about the cost-effectiveness of the HPV vaccination program, social insecurity in conflict zones, and cultural norms or religious beliefs. Lack of knowledge about the HPV vaccine was one of common barriers in EMR countries. Lack of knowledge creates a vacuum and encourages the spread of misinformation on social networks (54). Educational initiatives aimed at women may be most successful if designed to increase awareness of their susceptibility to HPV infection and transmission. School-based meetings would serve as a useful sensitization strategy to enhance the quantity and quality of knowledge about cervical cancer and the HPV vaccine. Vaccine safety is one of the prime concerns of parents and young persons. We need culturally sensitive training programs addressing parents and emphasizing the absence of side effects of the vaccine (55). Inclusion of the HPV vaccination in the curricula of undergraduate-level medicine and paramedics is also essential. A change in policies regarding the HPV vaccine is essential because we anticipate an increase in the burden of cervical cancer over the coming years (56).

Our analysis showed that the cost and cost-effectiveness of the vaccine are further barriers to its acceptance in the target population. Previous studies reported the highest vaccination coverage rates for HPV in countries where vaccines are funded by the national budget (57). Providing HPV vaccine

free of charge or at a low cost will be essential in low-income countries. In view of the fact that GAVI covers the provision and delivery of the HPV vaccine for low- and lower-middle income countries, upper- and middle-income countries are confronted with serious challenges in purchasing the vaccine (58).

Male guardianship is an issue in EMR countries. As the male-dominated culture requires the spouse's consent to a woman's vaccination, men's education should be included in the agenda of the health system. Women's access to the vaccination would depend on men being sufficiently informed about it (59). Advocacy programs will be needed for introducing the HPV vaccine. Addressing the burden of cervical cancer is a critical issue and requires a suitable cancer registry system (60).

Strong partnership and collaboration with all stakeholders will be needed to implement the HPV vaccination program (61). All of the involved parties should be aware of their clear roles from the point of distribution to implementation. In some countries, we need to identify specific needs, such as technical or financial support, a suitable plan of action, and appropriate monitoring.

Some factors, such as the level of income, knowledge, and the employment of mothers facilitate the acceptance of the HPV vaccine. Recommendations about the vaccine by health care authorities and its approval by decision-makers in each country would encourage mothers to accept the vaccine (41).

Conclusion

There is an urgent need for greater social awareness about the necessity of HPV vaccination. Addressing HPV vaccination in undergraduate medicine and the paramedic curriculum is an essential step towards improving HPV vaccination coverage in these countries. The burden of cervical cancer should be publicized on the basis of robust data proving the effectiveness and significance of HPV vaccination and justifying its cost-effectiveness. Policy-makers should be sensitized to the economic benefits of the vaccination. Ensuring the support of GAVI in eligible countries will be a crucial step in achieving high vaccination coverage. Non-GAVI countries should be encouraged to allocate national resources to HPV vaccination in an optimal way (62).

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The impact of developmental genes in non-syndromic cleft lip and/or palate

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Abstract

Non-syndromic cleft lip and/or palate (NSCL/P) is a congenital malformation with a prevalence of 1:700 births. It has a multifactorial etiology. Human craniofacial development takes place during the first 10 weeks of pregnancy. Normal craniofacial development arises from the convergence and fusion of the facial and palatal processes and involves interactions between genes that regulate cell growth, proliferation, differentiation, epithelial-to-mesenchymal transition, and apoptosis. Whole genome/exome analysis, and also genome-wide association studies give us to chance to identify the genetic factors which contribute to the development of NSCL/P. After detecting a cleft lip and/or palate on ultrasonography without associated anomalies, the patient should be evaluated in collaboration with a clinical geneticist, taking into account the many genes and environmental factors involved in NSCL/P etiopathogenesis, and a roadmap for possible genetic diagnosis should be drawn.

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Introduction

Non-syndromic cleft lip and/or palate (NSCL/P) is one of the most common congenital malformations with a prevalence of 1:700 (1,2). NSCL/P is a multifactorial condition caused by an inadequate partition of the nasal and oral cavities with no other anomaly (3,4). Orofacial clefts (OFC) are classified according to their facial position; unilateral or bilateral, involved parts; lip, palate, and lip and palate, and/or their underlying pathogenesis i.e. syndromic and non-syndromic.

Of all cases with cleft lip and/or palate (CLP), 30% are syndromic. The rest of the cases are NSCL/P, and of them 20% are familial and 80% are sporadic (2,5). As an isolated condition, 50% of all CLP patients are syndromic, the rest are sporadic (3). CLP is observed twice as frequently in males than in females (6). Unilateral clefts of the lip account for approximately 75% of all patients and among them the left side is affected twice as frequently as the right side (7).

The development of palate and lip involves a complex process including the organization of cells in tissues through cell growth, migration, differentiation, and apoptosis, which are controlled by gene expression and signaling molecules. Both genes and environmental factors, such as drugs and chemical exposure of the parent, as well as dietary habits, contribute to the occurrence of the disease pathogenesis (8). Hereditary factors are estimated to be 90% effective in the development of NSCL/P (9). Contribution of epigenetic factors and gene-gene/environment interactions make the pathogenesis of NSCL/P complex. This complex nature makes it difficult to understand the exact reason for the clinical condition. To diagnose genetic factors playing a role in disease pathogenesis, prenatally detected cases may be referred to genetic testing and counseling. Due to the complex nature of the disease, it is not always possible to define the exact gene/gene(s) involved. This information should be emphasized during pre- and post-test genetic counseling sessions. The aim of this study was to review the genes involved in NSCL/P.

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Embryogenesis

Human craniofacial development takes place during the first 10 weeks of pregnancy. The fourth and eighth gestational weeks are the time point where normal lip development occurs (10). The early formative systems of vertebrates are firmly controlled and closely monitored biologically, and hereditary and environmental components influence this sensitive interaction (8,11).

Neural crest cells (NCCs) differentiate to cranial processes by migrating (day 21) and differentiating to maxillary, lateral, and medial nasal processes (12). NCCs undergo epithelial-mesenchymal transition before moving to the craniofacial region and constitute the antecedents of the processes that will develop basic facial structures (13).

Head and neck develop from two-sided transient outgrowths called pharyngeal arches, which take 23-24 days and marks the beginning of early facial development. These swellings of tissue are coated by ectodermal epithelial cells, with a center of NCC-determined mesenchyme (14). A medial outgrowth of the first frontonasal prominence (FNP) is involved in the upper lip, roof of the palate, and lower jaw development (14). The FNP is responsible for the development of bilateral nasal pits and extends into the primitive oral cavity (12). The development of the FNP continues by dividing into paired medial and lateral nasal processes. They fuse with the maxillary process to form an intact upper lip (12).

As palate development is an early event during embryogenesis and takes a relatively long time, probable exposure to teratogens increases the risk of CLP formation in mammalian embryos (1). The palate develops in two parts. By the sixth week of development, the primary palate formation occurs from the bilateral medial nasal processes (15). Between the sixth and twelfth weeks, the rest of the palate, termed the secondary palate, develops. At this stage, palatal shelves outgrow from the inner oral side of the maxillary processes (16). The tongue is required for true mammalian hard palate fusion (17). Mammalian palatal bone ossification is followed by bone fusion (16). Palatal bone develops through intramembranous ossification, in which osteoblasts directly lay down the bone matrix. Lip, palate, and nose deformities are caused by a disruption in normal development. The extent of the defect is dependent on the disruption time, severity and amount. For the formation of the primary palate and central lip, rapid cell division is required in the lateral nasal process region. The developing embryo is vulnerable to both genetic and environmental effects during this period (16).

Signaling pathways

With the development of technology, modern techniques including automated analyses have been used to study complex diseases. Whole genome/exome analyses, and also genome-wide association studies (GWAS) provide an opportunity to identify genetic factors that contribute to the development of NSCL/P. Different candidate genes have been reported in recent publications (1,5,18-20). GWAS also helps to identify rare, low-frequency coding variants (21,22). Besides point mutation, copy number variants (CNV), which disrupt the structure and also the regulatory region of the genes, can cause NSCL/P (23).

Signaling molecules and morphogens play a crucial role in mesenchymal proliferation and patterning during craniofacial development. These signaling molecules originate from the epithelial cells of the facial prominences and palate and create reciprocal epithelial-mesenchymal communications that is critical for palatal development (12). Wnt, *TGF/BMP*, Hedgehog, and Fgf-related signaling cascades are involved in these interactions, and mutations of the genes of these signaling pathways may be the underlying reason for CLP and CP (24).

MSX1, BMP4, BMP2, FGF10, and *SHOX2* are among the genes involved in anterior palate development. *BMP4* expression is regulated by a transcription factor called MSX1. *BMP4* regulates sonic hedgehog (Shh) expression in the palatal epithelium, and BMP2 expression takes place downstream of the Shh signaling cascades. Simultaneously, FGF10 uses the FGFR2 receptor to regulate the Shh signaling cascade in the palatal epithelium in a paracrine manner. Activated Shh promotes cell proliferation in the mesenchyme by using the BMP2 signaling cascade. These findings suggest that the growth of the anterior area of the palatal shelf is a very tightly controlled process, and BMP and FGF canonical pathways play a critical role in this process. However, less is known about the function of the genes expressed in the posterior area of the palate but it is understood that FGF8 is the first step in one of the pathways that promote the expression of PAX9 in the posterior area of the palatal mesenchyme. A deficiency in PAX9 results in a developmental defect of the palatal shelf and a cleft palate (CLP) (25).

Palatal shelf development defects are classified into five categories by Chai and Maxson (25): failure of palatal shelf formation due to mutations in *activin-βA* and *FGFR2;* a fusion of the palatal shelf with the tongue or mandible arising from *TBX22* mutations and loss of function mutations in *FGF10*; failure of palatal shelf elevation resulting from mutations in the *PAX9, PITX1,* and *OSR2* genes; failure of palatal shelves to meet after elevation as a consequence of mutations in *MSX1* and *LHX8, TGFBR2* in NCCs, or *Shh* in the epithelium; and persistence of medial edge epithelium caused by *TGFB3* and *EGFR* mutations (25).

Further hereditary investigations identified variants in the *MMP3, MMP25, TIMP2*, and *TIMP3* genes to be causative in the development of NSCL/P. Besides point mutations in coding regions, variants that affect functional promoter activity in *MMP3* and *TIMP2* have also been found to be related to NSCL/P (26,27).

As an oncogene, *FOS* promotes epithelial-to-mesenchymal transition, which is essential for craniofacial development (28). In embryonic development, apoptosis is an important mechanism for maintaining tissue homeostasis. *CASP8* is one of the vital genes involved in craniofacial development (29).

A gene list is provided in Table 1 related to NSCL/P and craniofacial development.

Epigenetics

Epigenetics is described as the regulation of gene expression through reversible chemical modifications without affecting the DNA sequence (61).

Among the best understood epigenetic modifications in animals are histone modifications, which regulate chromatin accessibility during transcription, and DNA methylation, which plays a critical role in many biological processes, and also contributes to the regulation of gene expression during palatal fusion (2,62).

The expression of the several genes that are associated with NSCL/P is controlled by epigenetic modifications. Epigenetically controlled genes include transcription factors (*LHX8, PRDM16, PBX1, GSC, VAX1, MYC*), growth factors and their modulators (*WNT9B, BMP4, EPHB2, BICC1, DHRS2*), and microRNAs (miRNAs) including *MIR140* and *MIR300* (63-67). Xu et al. (66) and Sharp et al. (68) reported methylation position variations in OFC subsets; and emphasized many methylation positions related to genes that differentiated between cleft lip with CLP, cleft lip only, and cleft palate only (CPO).

miRNAs have been reported to regulate the expression of 60% of genes encoding proteins (69), but abnormalities of expression are linked to a variety of diseases, including OFCs (70). An SNP in miR-140 was found to have a significant correlation with NSCL/P (71). Rattanasopha et al. (72) reported a role for miR-140 in PDGFRA regulation in association with human CPO. miR-140 was likewise found to control the expression of *BMP2* and *FGF9* genes in human palatal mesenchyme cells (73). These discoveries highlighted two significant focuses for craniofacial development: (a) Bmp signaling can be carried on by Smad factors and miRNA-17-92, and (b) miR-17-92 can have multiple effects by focusing on a few pathways, including TGF, FGF, Wnt, and others.

Environmental factors influence epigenetic modifications in both cells and organisms, which can result in different developmental outcomes (74). Van Rooij et al. (75) reported that maternal glutathione s-transferase genotype, and smoking as an environmental factor, increased the risk of CLP significantly. Joubert et al. (76) reported that maternal smoking was associated with differential methylation of some of the genes related to OFC, such as *MSX1, PDGFRA, GRHL3, ZIC2*, and *HOXA2*. Jugessur et al. (77) reported that alcohol dehydrogenase gene *ADH1C* variants are associated with clefting.

Human studies have also found that dietary folate plays a role in epigenetic-mediated CL/P (64). Gonseth et al. (64) conducted an epigenome-wide association study to investigate the correlation between epialleles and OFCs in the United States, before setting up mandatory folate treatment in 1998.

Prenatal evaluation

During diagnostic ultrasonography, the defined cleft lip is a direct imperfection stretching out between the lip side and the nostril. CLP with cleft lip might extend between the alveolar side and hard palate, reaching the nasal and oral cavities, and may also extend to the orbits. Diagnosis requires the use of both the transverse and coronal planes. During visualization of cleft and palate, color Doppler might be valuable in showing flow across the palate. The diagnosis of isolated CP is difficult. Even between 11 and 13 gestational weeks, diagnosis of CLP may be possible but mostly CLP is diagnosed by detailed ultrasound examination at 18-22 gestational weeks. Retronasal triangle and maxillary gap views should be obtained during ultrasonographic evaluation of the fetus in screening for OFCs. Magnetic resonance imaging may be an adjunct to prenatal diagnosis of CLP. After the prenatal ultrasound diagnosis of CLP, other system anomalies should be screened and invasive testing for karyotyping and microarray testing should be offered. Prenatal consultation with a multidisciplinary team, including clinical geneticists, should be performed during prenatal evaluation (78). Clinical geneticists take a detailed pedigree and family history followed by reviewing the ultrasonography findings. The finding can be isolated or associated with a specific syndrome (79). After genetic counseling and risk calculation, clinical geneticists decide about appropriate genetic tests (78). Karyotype analysis is necessary to exclude trisomy 13 and other chromosome abnormalities for fetuses with multiple abnormal ultrasonographic findings (80). If there is a suggestion of a specific syndrome due to the associated anomalies, targeted genetic studies including fluorescent in situ hybridization or multiplex ligation-dependent probe amplification can be performed before the microarrayor testing may proceed directly to microarray following the karyotyping. If these test results are normal, whole exome sequencing can be the next step to detect point mutations.

Prognosis relies upon the presence and kind of related abnormalities. If it is isolated, the prognosis is good and normal survival can be achieved with appropriate management. Surgical intervention is frequently performed between postnatal 3-6 months. The recurrence risk can be defined as 5% if one sibling or parent is affected and 10% if two siblings are affected in isolated cases. If the genetic alteration is disclosed

Table 1. A gene list related to NSCL/P and craniofacial in Table 1

Figure 1	Gene name and symbol	Chromosome locus	Mechanism	Phenotype	Reference
1	Orofacial cleft 1 *(OFC1/OFCC1)*	6p24.3		CL ± P CPO	(30)
2	Transforming growth factor-alpha/orofacial cleft 2 *(TGFA/OFC2)*	2p13	*TGF-α* polymorphisms have controversial results in previous studies suggesting the gene as a neighbor gene to the disease locus rather than a disease-causing gene	CL ± P	(31)
3	BCL3 transcription coactivator/orofacial cleft 3 *(BCL3/OFC3)*	19q13	*BCL3* plays a role in cell adhesion. Its downregulation results in disruption of facial development	CL ± P	(32)
4	Orofacial cleft 4 *(OFC4)*	4q21-q31	-	CL ± P	(33)
5	Msh homeobox 1/orofacial cleft 5 *(MSX1/OFC5)*	4p16.1	*MSX1* is a transcriptional repressor playing role in mesenchymal cell proliferation	CL ± P	(34)
6	Interferon regulatory factor 6/orofacial cleft 6 *(IRF6/OFC6)*	1q32.3-q41	Determines the keratinocyte proliferation/ differentiation switch It is assumed that the protein may have a transcriptional activator role Protein play role in midfacial clefting	CL ± P	(35)
7	Poliovirus receptor related1/ orofacial cleft 7 *(PVRL1/OFC7)*	11q23.3	*PVRL1* encodes a cell adhesion family protein and plays role in the development of palatal shelves	CL ± P	(36)
8	Tumor protein p63/orofacial cleft 8 *(TP63/OFC8)*	3q27	As a transcription factor plays a key role in epithelial lineage progression during and after development	CL ± P	(37)
9	Orofacial cleft 9 *(OFC9)*	13q33.1-q34		CL ± P	(38)
10	Small ubiquitin-like modifier 1/orofacial cleft 10 *(SUMO1/OFC10)*	2q33	Plays role in the control of nuclear transport, transcriptional regulation, apoptosis, and protein stability	CL ± P	(39)
11	Bone morphogenetic protein 4 /orofacial cleft 11 *(BMP4/OFC11)*	14q22	A regulatory molecule of several developmental processes including facial development	CL ± P	(40)
12	Orofacial cleft 12 *(OFC12)*	8q24.3	The gene contains cis-acting enhancers that direct Myc expression during facial development	CL ± P	(41)
13	Orofacial cleft 13 *(OFC13)*	1p33		CL ± P	(42)
14	Orofacial cleft 14 *(OFC14)*	1p31		CL ± P	(43)
15	Distal-less homeobox 4/ orofacial cleft 15 *(DLX4/ OFC15)*	17q21	As a transcription factor plays a critical role in the craniofacial development	CL ± P	(44)
16	Methylenetetrahydrofolate reductase *(MTHFR)*	1p36	Folate pathway	CL ± P	(45)
17	Cysteine-rich secretory protein LCCL domain containing 2 *(CRISPLD2)*	16q24.1	It shows its effect on facial development by disrupting the binding of regulatory elements and inhibiting protein expression. The facial developmental processes	CL ± P	(46)
18	CLPTM1 regulator of GABA type A receptor forward trafficking *(CLPTM1)*	19q13.32	Functioning as the regulator of GABA type A receptor	CL ± P	(39)
19	Fibroblast growth factor receptor 2 *(FGFR2)*	10q26.13	Play role in craniofacial osteogenesis and suture homeostasis	CL ± P	(47)
20	Frizzled-related protein *(FRZB)*	2q32	This is a secreted protein. It is involved in the regulation of bone development	CL ± P	(48)

21	Sprouty RTK signaling antagonist 1 *(SPRY1)*	4q28.1	Primary paralog of SPRY2 The encoded protein inhibits the FGF and MAPK pathways	CL ± P	(49)
22	Mitogen-activated protein kinase 3 *[MAPK3 (ERK1)]*	16p11.2	Disruption of this gene leads to OFCs in animal models	CL ± P	(49)
23	TUB-like protein 4 *(TULP4)*	6q25.3		CL ± P	(50)
24	SATB homeobox 2 *(SATB2)*	2q33.1	Communicates with transcription factors that regulate craniofacial development	CP	(51)
25	Meis homeobox 2 *(MEIS2)*	15q14	Transcription regulator contributes to developmental programs	CP	(51)
26	Stearoyl-CoA desaturase 5 *(ACOD4/SCD5)*	4q21.22	Stearoyl-CoA desaturase is located at the integral membrane of the endoplasmic reticulum	CL	(33)
27	Transforming growth factor beta 3 *(TGFB3)*	14q24	Tgf signaling contributes to the formation of secondary palate fusion	CL ± P	(52)
28	ATP binding cassette subfamily A member 4 *(ABCA4)*	1p22	Encodes an ATP-binding cassette transporter	CL ± P	(53)
29	MAF bZIP transcription factor B *(MAFB)*	20q12	Involved in the development and differentiation of keratinocytes	CL ± P	(53)
30	Bone morphogenetic protein 2 *(BMP2)*	20p12.3	Bmp2 and Bmp4 signaling is important for craniofacial patterning	CL ± P	(39)
31	Forkhead box E1 *(FOXE1)*	9q22	It contains a DNA- binding forkhead domain	CL ± P	(54)
32	T-box transcription factor 1 *(TBX1)*	22q11.21	Takes part in early progenitor cells relevant for palate development	CL ± P	(55)
33	Fos proto-oncogene, AP-1 transcription factor subunit *(FOS)*	14q24.3	Control epithelial-to-mesenchymal transition, a critical process during craniofacial development	CL ± P	(28)
34	Matrix metallopeptidase 2 *(MMP2)*	16q12.2	Plays role in extracellular matrix remodeling and subsequent fusion of the palatal shelves	CL ± P	(56)
35	Caspase 8 *(CASP8)*	2q33.1	Takes part in apoptosis, which is crucial to sustaining tissue homeostasis during embryonic development	CL ± P	(29)
36	Ventral anterior homeobox 1 *(VAX1)*	10q25.3	Transcriptional regulator containing a DNA- binding homeobox domain	CL ± P	(1)
37	SMAD-specific E3 ubiquitin-protein ligase 1 *(SMURF1)*	7q22.1	Negative regulator of BMP signaling pathway and controls cell motility, and polarity	CL ± P	(51)
38	Sprouty-related EVH1 domain containing 1 *(SPRED1)*	15q14	Negative regulator of the FGF and MAPK pathways	CL ± P	(51)
39	Grainy head-like transcription factor 3 *(GRHL3)*	1p36.11	Needed for periderm differentiation and assumes a part in palate formation	CP	(57)
40	T-box transcription factor 22 *(TBX22)*	Xq12	Plays role in the adhesion of opposing palatal shelves. It involves palatogenesis and plays a role as a transcriptional repressor	CP	(58)
41	Wnt family member 3 *(WNT3)*	17q21.31-q21.32	Epistatic interaction with COL2A1, as well as interactions with *FGFR1* and *MTHFR* genes in humans	CL ± P	(59)
42	SH3 and PX domains 2A *(SH3PXD2A)*	10q24.33	Involved in cell migration and matrix degradation	CL ± P	(60)
43	Paired box 9 *(PAX9)*	14q13.3	Transcription factor, with the critical role during fetal development	CL ± P	(35)
44	Paired box 7 *(PAX7)*	1p36.13	Transcription factor, with the critical role during fetal development	CL ± P	(41)

NSCL/P: Non-syndromic Cleft lip and/or palate, CL ± P: Cleft lip and/or palate, CPO: Cleft palate only

during genetic work up, genetic counseling should be given to the family about the recurrence risk after trying to determine the inheritance pattern together with the parental genetic evaluation.

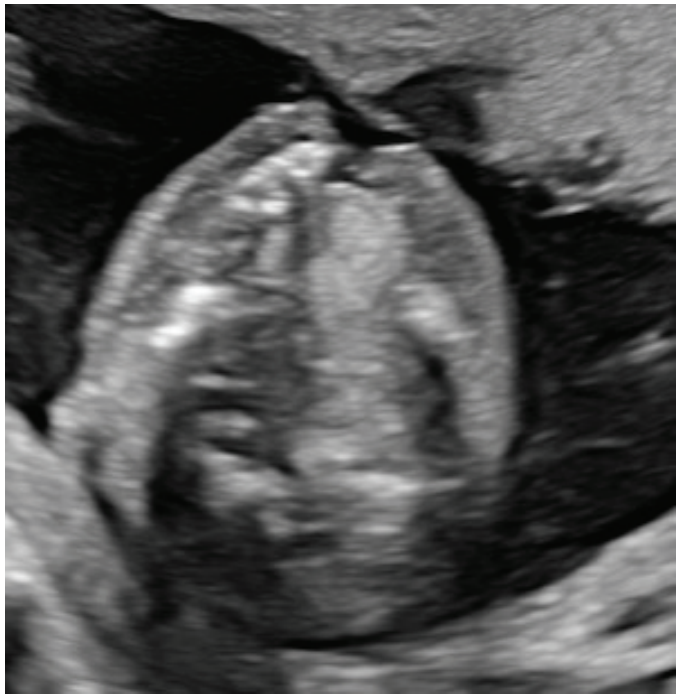

Figure 1. A fetus with a unilateral cleft lip and palate at the sixteenth gestational week

Conclusion

Pathogenesis of OFCs is complex and may frequently include hereditary and environmental interactions that are yet to be fully understood. As the condition is complex, epigenetic modifications may also contribute to the clinical condition if there is no defined genetic reason.

When a cleft lip and/or palate is detected by ultrasonography, in the absence of associated anomalies, the patient should be evaluated in consultation with the clinical geneticist, taking into account many genes and environmental factors involved in NSCL/P etiopathogenesis. A roadmap for possible prenatal genetic diagnosis should be devised, as genetic testing is an important component of pre- and post-natal management of cases.

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Pediatric and adolescent gynecology- a current overview

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Abstract

In pediatric and adolescent gynecology we encounter a number of diseases that occur solely during a specific phase of physical development. The diseases need some experience in the field, as well as an accurate diagnosis and are therefore often diagnosed somewhat late. The separation and traction technique is a painless method of inspecting the child's genitals. It is also effective and easy to perform. In contrast to a routine investigation in adults, very specific diagnostic questions require the insertion of a speculum, vaginoscopy, taking swabs for analysis, ultrasound investigations, or blood sampling in children. A number of diseases that occur frequently in prepubertal girls will be discussed. The etiology, clinical characteristics, treatment and prognosis of the following diseases will be addressed in detail: vulvovaginitis, lichen sclerosus, labial adhesions, ovarian torsion, abnormal uterine bleeding, uterine fibroids, and hypertensive disorders of pregnancy.

Keywords: Methods of investigation, phases of development, vulvovaginitis, lichen sclerosus, labial adhesions

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Introduction

A number of diseases are encountered in pediatric and adolescent gynecology, depending on the child's stage of development and the level of estrogenization. A few specific gynecological diseases concern only children, whereas others occur in adults as well, but are subject to a different pathogenesis and treated differently in children. Thus, the treatment of gynecological diseases in children and adolescents requires specialized knowledge of the subject.

Depending on their age, children and adolescents present with a variety of problems and questions in a doctor's medical office. The reason for seeking medical advice for prepubertal children are usually findings or problems in the outer genitals, such as vulvovaginitis, labial adhesions, lichen sclerosus, or unclear pruritus. In adolescents and pubescent individuals, the main focus of treatment is disorders of pubertal development, menstrual symptoms, menstrual disorders, especially primary and secondary amenorrhea, contraception, sexual relationships, and sexuality.

Many children are anxious and withdrawn at their first visit, and even subsequent visits, to the gynecologist. It is extremely important to gain the child's trust.

The following should be kept in mind from the very start:

The waiting room should be provided with a play corner and aligned to the needs of children. It would be advisable to keep the consultation hours for pediatric and adolescent gynecology at the start or the end of the regular consultation hours. Depending on the reason for the visit, the treatment of children and adolescents is much more time consuming than that of an adult. About 30-60 minutes should be allocated for the treatment of a child or adolescent.

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The pediatric gynecological investigation

Depending on the issue in question, the pediatric gynecological examination may be a whole-body investigation, as in cases of a suspected general disease or sexual violence. In the event of specific questions concerning the genitals alone, it may not be necessary to inspect the entire body. The child's medical checkup booklet should be reviewed. The patient's current height and weight should be noted on the appropriate percentile curve. A pleasant atmosphere should be created during pediatric and adolescent gynecological consultation hours. Treatment should be provided in a calm environment and without haste. The procedure should be explained using age-appropriate language to the child before it is performed. The process, purpose, and potential limits of the investigation should be explained to the parents or the accompanying person. If the child is defensive or refuses to undergo the investigation, she should not be compelled or held forcibly. The doctor's assistant or (pediatric) nurse who accompanies the investigation may try to divert the child's attention with water-filled syringes or an inflated glove. If this is not successful, the investigation may either be deferred to a later point in time or, depending on the urgency of the question and the severity of symptoms (such as starting sepsis in case of a suspected foreign body in the vagina), one might consider short-acting anesthesia. Handing out a small gift to the child at the time of her departure or after the investigation has proved to be a useful measure. A treasure chest filled with soap bubbles or small toys could serve as a motivation for the upcoming investigation.

Three positions of examination may be used for the inspection of the external genitalia:

- Supine with drawn legs; the "frog" position
- In the mother's or caregiver's lap
- On the gynecological chair in lithotomy position and/or knee-elbow position

Inspection of the external genitalia plays a key role in the gynecological investigation. The following entities must be evaluated: the labia majora and minora, clitoris, external urethral meatus, anus, perineum, posterior commissure, navicular fossa, the hymen and its margin, vaginal introitus, and the distal third of the vagina. While the speculum is mandatory for adequate evaluation of the vagina in adults, usually the so-called separation and traction technique is sufficient for prepubertal girls. The physician takes a light hold of the outer aspect of the labia majora or perianal region and exerts a slight pull in lateral-caudal direction. The genitals are thus unfolded. The abovementioned anatomical structures can be assessed without further aids (Figure 1).

Depending on the issue of investigation or symptoms, it may be helpful to perform more detailed investigations; however, these are definitely not a part of the routine examination (1):

Figure 1. The normal vulva. The separation and traction technique serves to view and inspect the external genitals

- Vaginal swab (bacteriology, virology)
- Microscopic investigation of vaginal secretion
- Vaginoscopy (foreign body, injuries, fistulae)
- Blood sampling (STD, inflammatory values, endocrinological diagnosis)
- Ultrasound of the abdomen (with a filled bladder)

Phases of development

Depending on estrogen levels, the development of a girl may be divided into various phases, each accompanied by specific pathologies (2). Table 1 shows the various phases of development, the corresponding age, hormonal characteristics, and respective disease syndromes.

A number of diseases that occur in girls of prepubertal age are listed below.

Vulvovaginitis

Vulvovaginitis is the most common disease in the non-estrogenized environment of the pediatric genitals. Inflammatory changes in the vulva are rather rare in puberty, but may occur in conjunction with a vaginal candida infection.

Etiology

Non-specific vulvovaginitis occurs in about 80% of cases. In other words, no specific pathogen is found. This condition is referred to as irritant contact dermatitis. In specific vulvovaginitis, on the other hand, one is able to isolate a specific pathogen. Quite often the patient undergoes a protracted period of unsuccessful treatment with local agents, especially antifungal substances. Vulvovaginits is frequently aggravated by poor hygiene, especially at an age when girls start to go to the toilet independently. Bacteria, such as *Escherichia coli, Enterococci, staphylococci, Proteus mirabilis* or *Pseudomonas aeruginosa* are found in many cases (3-5). The genitals of prepubertal girls have a predilection for vulvovaginits due to the absence of a labial fat pad, the thin and sensitive skin of the vulva, proximity to the anus, and a wide introitus. The physiological estrogen deficiency and an alkaline intravaginal pH favor the emergence of vulvovaginitis. In some cases it may coincide with respiratory infections; the path of transmission is usually an oral-genital smear infection (6). Obesity, tight clothing, or underclothes made of synthetic fiber favor the emergence and persistence of vulvovaginitis.

Clinical symptoms

The labia majora and minora, and in some cases the perineum and the perianal region, are reddened to a greater or lesser degree with vulvovaginitis. The patients present with a dried secretion, known as smegma and consisting of desquamated cells and/or urine residues, in the interlabial aspect. Whitish or yellowish-green vaginal discharge is the most frequent symptom of vulvovaginitis. The condition may be divided into an acute and a chronic stage. In the acute stage the vulva and the vaginal introitus are inflamed and reddened; the redness is marked by blurred margins. The labia minora and hymen are swollen due to edema. Smega, or in some cases stool residues, are found in the interlabial or in the perianal aspect. The chronic stage is marked by pink coloring with intermittent whitish zones and scratch marks. The vulva is significantly dry. The mildly reddened and dry, scaly region of the vulva usually includes the anus and has a rather clearly delineated margin.
Figure 2a shows the acute stage of vulvovaginitis while Figure 2b depicts chronic disease. The patients complain of an itching and burning sensation in the external genitals, especially during micturition.

Diagnosis

The first step is an exact medical history about the duration of symptoms, followed by documentation of the following:
- Previous therapies, such as antifungal agents and/or creams containing cortisone
- Comorbidities such as dermatosis

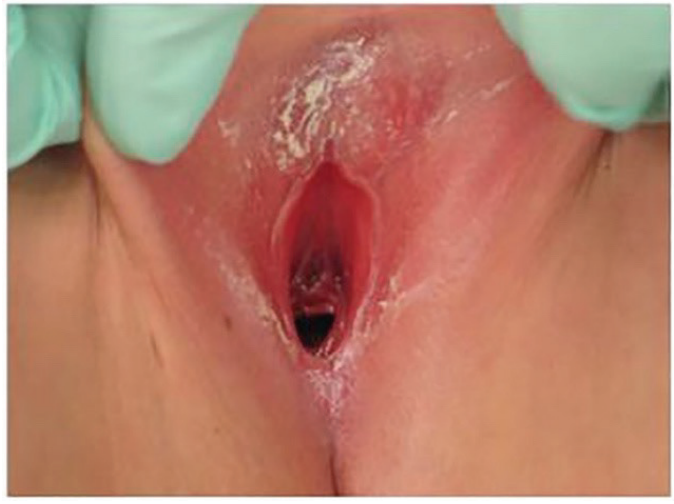

Figure 2a. Acute vulvovaginitis with marked reddening of the genitals

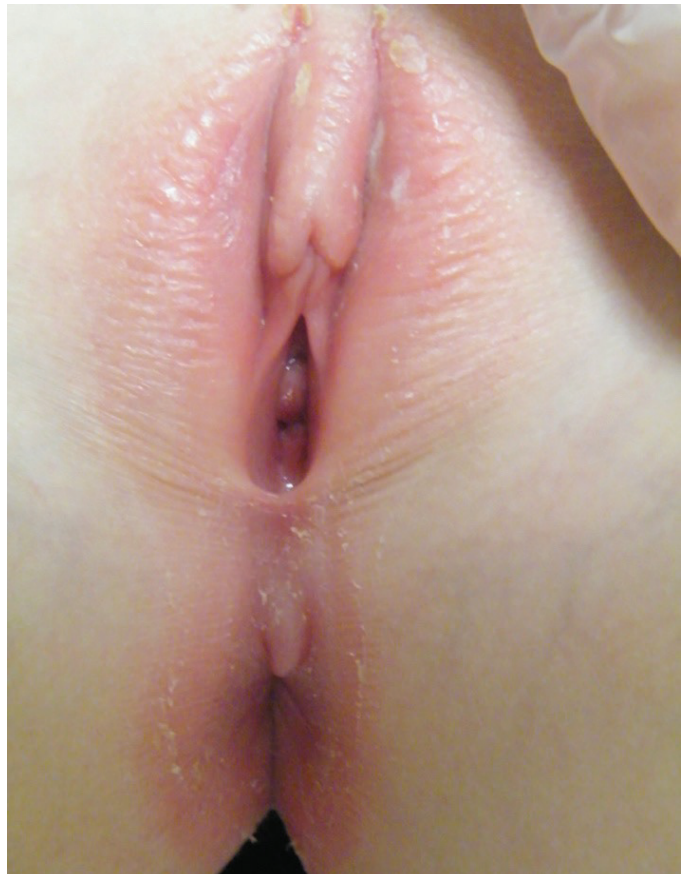

Figure 2b. Chronic vulvovaginitis with moderately reddened and dry, scaly areas

- Hygiene habits
- Current use of external agents
- Possibility of trauma or insertion of a foreign body

The following should be given attention during the inspection:
- Tanner stages (signs of puberty)

- Signs of dermatosis
- Detailed local findings (redness, scratch marks, bloody or putrid vaginal discharge, level of hygiene)

A rectal palpation and a vaginoscopy should only be performed in case of a suspected foreign body, but should be a routine procedure in patients with a bloody vaginal discharge.

Microbiological investigation of a swab provides evidence of specific pathogens only in about 50% of cases (7). In case of clinical symptoms, such as a white vaginal discharge or a distinct redness, a smear should be obtained. Care should be taken to ensure that the smear is taken from the vagina and not the introitus. Furthermore, as injury to the hymen would be very painful for a girl, the hymen should be left untouched as far as possible.

In terms of the differential diagnosis of non-specific vulvovaginitis, the following causes should be ruled out:

- Vulvovaginits due to specific pathogens (group A streptococci, trichomonas, chlamydia, genital herpes, genital warts, gonorrhea)
- Foreign body in the vagina (blood, putrid discharge)
- Dermatosis (lichen sclerosus, psoriasis, neurodermitis, Behçet's disease)

Pathogens are found in about a third of smears taken from symptomatic girls. The most common of these (about 60% of cases) are group A hemolytic streptococci (pyogenic streptococci or "anal scarlet fever") (4). This germ is considered highly pathogenic and should be treated systemically with penicillin.

Further pathogens include *Haemophilus influenzae, Klebsiella pneumoniae* and *Streptococcus pneumoniae* (7). Depending on the severity of symptoms, the patient may be given specific (oral) systemic therapy after an antibiogram has been obtained.

A candida infection is observed exclusively in the estrogenized epithelium (i.e. during puberty) or at the age when diapers are used.

Candida albicans is rarely isolated as a pathogen (1%) in girls with vulvovaginits prior to puberty (3,4,8). It should be noted that 12.5% of 3- to 9-year-old girls are asymptomatic carriers of candida. Pathological growth of candida with symptoms usually occurs only in cases of immunodeficiency, malignant disease, preterm birth, previous antibiotic treatment, or estrogenization. Nevertheless, at least one antifungal therapy had been administered in more than 80% of children with non-specific vulvovaginits (8).

Antifungal agents and antibiotics (local or systemic) are not indicated in unspecific vulvovaginitis.

Therapy

- Omit all external irritants, such as bath additives or washing lotions; dispense with wet wipes, wear comfortable clothing; adhere to consistent and careful genital hygiene, wash only with plain water, appropriate voiding behavior.
- Sitz baths with sodium chloride; local application of a neutral lipid-containing cream.
- Oral antibiotic treatment after obtaining an antibiogram; only if there is evidence of pathogens (as detailed above).

Lichen sclerosus

Lichen sclerosus is a chronic inflammatory autoimmune dermatosis with a predilection for the skin in the anus and the genital tract. About one in every 900 girls is affected by this autoimmune disease (9). The mean age of disease onset is 5 years, but the mean age at diagnosis is about 7 years (9). The disease is marked by two peaks, namely before puberty and after menopause.

Etiology and pathogenesis

The etiology of lichen sclerosus is largely unknown. A number of causes with an autoimmune component have been suggested. The autoimmune component induces a dysregulation of the immune system. In 4-14% of cases, it is associated with other autoimmune diseases, such as Hashimoto thyroiditis, vitiligo, alopecia areata, pernicious anemia, rheumatoid arthritis, or diabetes mellitus. A family history of autoimmune disease in parents or grandparents has been observed in more than 50% of cases (9,10).

Clinical symptoms

The principal symptoms are pronounced itching, especially in the evening and at night before falling asleep, as well as pain and a burning sensation in the vulva. Secondary symptoms include constipation, perianal symptoms with painful fissures, dysuria, and superficial bleeding. The diagnosis is frequently delayed by several years because of inappropriate treatment due to a suspected candida or bacterial infection. In a large number of cases, the patient will have undergone various treatments with local and systemic antibiotics and antifungal agents. When a patient presents with lichen sclerosus, sexual abuse is suspected in more than 70% of cases (9).

The local appearance of lichen sclerosus is a typical figure of eight, around the vulva and the anus. The skin is red, stretched, and has a porcelain-like shine. In the early stage one frequently finds perilabial and transverse grooves. Indurations/sclerosis and hyperkeratosis may also be observed. Patients in advanced stages may present with a whitish atrophy. Further characteristics include superficial ecchymosis (bleeding), hyperpigmentation or depigmentation, and rhagades. Figures 3a and b show the typical appearance of lichen sclerosus.

Complications occur, especially in advanced stages or in cases of non-diagnosed or inappropriately treated disease. These

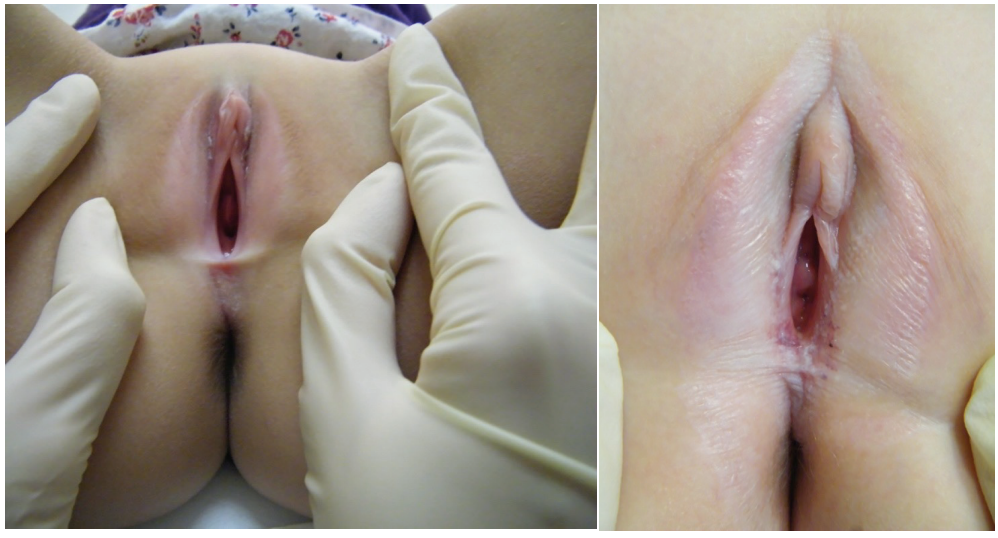

Figure 3a, b. Lichen sclerosus with the white discoloration characteristic of the disease, in a figure of eight around the vulva and anus

include atrophy, scarring, destruction of vulvar architecture, and chronic pain/vulvodynia (9).

Diagnosis

The diagnosis is derived from typical clinical findings and the symptoms described by the patient. In contrast to adults, the physician should never perform a biopsy of the whitish vulva in children to confirm the diagnosis.

Therapy

The first step is to institute or follow so-called "basic measures". These include the avoidance of local irritation, such as tight underclothes or underclothes made of synthetic fiber, careful genital hygiene, the use of non-perfumed, preservative-free detergents, and washing with plain water.

Specific first-line therapy consists of de-escalating treatment with ultrapotent topical corticosteroids (9,11,12), such as Clobetasol cream for 12 weeks. The following regimen has proven to be effective:

Week 1-4: once daily

Week 5-8: every two days

Week 9-12: twice a week

Corticosteroid therapy may be administered in shorter intervals. Regardless of the duration of treatment, it should always be tapered off slowly.

Maintenance therapy with local agents (such as Linola fat cream or Deumavan cream) and these basic measures should be used after, or even during, corticosteroid therapy.

Failure of corticosteroid therapy is extremely rare. If the symptoms do not improve despite cortisone treatment, the diagnosis should be reviewed first and possible differential diagnoses should be taken into account and/or ruled out, such as other types of dermatosis, atopic eczema, vitiligo, and/or infection. Once the corresponding differential diagnoses have been ruled out, the physician should check the parents' compliance.

Second-line therapy consists of topical calcineurin inhibitors from the group of immunosuppressants or immunomodulators (12).

- Pimecrolimus 1% cream for a maximum of 12 weeks or
- Tacrolimus 0.03% ointment for three weeks

Both substances are for off-label use.

In the event of recurrence, corticosteroids should be used again for seven days. The second treatment should be started no earlier than three months after conclusion of the last course. If the vulva is atrophic due to preceding corticosteroid therapy, it may be advisable to use calcineurin inhibitors instead.

Prognosis

Sustained complete remission is achieved in a mere 22% of children (13). Lichen sclerosus diagnosed before puberty reappears after puberty in 75% of cases (14,15). Fortunately, in children this condition is not associated with squamous cell carcinoma (SCC) or other neoplasms. In contrast, the risk of an adult developing a SCC in the context of lichen sclerosus is <5% (16,17).

Note: Delayed or inappropriate treatment of lichen sclerosus may destroy vulvar anatomy permanently.

Labial adhesions

Definition

Labial adhesion is defined as a fusion of the labia minora, which may be partial, subtotal, or complete. The adhesion

forms a thin and partly translucent membrane that closes the vulva.

Etiology and epidemiology

Labial adhesions may occur as a result of the physiological estrogen deficiency in prepubertal girls. Vulvitis, poor hygiene, or the use of wet wipes may also promote its emergence.

About 1.8% of girls between the ages of three months and four years are affected by this condition. The peak incidence is between the ages of 13 and 23 months (18).

Clinical symptoms

The disease is most frequently asymptomatic and is diagnosed in the course of a routine investigation. Depending on the severity of the adhesion, the labia will be adherent from the midline to the clitoris. Quite often, there is just a tiny opening above the urethra. The introitus and the hymen are covered by a parchment-like membrane. Figures 4a and 4b show the typical appearance of labial adhesions.

Complications

- Aberrant urinary stream
- Dribbling of urine after micturition; urine accumulates in the intravaginal aspect
- Asymptomatic bacteriuria (20%), urinary tract infection (20-40%)
- Very rare: obstruction with bladder distension and hydronephrosis

Therapy

Therapy recommendations are not based on randomized studies but only on retrospective data.

Basic therapy consists of careful and consistent hygiene, washing the genitals with warm water only, and the omission of external irritants (perfumed wet wipes, washing lotions, creams). Twice daily application of Linola fat cream or Deumavan cream is recommended.

Specific therapy consists of de-escalated application of an estriol cream (such as Oekolp or Ovestin) for four weeks, applied directly on the line of fusion with a cotton swab for 10 seconds under slight pressure. Care should be taken to ensure that the layer of cream is not too thin, as this may be ineffective. This should be followed by local application of a neutral lipid-containing cream.

Surgical or mechanical treatment, which would involve cleavage of the membrane, should be considered only when conservative treatment has failed and clinical symptoms persist, such as dribbling of urine or recurrent urinary tract infection. After treatment with estriol and under local anesthesia with EMLA cream, the line of fusion is separated mechanically. Depending on the child's behavior, it may be necessary to administer brief anesthesia.

Progress and prognosis

Spontaneous remissions within one year have been reported in 80% of cases (19).

If micturition is not hindered, one could dispense with treatment. The parents must be informed about the benign nature of labial adhesions and their self-limiting nature with the onset of endogenous estrogen production during puberty.

Success rates of 47-100% have been reported for estriol therapy (20,21); most studies report success rates in excess of 90%.

A wide range of recurrence rates have also been mentioned for estriol therapy (0-40%) (20,21). However, the majority of studies mention low recurrence rates (0-11%).

One alternative is a 0.05% betamethasone cream applied twice daily for two weeks. Similar results have been reported for this treatment (22), and in some cases even faster remissions, than those achieved with estriol (23). Betamethasone may be considered in cases of symptomatic recurrence or failure of estriol therapy.

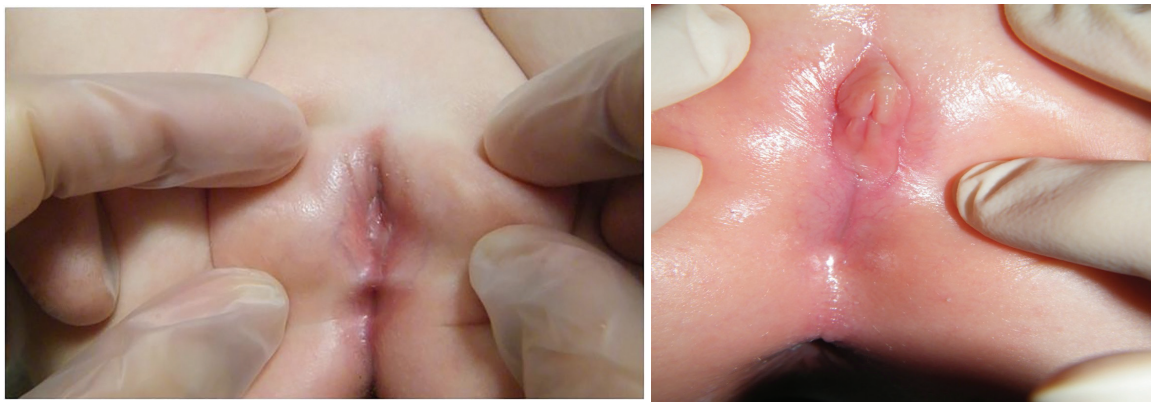

Figure 4a, b. Labial adhesions. Fusion of the labia minora, which form a thin membrane covering the introitus and the hymen

Table 1. Phases of development and typical diseases associated with different levels of estrogenization

Phase	Characteristics	Diseases
Neonates/infants	Active hypothalamic-pituitary-adrenal (so-called mini-puberty); elevated E2; elevated androgens	Labial adhesions, malformations, ovarian cysts
Pre-pubertal phase	The hypothalamic-pituitary-adrenal axis is subject to inhibitory influences → estrogen deficiency, alkaline vaginal pH, genital atrophy; activation of NNR from the age of 5 years → rise in DHEAS	Vulvovaginitis, lichen sclerosus, including dermatosis, labial adhesions, premature (partial) development: growth disorders
Onset of puberty	Activation of the hypothalamic-pituitary-adrenal axis	Premature onset of puberty, pain in the lower abdomen; vulvovaginitis
E2: Estradiol, NNR: Adrenal cortex, DHEAS: Dehydroepiandrosterone sulfate		

In addition to vulvovaginitis, lichen sclerosus and labial adhesions, which have been discussed in great detail, another four clinical pictures will be presented below. They are also important in pediatric and adolescent gynecology and their diagnosis and treatment should be known.

Ovarian torsion is a surgical emergency and occurs in 2.7% of all pediatric/adolescent patients (24). The vague clinical presentation and variable imaging findings make this diagnosis challenging. The patient suffers from acute abdominal pain which leads to an urgent investigation and often consecutive operative management with laparoscopic adnexal detorsion. The ovary should be preserved, even in case of a necrotic-appearing ovary, because studies persistently show follicular development and ovarian function after a short time period after detorsion and no increased patient morbidity. Furthermore, oophoropexy may be considered in case of severe necrosis (24,25). The appearance of the ovary does not correlate with long-term ovarian viability or function. The consensus recommendation for imaging surveillance following ovarian detorsion is an ultrasound at three months postprocedure but sooner if there is a concern for malignancy (26).

Abnormal uterine bleeding (AUB) is defined as any atypical genital bleeding originating from the uterine cavity, but without the characteristics of a normal menstrual period. This bleeding disorder can appear equally in adolescents and adults. AUB is a common problem which has significantly adverse effects on an affected adolescent's quality of life (27). The most common underlying condition in AUB in adolescence is anovulation. Although about 95% of AUB could be considered as a dysfunctional disorder, this clinical picture requires well-defined diagnostic procedures in order to detect a possible physical cause or ruling out complex or systemic diseases, including oncological ones (28). A complete gynecological evaluation (if possible) and a full physical examination are useful to detect any kind of general disease which can compromise the hormonal reproductive system. Auxiliary tools such as gynecological ultrasonography for pelvic examination are allowed in sexually-active women, otherwise abdominal or transrectal ultrasonography could be considered, if needed. Although observation is sufficient in the mild form of AUB, the first-line treatment consists of combined oral contraceptives and, when they are contraindicated, progesterone alone, medicated intrauterine devices, GnRH-analogues, or desmopressin are the most common second-line treatments (27,28).

Uterine fibroids are benign tumors originating from the smooth muscle of the myometrium and affect women mainly during their reproductive years. The symptoms and their severity may differ, depending on the size and location of the fibroids. The most common presenting symptom is heavy menstrual bleeding, which may lead to anemia, fatigue, or painful periods. Other possible symptoms include lower back pain, pelvic pressure or pain, and pain during intercourse (29). Fibroids are diagnosed in up to 70% of white women and increased age among premenopausal women is a risk factor for fibroids (29,30). Thus, this means that fibroids exist, but are relative rare in adolescence. Vitale et al. (31) wrote an interesting comment on "Laparoscopic Myomectomy of a Symptomatic Uterine Leiomyoma in a 15-Year-Old Adolescent". The authors support the use of robotic-assisted laparoscopic myomectomy, even in young patients, for better results in wound healing, anatomical reconstruction and fertility outcome later in life (31). Consequently, even in the rare case of symptomatic fibroids in adolescents, the treatment strategies are analogous to that of adults.

Another topic that should be discussed and should be kept in mind is the association between adolescent pregnancy and severe outcomes, such as preeclampsia, preterm premature rupture of the membrane, maternal anemia, sexually transmitted diseases, postpartum depression, and maternal death, and adverse neonatal outcomes, including low birth weight, prematurity, stillbirth, early neonatal demise, and low Apgar score (32). Rates of adolescent pregnancy are increasing in developing countries, with higher occurrences of adverse maternal and perinatal outcomes (33). The prevalence of preeclampsia and eclampsia should be especially noted. Macedo et al. (34) performed a systematic review and meta-analysis concerning this topic and included 30 countries and

291,247 adolescents between 1969 and 2019. The authors showed an overall prevalence rate of preeclampsia and eclampsia in adolescents of 6.7% [95% confidence interval (CI) =5.8-7.6]. Subgroup analysis revealed association of preeclampsia and eclampsia (p=0.050) and eclampsia (p=0.0113) with country income, and the highest prevalences were found in low- and medium-income country groups (11.5%, 95% CI =7.8-15.8 and 10.6%, 95% CI =6.05-16.2) (34). Consequently, a strategy of close-meshed care and precaution is indispensable in cases of adolescent pregnancy.

Conclusion

The investigation should be explained calmly to the child and/or the accompanying person. The examination should be conducted in a quiet atmosphere with no exertion of force or compulsion on the patient. If necessary, another appointment could be fixed for a later point in time. Antifungal agents or antibiotics (local or systemic) should not be used in cases of non-specific vulvaginitis. Lichen sclerosus, when the diagnosis is delayed or inappropriately treated may destroy vulvar anatomy permanently (note: mean age at disease onset 5 years, mean age at diagnosis 7 years). Labial adhesions are a benign self-limiting disease. In cases of undisturbed micturition, one may dispense with treatment altogether. Ovarian torsion is a surgical emergency and should be treated with laparoscopic adnexal detorsion and, if necessary, oophoropexy. AUB is defined as any atypical genital bleeding originating from the uterine cavity. The most common underlying condition in AUB in adolescence is anovulation. Adolescent pregnancies are associated with severe outcomes, like preeclampsia and eclampsia. Uterine fibroids are quite rare in adolescents but may lead to the same symptoms as adults suffer from. Laparoscopic myomectomy or even robotic-assisted laparoscopic myomectomy are advisable therapy options.

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What is your diagnosis?

A 38-year-old lady presented with a painful swelling in the umbilicus, together with a history of increased pain and bleeding from the swelling at the time of menstruation for the last seven months. Her menstrual cycles were regular, with average flow and no dysmenorrhea. She had two living children, both were delivered vaginally. There was no history of pelvic pain, infertility, treatment for infertility, pelvic/abdominal surgery, or caesarean section. Examination revealed a 1.0x0.5 cm firm, tender, reddish-blue colored nodular swelling in the abdominal wall, located just inferior to the umbilical ring with well-defined margins and a regular surface (Figure 1). Pelvic examination was essentially normal with a multiparous-sized uterus that was anteverted, mobile, and non-tender. Both fornices were free and non-tender. The rectovaginal septum was free and there were no nodules in the pouch of Douglas.

Ultrasound revealed a well-defined, hetero-echoic lesion with a peripheral rim of colour lying infra-umbilically, superficial to the rectus sheath. The same lesion appeared hyperintense on T1/T2 magnetic resonance imaging (MRI) with post-contrast enhancement. The abdomen and pelvis were found to be normal on MRI.

The patient was taken up for surgical excision of the nodule. Radical omphalectomy was performed. A peri-umbilical incision was made. Umbilicus, underlying nodule, and the surrounding area of fibrosis were dissected with a 5 mm clear margin using diathermy (Figure 2). The patient is on follow-up and is free of the disease at 18 months after surgery.

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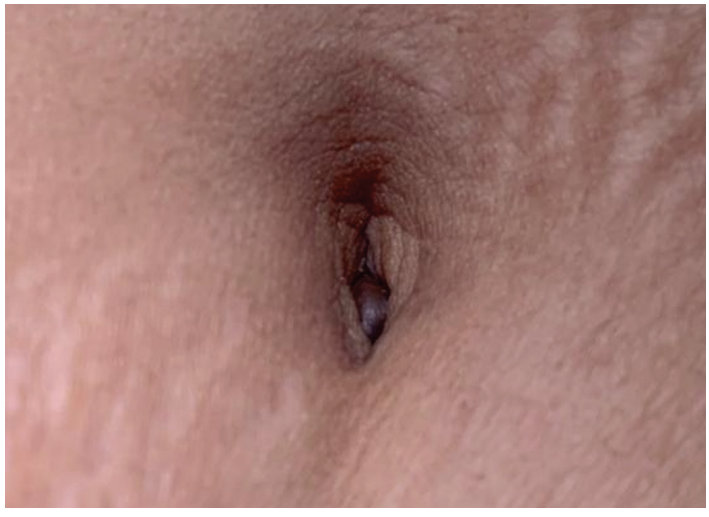

Figure 1. Clinical picture of umbilical endometriosis: bluish-purple firm umbilical swelling or nodule with associated cyclical pain

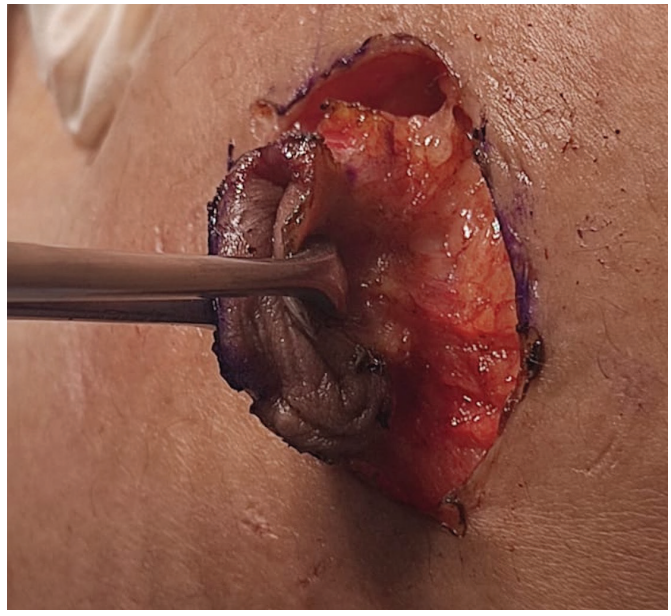

Figure 2. Radical omphalectomy

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Answer

Histopathological examination of the umbilical nodule revealed plenty of endometrial glands surrounded by compact stroma and intervening hemorrhage, confirming the diagnosis of umbilical endometriosis (PUE) (Figure 3). This was a rare case of Villar's nodule or PUE without concomitant pelvic endometriosis and in the absence of previous pelvic surgery.

PUE is extremely rare, making up 0.5-1% of all ectopic endometriosis cases (1). Extension of the endometrial cells to the umbilicus via the round ligament or the omphalo-mesenteric canal may explain the occurrence of PUE. Hematogenous or lymphatic transport of the endometrial cells is another possible mechanism that supports the existence of PUE (2).

PUE usually presents around 35-40 years of age. Clinical presentation includes a bluish-purple, firm, umbilical swelling or nodule with associated cyclical pain. Patients can even show catamenial bleeding from the umbilicus concomitantly with the menstrual cycle (3). Diagnosis is suspected when there is a typical history of an increase in pain/bleeding through the nodule at the time of menstruation. However, diagnosis can be confirmed only by histopathological examination (1,2).

Imaging modalities, such as ultrasound, MRI, or computed tomography scan are not superior in terms of sensitivity to the clinical scenario and examination findings (4). However, imaging can help assess the anatomical relationship of the nodule with the surrounding tissues and to rule out other differential diagnosis of umbilical lesions, like a desmoid tumor, lipoma, Sister Mary Joseph's nodule, teratoma, trichobezoars, umbilical concretions, and hernia, for example (5). Imaging can also help to investigate the anatomical relationships of the nodule with the surrounding tissues (5).

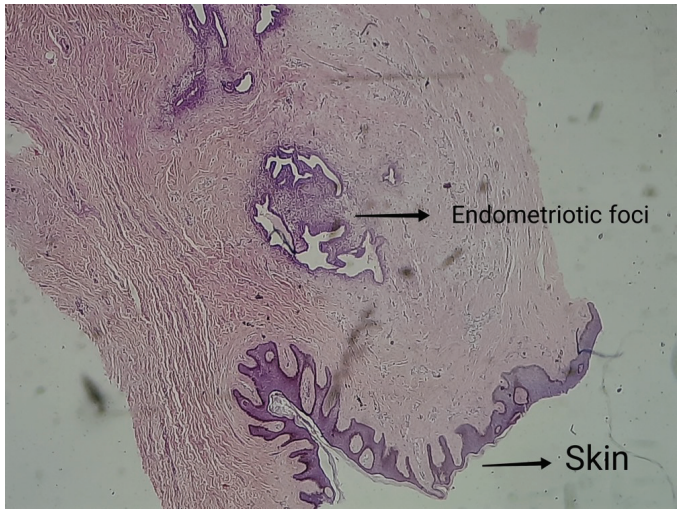

Figure 3. Sections from umbilical mass showing stratification squamous epithelium with the subepithelium showing endometrial glands and stroma

Surgery remains the mainstay of treatment. Medical management using progestins, danazol, and/or gonadotrophin releasing hormone agonists may be tried, but recurrence rates are high (5). Radical omphalectomy is the most frequently performed surgery for umbilical endometriosis. This involves the removal of the umbilicus with the nodule along with plastic reconstruction. Partial omphalectomy is local resection of the endometrial nodule with umbilical sparing. It is important to ensure disease-free margins of at least 3 mm to prevent local recurrences. Sometimes a deep-seated nodule may also necessitate removal of the underlying rectus sheath, which may require anatomical repair or mesh placement.

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What is your diagnosis?

A 39 year-old woman, P2L2, presented to the emergency department with vaginal bleeding. She was diagnosed as having a molar pregnancy one and half months previously, for which surgical evacuation was performed at a private clinic. Beta-human chorionic gonadotropin (β-hCG) level before evacuation was 40,738 mIU/mL and follow-up values followed a declining trend from 3,820 mIU/mL 48 hours after evacuation, to 1,342 mIU/mL after one week. Histopathological examination (HPE) ruled out any possibility of malignancy. During follow-up, she had an episode of severe vaginal bleeding on the fifteenth day after evacuation. Magnetic resonance imaging of the pelvis suggested uterine arteriovenous (A-V) malformation with the possibility of residual gestational trophoblastic disease (GTD), in view of medical history and raised β-hCG (1,083 mIU/mL) (Figure1a-c). She received a blood transfusion and single-agent chemotherapy (methotrexate) at her primary centre, however, her β-hCG values continued to fall. After one month, she had another episode of severe vaginal bleeding and was referred to our centre. Here, her vitals were stable and systemic examination showed no abnormality. Gynecological examination revealed normal vulva, vagina, cervix, and soft and enlarged uterus of 14 weeks size. β-hCG was 23.55 mIU/mL. Transvaginal sonography showed thin endometrium and a cystic lesion in the uterine fundus invading the anterior myometrium, which was hyper-vascular on colour Doppler, suggestive of A-V malformation (Figure 2a,b). Therefore, bilateral uterine artery embolization (UAE) was performed. The patient was then discharged in a stable condition. After one week of UAE, she again presented with severe vaginal bleeding. She had pallor, pulse rate of 110/minute, and blood pressure of 90/60 mmHg. After thorough discussion, a plan for emergency hysterectomy was made, and written informed consent was taken.

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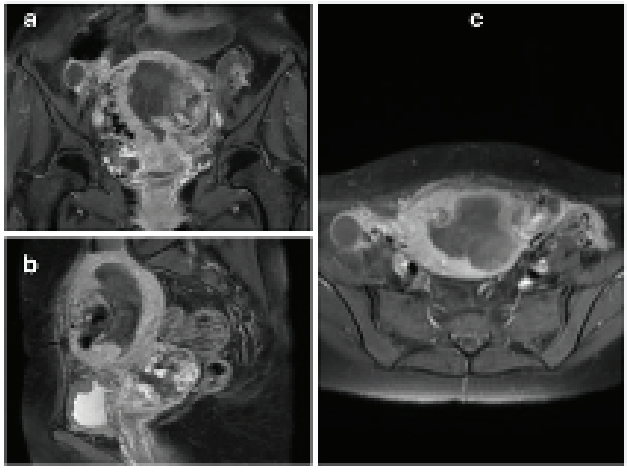

Figure 1. MRI of pelvis showing an enhanced bunch of vascular flow, void size ~53.8x88.8x79.3 mm, involving the anterior aspect of the fundus of the uterus, compressing and displacing the irregular endometrium posteriorly: a) sagittal; b) coronal; and c) axial view
MRI: Magnetic resonance imaging

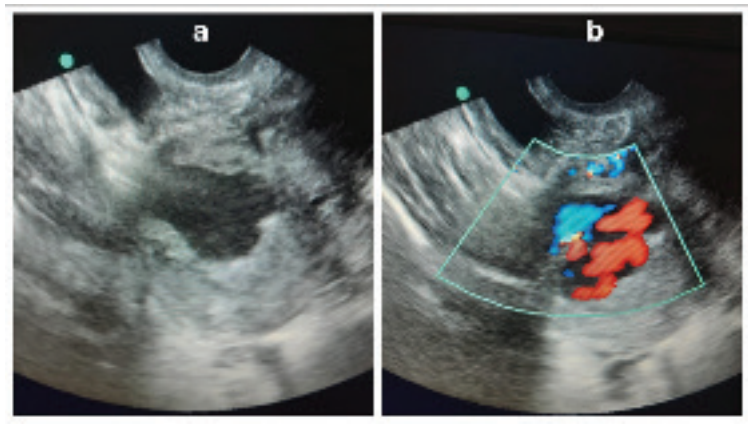

Figure 2. Transvaginal sonography. (a) This showed thin endometrium and cystic lesion in the uterine fundus invading the anterior myometrium. Color Doppler (b) showed hyper-vascularity, suggestive of A-V malformation

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Answer

Total abdominal hysterectomy was performed under general anaesthesia. Intra-operatively, the uterus was 14 weeks size, regularly enlarged, and bilateral fallopian tubes and ovaries were healthy. Cut section of the uterus showed an irregular endometrial cavity with thick-walled cysts involving endomyometrium filled with blood clots, but the uterine serosa was intact (Figure 3). The postoperative course was uneventful. Her β-hCG was undetectable (<5 mIU/mL) on day 1 after surgery. The final HPE was suggestive of placental site trophoblastic tumor (PSTT) (Figure 4a-d). After this HPE diagnosis, no distant metastasis was found on CECT abdomen, pelvis and thorax. She had FIGO stage 1 disease and The International Federation of Gynecology and Obstetrics/World Health Organization prognostic score was 0. Postoperative management was discussed with medical oncology and the decision to follow-up by monitoring β-hCG was taken. Currently, she is on monthly β-hCG follow-up and has had no relapse for the last six months.

Discussion

PSTT is a rare malignant tumor with an incidence of between 1/50,000-1/100,000 pregnancies and 0.23-3% of all GTDs (1). PSTT often occurs after a normal term pregnancy (61%), or less commonly after molar pregnancy (12%), and occasionally after miscarriages, ectopic pregnancies, stillbirths, and preterm deliveries (2). In this case, PSTT was diagnosed a few weeks after molar pregnancy. Sometimes, PSTT is diagnosed even after years of antecedent pregnancy. A median delay of 13 months (range 0-240) was reported by Alexander et al. (3).

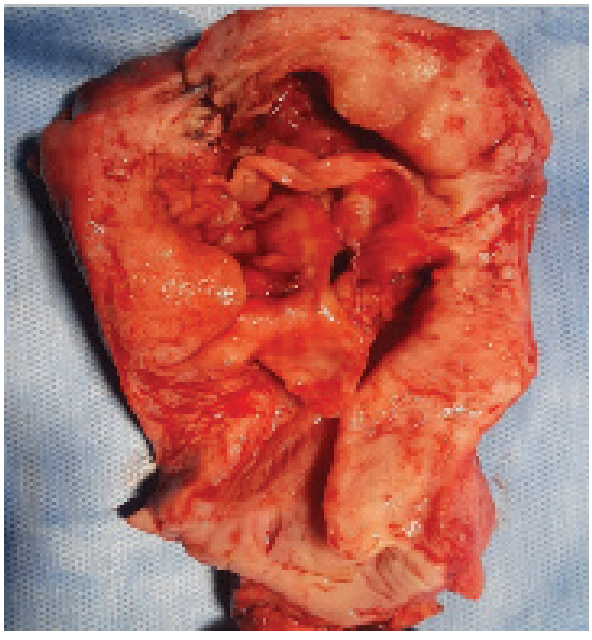

Figure 3. Irregular endometrial cavity with thick walled cyst involving endomyometrium in the cut specimen of uterus

The diagnosis of PSTT is usually difficult as it lacks specific and sensitive tumor markers, and radiological diagnostic criteria, and can be confirmed on HPE only. PSTT originates exclusively from the proliferation of the intermediate interstitial trophoblast and is characterised by the absence of villi and a mild mitotic activity (4). β-hCG level remains low in PSTT due to the absence of syncytiotrophoblast (5). Apart from HPE, immunohistochemical markers play an important role in differentiating it from other intermediate trophoblast tumor-like epitheloid trophoblastic tumor. Human placental lactogen, epidermal growth factor, and vascular endothelial growth factor stains strongly positive, β-hCG generally stains weakly and is focally positive, cytokeratin stains diffuse positive, and human epidermal receptor 2/neu and cluster of differentiation 117 stain negative in PSTT (6,7).

Surgery remains the cornerstone of management, with primary hysterectomy being the optimal therapy (8,9). Ovaries should be conserved unless there is a family history of ovarian cancer or the patient is post-menopausal. PSTT tends to metastasise through lymphatic vessels with a reported incidence is 5.9% (6). Therefore, lymphadenectomy is recommended in stage I PSTT with >50% myometrium invasion and in advanced stages (II or more) (6). The impact of complete abdominal and pelvic lymphadenectomy on overall survival is yet to be elucidated. Some patients with metastatic disease or a high mitotic index might require adjuvant platinum based multi-agent chemotherapy.

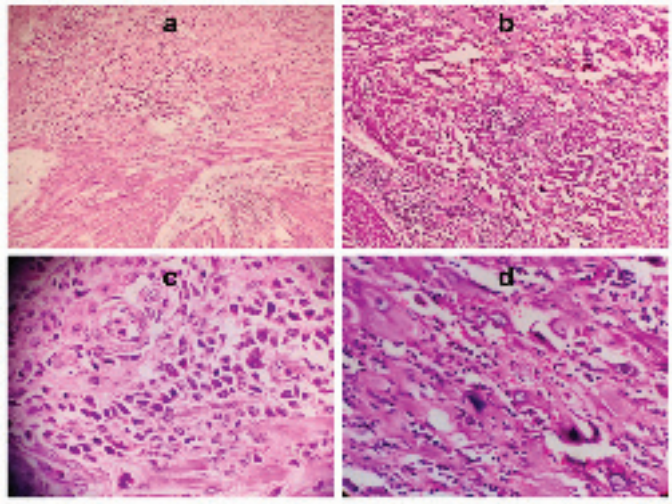

Figure 4. a) Endomyometrium showing areas of necrosis and hemorrhage (H&E, x200); b) Sheets and cords of mononucleated tumor cells infiltrating through the myometrium (H&E, x200); c) Higher magnification shows round to polyhedral intermediate trophoblastic cells with high nucleocytoplasmic ratio and eosinophilic cytoplasm *(H&E, x400); d) Scattered multinucleated bizarre cells (H&E, x400)*

PSTT is mostly confined to the uterus (stage I) and has a good prognosis. However, extra-uterine disease leads to a poor prognosis (10). Additionally, it has a poor prognosis in contrast to other GTDs, which are exquisitely chemosensitive. PSTT is relatively unresponsive to chemotherapy (11).

In our case, the disease was confined to the uterus and there was no evidence of metastasis. A total hysterectomy was performed, lymph node sampling could not be done as we did not suspect a malignancy, and the disease was limited to the uterus. The patient is being closely followed up with β-hCG and routine clinical checkups on regular basis.

Unlike other GTDs, follow-up in PSTT cannot be done with β-hCG alone, particularly in cases with very low β-hCG at presentation. Clinical examination and imaging to be considered to detect recurrence. Lok et al. (12) suggested β-hCG level weekly monitoring for six weeks (after normalisation), followed by monthly for 12 months and then less frequently for 10 years.

In this case there was a diagnostic challenge; PSTT or uterine A-V malformation. History, examination, and imaging findings showed that distinguishing between PSTT and A-V malformation is clinically challenging. Therefore, in these cases we suggest a strong suspicion should be kept for PSTT.

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Tips and tricks for adnexal lifting and mobilisation during laparoscopic cystectomy

To the Editor,

We read the article entitled "Ovarian suspension loop: an assembled device for ovarian lifting and immobilization during laparoscopic cystectomy" by Tahermanesh et al. (1) with a great deal of interest. During laparoscopic cystectomy, lifting and immobilisation of the adnexa is essential. This should be performed in way that minimizes injury to the utero-ovarian and/or infundibulo-pelvic ligament or the ovary and tube themselves. We would like to congratulate the authors for their brilliant idea, as their technique is cheap, easily reproducible and safe.

We would also like to highlight similar approaches in order to achieve lifting and immobilization of the adnexa. Recently, Chatzipapas et al. (2) proposed laparoscopic cystectomy in a bag using temporary sutures that pass through the abdominal wall and under the brim of the Endo Bag™. By this means, the cyst-harboring adnexa is stabilized by placing the suture string below it. Moreover, in laparoscopic ovarian cystectomy, suturing the infundibulo-pelvic ligament is a good idea, but by using an Endo Bag™ for this purpose the overall cost of the treatment is increased. If the patient is not willing to bear the extra expenses, then the use of umbilical tape can be proposed, which in comparison with an Endo Bag™ is much cheaper. Similarly, Thompson et al. (3) used the Carter-Thompson CloseSure System for ovarian suspension. Another option could be the use of T'LIFT adnexa retraction and suspension (4). Interestingly, a recent study reviewed the safety and efficacy of temporary ovarian suspension using absorbable sutures (5). No intraoperative complications, including bleeding, infection, haematoma or bowel herniation, were reported in the study, while fertility and delivery rates were not affected (5). It should also be highlighted that the adnexa can be identified in its anatomical location after the suture's absorption.

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Selection bias, a caveat in gestational weight gain research

To the Editor,

In epidemiological studies on gestational weight gain (GWG), the selection bias burden due to a mismatch between the selected and eligible target population remains unclear and underexplored. It is, therefore, critical to explore the plausible sources of selection bias to ensure rigor in epidemiological estimates determining associations between GWG and other parameters of interest. GWG is the difference between pre-delivery weight and first trimester or pre-pregnancy weight, which has emerged as a burning research topic due to its independent association with adverse perinatal outcomes, such as large for gestational age and macrosomia (1). Selection bias can happen due to the pathophysiological and clinical complexities associated with GWG. This letter highlights some of these scenarios that require a calibrated study population selection approach to minimize the selection bias risk in future GWG studies.

I begin with the Institute of Medicine 2009 guideline, (2) a popular prepregnancy body mass index-based recommendation of GWG ranges and patterns, widely used in population-based epidemiological studies. It's critical to identify and exclude pregnant females with the following characteristics from the eligible study population, as this guideline may not apply to them due to inadequate evidence: Aborigines; preeclampsia; gestational diabetes mellitus; different obesity subclasses; and triplet and higher-order pregnancies (2-4). Besides, some physicians believe that the recommendations for overweight and obese women are too high (4).

Then, what are the conditions or situations in which GWG measurements are at risk of reverse causation bias? for example in gestational diabetes mellitus (GDM), a late metabolic complication of pregnancy characterized by hyperglycemia, GDM treatment with a calorie-restricted diet, for instance, can alter the GWG course. Besides, variation in the treatment can cause differences in GWG patterns among patients suffering from the same ailment. For example, while weight loss may occur in GDM patients compliant with non-pharmacological interventions, the opposite can happen in insulin-treated GDM patients. Pre-existing health conditions can also determine the GWG pattern because of the disease course itself or its treatment, as may be seen in thyroid dysfunction and Stein-Leventhal syndrome.

Next, it's essential to distinguish pregnancies prone to GWG fluctuations. For instance, women with preeclampsia, a pregnancy-induced hypertensive condition associated with proteinuria, may present with decreased weight gain in early pregnancy due to inadequate intravascular plasma volume expansion and increased weight gain in late pregnancy because of excessive vascular permeability and edema (due to oncotic pressure drop) (2).

Other factors which can influence GWG measurements during a prospective longitudinal follow-up of a pregnant cohort include abnormal amniotic fluid volumes (e.g., oligohydramnios), shorter or longer duration of pregnancy (e.g., preterm delivery), social factors (e.g., smoking), and genetic makeup of the mother (5).

Taken together, all these factors highlight the importance of selection bias evaluation in GWG studies. Therefore, cautious, well-rationalized, and knowledge-based research protocols are required for GWG research to produce unbiased, robust, and generalizable research findings.

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Hemoperitoneum from adenomyoma in unscarred non-gravid uterus

To the editor,

Adenomyosis is characterized by the presence of endometrial gland and stroma in the uterine musculature. It is reported to affect 25-60% of reproductive age women (1). Affected women usually present with dysmenorrhea, heavy menstrual bleeding or chronic pelvic pain. Internal bleeding, hemorrhage and hemoperitoneum due to adenomyosis in non-gravid uterus are all extremely rare and can be easily overlooked in an emergency situation. Furthermore, delay in diagnosis can increase the risk of morbidity and mortality. Hence, acute abdomen in the context of hemoperitoneum in reproductive age women requires a prompt diagnosis.

We report a case of a 34-year old woman who presented with complaints of lower abdominal pain, along with bleeding per vaginum for two days. Over the next several hours, she experienced repeated episodes of pain of variable intensity. Past medical history was unremarkable except for receiving treatment for pelvic inflammatory disease (PID) and ovulation induction drugs in the past. She denied any previous abdominal surgeries.

On examination, she was clinically unstable (temperature normal, moderate pallor, blood pressure 94/56 mmHg and heart rate 104 beats per minute). Abdominal examination revealed tenderness over the right iliac fossa with guarding. On pelvic examination, forniceal fullness was noted bilaterally with slight vaginal bleeding.

Urinary pregnancy test came back negative. Laboratory results showed low hemoglobin at 8.7g/dL, undetectable beta-Human chorionic gonadotropins (β-hCG) at <2 mIU/mL and normal coagulation profile. Abdominal ultrasound revealed a bulky uterus with 4x3.2 cm isoechoic structure with a cavity adjacent to the right coronual region suggestive of rudimentary horn, and bilateral ovaries adherent to the postero-lateral wall of uterus. A massive amount of free fluid was noted in the pelvic cavity containing a few echogenic foci, suggestive of internal bleeding. So, under the impression of hemoperitoneum of uncertain origin, we proceeded to undertake a diagnostic laparoscopy.

On laparoscopy, the uterus and the bilateral tubo-ovarian complex were firmly adhered to lower anterior abdominal wall. An organized mass of size 7x5 cm was noted attached to the fundus of the uterus and blood was seen trickling from the mass (Figure 1A). The mass was excised and sent for histopathological examination. Hemoperitoneum of about 1,500 mL was evacuated and adhesiolysis was performed to restore the anatomy.

The patient's postoperative period was uneventful and she was discharged on the second postoperative day. Histopathological examination of the excised mass reported adenomyoma (endometrial glands embedded in myometrium) (Figure 1B).

Hemoperitoneum in the context of acute abdomen necessitates prompt action. Common aetiologies are ruptured ectopic pregnancy, corpus luteal cyst, ovarian cyst, vascular rupture and hepatic or splenic rupture (2). Adenomyoma rarely present with hemoperitoneum, especially in non-gravid uterus, and only a few cases have been reported in the literature (1,3). Although the exact cause of vessel rupture remains unclear, venous congestion along with the increased friability of vessels associated with chronic PID or endometriosis have been proposed as the predisposing factors (4). Sudden increase in venous pressure due to uterine contraction or increased intra-abdominal pressure may trigger the rupture of these friable vessel (5). In the present case, we assume that PID (evident from the presence of intra-pelvic dense adhesions and past history of PID) in conjunction with the adenomyosis may have contributed to the rupture of vessels. Preoperative diagnosis was difficult, given the rarity of the entity. Ultrasound remains the first imaging modality for evaluation, though in this case the ultrasonographic diagnosis was unexplained hemoperitoneum.

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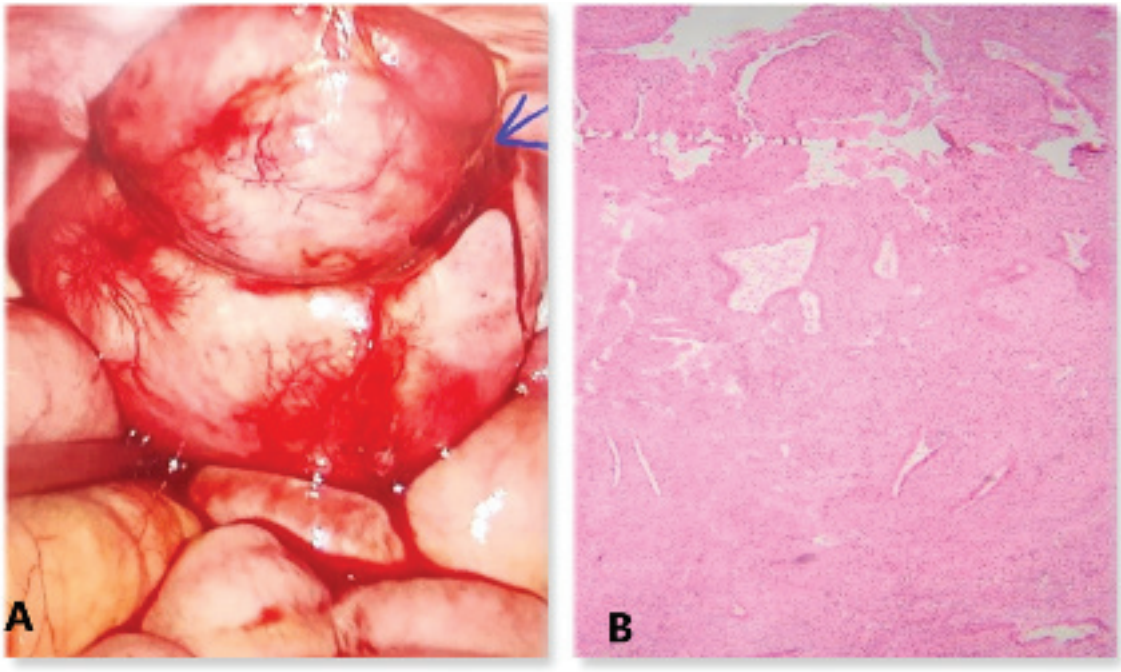

Figure 1. (A) A 7x5 cm organized mass attached to the fundus of the uterus, arrow showing trickling of blood from the mass. (B) Hematoxilin and eosin stained section from tumor tissue showing endometrial glands embedded in the myometrium (x100)

Computed tomography would be helpful in identifying bleeding vessels and to exclude other intra-abdominal pathology. Surgery, either hysterectomy or adenomyomectomy, remains the mainstay of treatment. Zhang et al. (3) reported a similar case of hemoperitoneum caused by bleeding from adenomyosis which was managed with hysterectomy. However, in the present case we performed uterine preserving surgery as the patient was young and desirous of pregnancy.

Though extremely rare, the possibility of adenomyoma should be kept in mind, while evaluating cases of hemoperitoneum in reproductive age women. Early surgical intervention is recommended to establish the diagnosis, control the hemorrhage and decrease the associated morbidity and mortality.

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Laparoscopic radical hysterectomy and total vaginectomy for vaginal malignant melanoma with cervical metastasis

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Abstract

The presented case is a 63-years-old multiparous woman admitted with the complaint of postmenopausal bleeding. On gynecologic examination multifocal lesions were detected, including 1 cm on lateral vaginal wall, 4 cm on posterior vaginal wall and 0.5 cm on the left lateral part of the cervix. Histopathology examination gave a diagnosis of epithelioid malignant melanoma. Consequently, laparoscopic radical hysterectomy and total vaginectomy with bilateral pelvic and inguinofemoral lymph node dissection were planned. On both sides, pararectal and paravesical spaces were created and the ureter was identified. Then, the vesicouterine and vesicovaginal spaces were developed. Uterine artery and superior vesical artery were coagulated, cut and the lateral parametrium was prepared. The left ureter was dissected and the ureteral tunnel was unroofed up to the bladder entrance. Subsequently, the anterolateral parametrium was transected. Then, the infundibulopelvic and sacrouterine ligaments were sealed and transected. At this time, the rectovaginal space was developed. Bilateral paracolpos were transected. The endopelvic fascia with the levator muscles were sealed and cut circumferentially. Anteriorly, the pubovesicocervical fascia was transected and the bladder was mobilized up to the uretrovesical junction. Thereafter, through a vaginal approach, the cervix and vagina were inverted by grasping the cervix with a tenaculum. An incision on the posterior vaginal wall at the introitus was made and the urogenital diaphragm was dissected to connect with the pelvic cavity. The vaginal entrance was cut circumferentially and the surgical specimen was extracted. In conclusion, laparoscopy can be considered as a feasible approach for radical hysterectomy and total vaginectomy in selected patients.

Keywords: Laparoscopic radical hysterectomy, total vaginectomy, vaginal malignant melanoma

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Introduction

Genital malignant melanomas constitute 1.6% of all melanomas occurring in women. Vaginal malignant melanomas constitute 0.3% of all malignant melanomas and less than 3% of all vaginal carcinomas (1). It is predominantly observed in the sixth and seventh decades of life (2). Surgery (vaginectomy, hysterectomy and lymphadenectomy) is the cornerstone of treatment (3). The primary goal of these excisional procedures is to avoid local recurrence, which mainly occurs in the vagina (3). The present case was a 63-year-old multiparous woman admitted with the complaint of postmenopausal bleeding. On gynecologic examination, multifocal lesions were observed, including 1 cm on the lateral vaginal wall, 4 cm on the posterior vaginal wall and a 0.5 cm lesion on the left lateral part of the cervix. The pelvic organs were evaluated as normal with bimanual examination and transvaginal ultrasonography. A whole-body positron emission tomography-computed tomography scan was performed and no distant metastasis or tumor infiltration was evident. Histopathological assessment confirmed a diagnosis of epithelioid malignant melanoma.

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Consequently, laparoscopic radical hysterectomy and total vaginectomy with bilateral pelvic and inguinofemoral lymph node dissection were planned. At laparoscopy a 10 mm trocar was inserted via intraumbilical vertical incision. Three 5 mm ancillary trocars were used; one on the left lateral of the umbilicus, and two in the left and right lower abdominal quadrants. After entrance to the abdomen, all the pelvic structures and abdomen were explored. On both sides, the broad ligament was incised, pararectal and paravesical spaces were created and the ureter was identified. The uterovesical peritoneum was opened, vesicouterine and vesicovaginal spaces were developed. Uterine artery and superior vesical artery were coagulated, cut and the lateral parametrium was prepared, on both sides. The right ureter was dissected and ureteral tunnel was unroofed up to the bladder entrance. Subsequently, the anterolateral parametrium was transected. Then, the infundibulopelvic and sacrouterine ligaments were sealed and transected. Then the rectovaginal space was developed and the rectovaginal septum was transected and dissected to the pelvic floor. Bilateral paracolpos were transected. The endopelvic fascia with the levator muscles were sealed and cut circumferentially. Anteriorly, the pubovesicocervical fascia was transected and the bladder was mobilized up to the uretrovesical junction. Ultimately, when the pelvic diaphragm was sealed and cut to the perineum and vestibulum, the laparoscopic phase was completed. Using a vaginal approach, the cervix and vagina were inverted by grasping the cervix with a tenaculum. An incision on the posterior vaginal wall at the introitus was made and the urogenital diaphragm was dissected to connect with the pelvic cavity. Thereafter, the vaginal entrance was cut circumferentially under finger guidance, taking care to preserve the urethra, and the surgical specimen was extracted (Figure 1). Finally, the vaginal entrance was sutured. The surgical procedure is presented in Video 1.

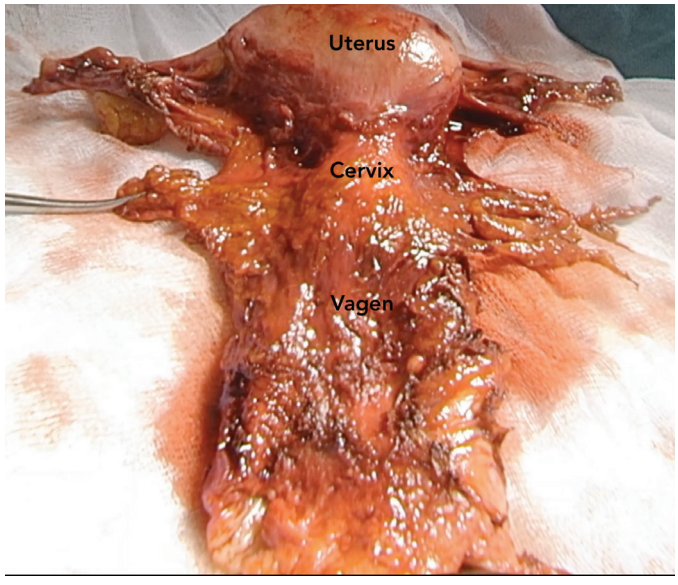

Figure 1. Surgical specimen

The patient was discharged on postoperative day 7 without any complications. Seventeen inguinofemoral and 30 pelvic lymph nodes were resected and none were metastatic. Surgical margins were negative within >10 mm. No adjuvant treatment was recommended. In conclusion, laparoscopy can be considered as a feasible approach for radical hysterectomy and total vaginectomy in such appropriate patients.

Video 1. Laparoscopic radical hysterectomy and total vaginectomy for vaginal malignant melanoma with cervical metastasis

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology)

March 16-18, 2023	9th Congress of the Society of Endometriosis and Uterine Disorders (SEUD), Abu Dhabi, UAE
March 19-22, 2023	Society for Gynecologic Surgeons (SGS) 49th Annual Meeting, Tucson, Arizona, United States
March 21-25, 2023	Society for Reproductive Investigation (SRI) 70th Annual Scientific Meeting, South Brisbane, Quennsland, Australia
May 02-06, 2023	15th World Congess on Endometriosis, Edinburgh, Scotland Great Britain
May 03-05, 2023	14th European Congress on Menopause and Andropause, Florence, Tuscany, Italy
May 04-07, 2023	ASCCP 2023 Scientific Meeting, Houston, Texas, United States
May 07-10, 2023	World Congress of Perinatal Medicine, Milano, Italy
May 19-21, 2023	American College of Obstetricians and Gynecologists (ACOG) 2023 Annual Clinical and Scientific Meeting, Baltimore, Maryland, United States
May 21-25, 2023	American Society for Reproductive Immunology (ASRI) Annual Meeting 2023, Santa Fe, New Mexico, United States
May 24-27, 2023	International Society of Gynecological Endocrinology 21st World Congress, Bali, Indonesia
June 06-09, 2023	The Society of Obstetricians and Gynecologists of Canada Annual Clinical Scientific Conference, Ottawa, Ontario, Canada
June 21-24, 2023	International Urogynecological Association (IUGA) 48th Annual Meeting, The hauge, South Holland, Netherlands
June 25-28, 2023	European Society of Human Reproduction and Embryology (ESHRE) 39th Annual Meeting, Copenhagen, Denmark
September 10-13, 2023	International Federation of Fertility Societies (IFFS) World Congress, Athens, Greece
October 14-18, 2023	American Society for Reproductive Medicine (ASRM) 79th Annual Meeting, New Orleans, LA, United States
October 16-19, 2023	33rd ISUOG World Congess, Seoul, South Korea
October 18-22, 2023	19th World Congress on Menopause, Melbourne, Australia
November 05-07, 2023	International Gynecologic Cancer Society (IGCS) 2023 Meeting, Seoul, Souh Korea
November 05-09, 2023	The 52nd American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Nashville, Tennessee, United States
November 23-25, 2023	The 31st World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Vienna, Austria

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2022.com)

March 05-08, 2023	17. Uludağ Jinekoloji ve Obstetrik Kış Kongresi ve 1. Marmara Kadın Sağlığı Kongresi, Bursa, Türkiye
March 16-19, 2023	CİSEF 3. Uluslararası Cinsel Sağlık Kongresi, Antalya, Türkiye
March 17-18, 2023	11. İstanbul Kadın Doğum Günleri, İstanbul, Türkiye
April 17-21, 2023	20. Ulusal Jinekoloji ve Obstetrik Kongresi, K.K.T.C.
June 02-06, 2023	6. Karadeniz Jinekoloji ve Obstetrik Kongresi, Trabzon, Türkiye
June 09-11, 2023	1. Akdeniz Jinekoloji ve Obstetrik Kongresi, Adana, Türkiye
June 21-24, 2023	6. Minimal İnvaziv Jinekolojik Cerrahi Kongresi, İstanbul, Türkiye
October 05-08, 2023	5. Jinekoloji ve Obstetrikte Tartışmalı Konular Kongresi, Muğla, Türkiye
October 20-22, 2023	11. Ulusal Menopoz Osteoporoz ve Kadın Sağlığı Kongresi, İstanbul, Türkiye
October 25-28, 2023	Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği Ultrasonografi Kongresi, İstanbul, Türkiye
November 01-05, 2023	10. Üreme Tıbbı ve Cerrahisi Derneği Kongresi, Antalya, Türkiye
November 11-12, 2023	Çukurova Kadın Doğum Günleri, Adana, Türkiye
November 16-19, 2023	11. Üreme Sağlığı ve İnfertilite Kongresi, Antalya, Türkiye