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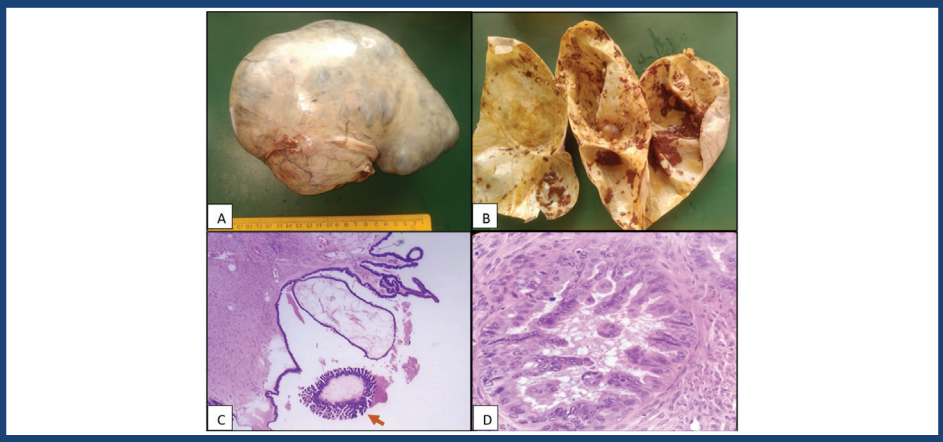

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Cover Picture: Mangla et al. What is your diagnosis?

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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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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

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Below the abstract provide 3 to 5 Keywords. Abbreviations should not be used as Keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the third 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 two reviews that we hope you will read with interest. Also you may have the oppurtunity to read the quiz. Here we share some of our favorite articles that were published in this issue of the journal.

Pelvic organ prolapse affects many women. Laparoscopic sacropexy is the gold standard therapy for apical prolapse. Banerjee and Noé originally introduced laparoscopic pectopexy as a more recent substitute for sacropexy in 2011. You will read an article comparing laparoscopic pectopexy with the standard laparoscopic sacropexy in women with symptomatic apical prolapse.

The most prevalent monogenic hemoglobin disorder in the world is beta-thalassemia. Repeated blood transfusions are used to treat this condition, which was once life-threatening. Hypogonadotropic hypogonadism may develop from the buildup of hemosiderosis in the hypothalamus and pituitary gland, which is also brought on by frequent blood transfusions. You will also read an article which evaluates the alterations in the ovarian reserve of beta-thalassemia patients over a time period of one year.

You will also have the opportunity to read a review discussing the importance of molecular classification for endometrial carcinoma.

Dear Participants,

I am very proud to say that "Congress on the Synthesis of Holistic and Modern Approaches in Gynaecology" was held in İstanbul on 2-3 June 2023 with a great success with more than 500 registered participants. Holistic medical practices in the area of obstetrics and gynecology were discussed at the congress and were enhanced by the insights, advice, and experiences of the entire faculty. I would like to thank all the participants once again for the time and dedication they gave to this event.

Dear Esteemed Readers, Authors and Reviewers,

I am proud to share the official Citescore value of the JTGGA for 2022. According to Scopus data for 2022, JTGGA's Citescore value has been determined as 2.1. This value demonstrates a noticeable progress compared to our previously announced Citescore value of 1.7 in 2021. Clarivate has also released the 2022 impact factor ratios of journals indexed in SCIE, SSCI, AHCI, and ESCI and updated its yearly Journal Citation Reports (JCR). More than 21,000 journals from 114 countries and 254 research categories are included in the JCR. Impact factor is a measure of the frequency with which the "average article" in a journal was cited in a given year or time period. As a result, the number of citations in the current year is divided by the number of source papers published in that journal in the previous two years to get a journal's impact factor (JIF). This year, with a JIF of 1.4, the JTGGA is ranked 98/128 in the obstetrics and gynecology category. We sincerely appreciate your support and confidence. We are advancing eagerly to keep expanding and providing value in the field we work in.
Please visit us online at www.jtgga.org and keep in touch with us by following us on Twitter @JtggaOfficial.
We are looking forward to receiving your valuable submissions, thank you in advance for your contributions.
Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of J Turk Ger Gynecol Assoc
President of TGGF

Comparison of laparoscopic pectopexy with the standard laparoscopic sacropexy for apical prolapse: an exploratory randomized controlled trial

Kavita Khoiwal, Kanhu Charan Dash, Amrita Gaurav, Jaya Chaturvedi
Department of Obstetrics and Gynecology, All India Institute of Medical Sciences (AIIMS), Rishikesh, India

Abstract

Objective: To compare laparoscopic pectopexy with the standard laparoscopic sacropexy in women with symptomatic apical prolapse.

Material and Methods: An interim analysis of an exploratory randomized controlled trial with the primary objective of comparing mesh fixation time and secondary objectives were to compare total operating time, blood loss, and intra-operative and post-operative complications. Additionally, patients completed the Prolapse Quality of Life (P-QOL) and Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12) questionnaires before surgery and during six months follow-up visit to evaluate the overall improvement in quality of life and sexual function. Patient Global Impression of Improvement (PGI-I) score was calculated on the 7-10th day post-operatively and then at six months to assess the level of improvement.

Results: The study included 30 patients; 15 underwent laparoscopic sacropexy, and 15 underwent laparoscopic pectopexy. Baseline characteristics were comparable in both groups. The mean duration of mesh fixation was significantly less with laparoscopic pectopexy (45.00±11.34 minutes) than laparoscopic sacropexy (54.67±9.35 minutes) (p=0.019). The total operating time and blood loss tended to be less in the pectopexy group, but not significantly so. Only one patient in the pectopexy group had a bladder injury. No patient in either group had any post-operative complications. One case in each group had a relapse of apical prolapse. All the domains of PISQ-12, P-QOL, and PGI-I scores improved significantly after both procedures.

Conclusion: Laparoscopic pectopexy is a safe, feasible, and comfortable alternative procedure to the standard sacropexy for apical prolapse. We noted significantly less mesh fixation time and less operating time, while blood loss tended to be less with laparoscopic pectopexy than with laparoscopic sacropexy. Post-operative parameters were comparable between techniques. Both corrective techniques for prolapse improved the PGI-I, P-QOL, and PISQ-12 scores. (J Turk Ger Gynecol Assoc 2023; 24: 144-51)

Keywords: Laparoscopic pectopexy, laparoscopic sacropexy, apical prolapse, mesh fixation

Received: 27 December, 2022 **Accepted:** 19 April, 2023

Introduction

Pelvic organ prolapse (POP) is a common disorder in women. According to Women's Health Initiative data, the prevalence of anterior wall prolapse is 34.3%, posterior wall prolapse is 18.6%, and uterine prolapse is 14.2% of women (1). The gold standard treatment for apical prolapse is laparoscopic sacropexy (2).

Laparoscopic pectopexy is a newer alternative to sacropexy, first described by Banerjee and Noé (3) in 2011. It is associated with fewer complications, shorter hospital stay, more rapid recovery, and safer operative field. In pectopexy, the mesh is fixed at the lateral areas of the bilateral ilio-pectineal ligaments and the apex of the vaginal vault or anterior cervical wall. The mesh follows the natural anatomical structure (round and broad ligament) to maintain the physiological axis, far from the ureter, bowel, and hypogastric vessels.

In laparoscopic sacropexy, the mesh is placed between the sacral promontory or anterior longitudinal ligament and the vaginal vault or the posterior cervical wall. It leads to narrowing of the pelvis, adhesions, or injury to the hypogastric nerves,

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which might cause chronic pain and defecation disorders (4). Pre-sacral bleeding is the most concerning intra-operative complication of sacropexy and may have life-threatening effects. Incidence of de novo defecation disorders and stress urinary incontinence (SUI) are greater with sacropexy (5). Furthermore, sacropexy becomes more challenging in obese women, due to enlargement of the sigmoid colon by fatty tissue, which can easily be obviated with the alternative technique; pectopexy.

The ilio-pectineal ligament is statistically much stronger than the sacrospinous ligament and the arcus tendinous of the pelvic fascia (6). The cranial anchor point for creating a physiological axis of the vaginal canal should be at the level of S2. The S2 level corresponds to the height of the lateral part of the ilio-pectineal ligament.

Keeping in mind the advantages of laparoscopic pectopexy, a study was planned in an Indian scenario to compare laparoscopic pectopexy with the standard laparoscopic sacropexy in women with symptomatic, apical prolapse. The primary objective was to compare the average time for mesh fixation, and the secondary objectives were to compare intra-operative parameters, and peri- and post-operative complications during laparoscopic pectopexy and laparoscopic sacropexy.

POP is known to significantly affects a woman's quality of life (QoL) and sexual health, which is expected to be improved by prolapse corrective surgery. Validated questionnaires, such as the Prolapse Quality of Life (P-QOL), Pelvic Organ Prolapse/ Urinary Incontinence Sexual Questionnaire (PISQ-12), and Patient Global Impression of Improvement (PGI-I) were used to investigate the effectiveness of both techniques (7,8).

Material and Methods

This was an exploratory, randomized, controlled trial conducted in the department of obstetrics and gynecology at a tertiary care center to compare laparoscopic pectopexy with the standard laparoscopic sacropexy in women with symptomatic apical prolapse. Prior permission for data analysis was obtained from the All India Institute of Medical Sciences (AIIMS) Institutional ethical board (AIIMS/IEC/20/823, date: 21.11.2020).

Women with apical prolapse of pelvic organ prolapse-quantification (POP-Q) > stage 2 (vault prolapse or uterine prolapse) who agreed to participate were included in the study. Both reproductive age-group women and postmenopausal women were included. Women with active pelvic inflammatory disease, history of vaginal prolapse corrective surgery, current pregnancy, history of premalignant or malignant diseases of uterus, cervix, or adnexa, any contraindication for laparoscopic surgery, patient unfit for anesthesia or not willing to comply with the protocol were excluded from the study.

The primary objective was to compare the average time for mesh fixation in laparoscopic pectopexy versus laparoscopic sacropexy. The secondary objectives were to compare intra-operative parameters such as operation time, blood loss, and peri- and post-operative complications during both techniques. Patients answered P-QOL and PISQ-12 questionnaires before the surgery and during six-month follow-up visits to evaluate the overall improvement in QoL and sexual functions. PGI-I score was calculated on the 7-10th day post-operatively and then at six months to assess the level of improvement.

Sample size calculation was performed using G Power 3.1.9.2. The sample size was calculated based on comparison of mean operation time in the two groups for a two-tailed test in a randomized controlled trial by Noé et al. (9). Using an alpha error of 0.05 and a power of 80%, the sample size was identified as 28 in each group. After assessment of eligibility criteria, patients were randomized by computer-generated random number allocation, and underwent the assigned surgical procedure. This report contains an interim analysis of 30 patients with apical prolapse (POP-Q > stage 2) who met the eligibility criteria over a period of 18 months (October 2020 to March 2022).

A detailed history was obtained, followed by a thorough examination, including POP-Q for prolapse staging. Routine pre-operative work-up was performed, and informed written consent was taken for surgery. Baseline characteristics, such as age, body mass index, parity, and socio-economic status, were recorded for all the patients.

Laparoscopic sacropexy (group A) was performed in 15 women [sacro-colpopexy (n=13), sacro-hysteropexy (n=2)]. In group B, 15 women underwent laparoscopic pectopexy [pecto-colpopexy (n=9), pecto-hysteropexy (n=6)]. The same surgeon performed all surgery. The surgery was documented using surgical notes and intra-operative videos. An additional procedure, such as anterior colporrhaphy, was performed in patients with stage 3 cystocele. Bilateral tubal ligation was performed simultaneously in patients with an intact uterus who opted for it as a permanent contraceptive method.

Surgical technique

All surgery was performed under general anesthesia, in the low dorso-lithotomy position with both arms next to the patient. After the creation of pneumoperitoneum with veress needle, a 10 mm supra umbilical port was inserted to introduce a 30-degree laparoscope. Under vision, three side ports were inserted; two 5 mm ports on the left for working instruments and one 5 mm on the right side for assistance. A uterine manipulator (in cases of the intact uterus) or a ring forceps with sponge (in hysterectomised patients) was introduced trans-vaginally at the beginning of the procedure for vaginal manipulation during surgery.

Laparoscopic sacropexy

During this procedure, the uterus (sacro-hysteropexy), or vaginal vault (sacro-colpopexy) was fixed to the anterior longitudinal ligament of the sacrum (S1-S2).

The peritoneum over the sacral promontory was opened, and the anterior longitudinal ligament was exposed. Then, the peritoneal incision was extended towards the pouch of Douglas up to the cervico-uterine junction in between the right ureter and rectum. A type-1, monofilament, macroporous, polypropylene mesh was used for uterine suspension. The single mesh (15x3 cm) was first fixed to the posterior cervix and uterosacral ligaments and then to the anterior longitudinal ligament at the level of S1-S2 with a 2-0 ethibond (polyethylene terephthalate) suture. The cervix level was checked by vaginal examination and its position was confirmed at or approximately 1 cm above the ischial spines. The peritoneum over the mesh was closed using a 2-0 vicryl (polyglactin) suture.

In cases of sacro-colpopexy, a Y-shaped mesh (15x3 cm) was prepared to cover the anterior and posterior walls of the vault, sutured with 2-0 ethibond. Then the vault was suspended to the sacral promontory, as described above.

Laparoscopic pectopexy

During this procedure, the uterus (pecto-hysteropexy), or vaginal vault (pecto-colpopexy) was fixed to the bilateral ileo-pectineal ligament.

Initially, the vesico-vaginal fold was opened, and the bladder was pushed down. The peritoneal layer parallel to the bilateral round ligaments was opened toward the pelvic sidewalls, one by one. The ileo-pectineal ligament (Cooper ligament) can be identified as a white glistening ligament adjacent to the insertion of the ilio-psoas muscle. The iliopectineal ligament was recognized at the base of the triangle, which is surrounded by the round ligament, external iliac vein (cranial/ventral), and obturator nerve (dorsal/caudal). The peritoneal layer was opened towards the vaginal apex on both sides, and the vaginal apex was prepared both anteriorly and posteriorly for the mesh fixation. With an intact uterus, the lower uterine segment was prepared anteriorly for mesh fixation. A polypropylene, monofilament mesh (15x3 cm) was fixed to the vaginal apex or anterior lower uterine segment and both iliopectineal ligaments in a tension-free manner with intracorporeal suturing, using a non-absorbable, ethibond 2-0 suture. Finally, the peritoneum over the mesh was covered with an absorbable 2-0 vicryl suture.

Outcome measures were mesh fixation time, total operating time, blood loss, and occurrence of major complications. The duration of mesh fixation was measured from the first to the last stitch for mesh attachment, and the duration of surgery was calculated as time taken from first skin incision to the last skin suture. Additionally, duration of hospital stay, hemoglobin (Hb) decline, and visual analog score (VAS) for pain was noted in the immediate post-operative period. Hb decline was calculated in both groups by subtracting post-operative Hb from pre-operative Hb.

Follow-up was maintained over six months in all patients, with data recorded at two time points, the first at 7-10 days and the second at six months post-operatively. Bladder and bowel dysfunctions, wound-related complications, new onset lower abdominal pain/ backache/ buttock pain, dyspareunia, relapse of apical prolapse > stage 2, de novo occurrence of anterior and/or lateral defect, and cystocele or rectocele were documented on each follow-up visit.

All of the patients underwent a PGI-I survey post-operatively on the first and last follow-up visits. Satisfaction with the surgery was queried on between seven and ten days post-operatively.

The validated P-QOL questionnaire was used both pre-operatively and post-operatively at six months in all women. The PISQ-12 questionnaire was used only in sexually active women with an intact uterus.

Statistical analysis

SPSS, version 21 (IBM Corp., Armonk, NY, USA) was used for data analysis. Descriptive statistics were documented as means ± standard deviations and median ± IQRs for continuous variables and frequencies and percentages for categorical variables. A $p<0.05$ was considered statistical significant.

Results

A total of 30 patients, 15 in each group, were included in this interim study. Both groups were comparable with respect to socio-demographic characteristics, as shown in Table 1. The mean age was 50.33±12.11 years (range: 33-70 years) in group A and 46.53±11.54 years (range: 30-61) in group B, which was similar in both groups.

Intra-operative parameters

Table 2 shows the intra-operative parameters of both groups. The mean duration of mesh fixation was significantly less in laparoscopic pectopexy than in standard laparoscopic sacropexy ($p=0.019$). Average blood loss and operating time tended to be less in laparoscopic pectopexy than sacropexy, but not significantly so. Only one patient, who underwent laparoscopic pectopexy, had a bladder injury, which was repaired intra-operatively (Clavien-Dindo complication classification 3b). No patient in any group required blood transfusion or conversion to another approach.

Post-operative parameters

Table 3 shows the post-operative parameters in the immediate post-operative period, and at the first and last follow-up visit.

Table 1. Socio-demographic characteristics of both groups

Parameters	Group A (n=15)	Group B (n=15)	p
Age (years)	50.33±12.11	46.53±11.54	0.506[1]
BMI (kg/m^2)	21.95±1.22	22.23±1.65	0.602[2]
Socio-economic status			
Upper	0 (0.0%)	0 (0.0%)	0.403[3]
Upper middle	5 (33.3%)	3 (20%)	
Lower middle	6 (40.0%)	6 (40.0%)	
Upper lower	4 (26.7%)	3 (20.0%)	
Lower	0 (0.0%)	3 (20.0%)	
Menopausal status			
Premenopausal	2 (13.3%)	6 (40.0%)	0.215[3]
Postmenopausal	13 (86.7%)	9 (60.0%)	
Parity			
Primigravida	0 (0.0%)	0 (0.0%)	1.000[4]
Multigravida	15 (100.0%)	15 (100.0%)	
History of any previous surgery	14 (93.3%)	9 (60.0%)	0.031[4]
POP-Q			
Stage 3	15 (100.0%)	9 (60.0%)	0.017[3]
Stage 4	0 (0.0%)	6 (40.0%)	
Pre-operative cystocele	5 (33.3%)	6 (40.0%)	0.705[4]
Additional procedure			
Anterior colporrhaphy	5 (33.3%)	6 (40.0%)	0.705[4]
Bilateral tubal ligation	0 (0.0%)	6 (40.0%)	<0.001[3]
Bladder rent repair	0 (0.0%)	1 (6.7%)	1.000[4]
PISQ-12 (pre-operative)	12.78±0.97	12.83±0.75	1.000[1]

Data are shown as mean ± standard deviation or frequency (%). [1]: Wilcoxon-Mann-Whitney U test, [2]: t-test, [3]: Fisher's exact test, [4]: Chi-square test, BMI: Body mass index, POP-Q: Pelvic organ prolapse-quantification, PISQ-12: Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire

Table 2. Intra-operative parameters of both groups

Parameter	Group A (n=15)	Group B (n=15)	p
Duration of mesh fixation (minutes)	54.67±9.35	45.00±11.34	0.019[1]
Blood loss (mL)	52.00±8.62	44.67±9.15	0.052[1]
Operating time (minutes)	107.67±17.8	96.00±9.86	0.053[1]
Occurrence of major complications	0 (0.0%)	1 (6.7%)	1.000[4]
Blood transfusion	0 (0.0%)	0 (0.0%)	1.000[4]
Conversion to other approach	0 (0.0%)	0 (0.0%)	1.000[4]

Data are shown as mean ± standard deviation or frequency (%). [1]: Wilcoxon-Mann-Whitney U test, [4]: Chi-squared test

Table 3. Post-operative parameters

Parameters	Group A (n=15)	Group B (n=15)	p
Immediate post-operative period			
Hemoglobin decline (g/dL)	0.84±0.52	1.21±0.66	0.098[2]
Pain (VAS score)	3.73±0.70	3.60±0.51	0.691[1]
Episode of constipation	0 (0.0%)	0 (0.0%)	1.000[4]
Urinary complaints	0 (0.0%)	0 (0.0%)	1.000[4]
Infection	0 (0.0%)	0 (0.0%)	1.000[4]
Duration of analgesic			
2 days	12 (80.0)	12 (80.0)	1.000[3]
3 days	3 (20.0)	3 (20.0)	
Duration of hospital stay (days)	3.60±0.74	3.40±0.51	0.539[1]
1st follow-up visit (7-10 days)			
Wound related complications	0 (0.0%)	0 (0.0%)	1.000[4]
Bladder and bowel dysfunction	0 (0.0%)	0 (0.0%)	1.000[4]
Low backache, lower abdominal pain, buttock pain	0 (0.0%)	0 (0.0%)	1.000[4]
PGI-I score at 1st follow-up visit (7-10 days)	2.53±0.83	2.40±0.51	0.693[1]
2nd follow-up visit (6 months)			
Wound related complications	0 (0.0%)	0 (0.0%)	1.000[4]
Bladder and bowel dysfunction	0 (0.0%)	0 (0.0%)	1.000[4]
Dyspareunia	0 (0.0%)	0 (0.0%)	1.000[4]
Mesh erosion	0 (0.0%)	0 (0.0%)	1.000[4]
Relapse of apical prolapse	1 (6.7%)	1 (6.7%)	1.000[3]
De novo occurrence of anterior and lateral de-fect cystocele	0 (0.0%)	0 (0.0%)	1.000[4]
De novo urgency and urinary incontinence	0 (0.0%)	0 (0.0%)	1.000[4]
De novo constipation and rectocele	0 (0.0%)	0 (0.0%)	1.000[4]
Satisfaction rate	14 (93.3%)	14 (93.3%)	1.000[3]
PGI-I score (6 months)	1.60±1.06	1.40±1.06	0.290[1]
PISQ-12 score (6 months)	18.89±1.17	18.33±1.86	0.534[1]

Data are shown as mean ± standard deviation or frequency (%). [1]: Wilcoxon-Mann-Whitney U test, [2]: T-test, [3]: Fisher's exact test, [4]: Chi-square test, VAS: Visual analog score, PGI-I: Patient Global Impression of Improvement, PISQ-12: Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire

In the immediate post-operative period, Hb decline, VAS score, requirement for additional analgesics, and hospital stay were similar in both groups. No patient had an episode of constipation, urinary complaint, or infection.

At the first visit, there were no wound-related complications, bladder and bowel dysfunction, lower abdominal pain, low

backache, or buttock pain in either group. At the six-month visit, there were no bladder and bowel dysfunction (constipation, dyschezia), dyspareunia, lower abdominal pain, low backache, buttock pain, mesh erosion, de novo occurrence of anterior and lateral defect cystocele, de novo urgency, de novo urinary incontinence or de novo constipation and rectocele in either group.

The overall patient impression of improvement by PGI-I score improved significantly over time in both groups. The mean PGI-I score decreased from 2.53±0.83 to 1.60±1.06 in group A (p-value=0.009) and from 2.40±0.51 to 1.40±1.06 in group B (p-value=0.006) from first visit to last visit post-operatively.

The PISQ-12 questionnaire was used in women younger than 45 years old with intact uterus, both pre-operatively and post-operatively at the 6-month follow-up visit. Nine patients in group A and six patients in group B completed the PISQ-12. In group A, the mean PISQ-12 score increased from 12.78±0.97 to 18.89±1.17 (p<0.001), while in group B, these scores were 12.83±0.75 and 18.33±1.86 (p<0.001), respectively at the same time points. All P-QOL domain scores improved post-operatively (p<0.001) in both groups, as shown in Table 4, which suggests all women had a better QoL after surgery compared to their pre-operative status.

Discussion

Various surgical procedures for POP correction have been described, which include sacrospinous fixation, sling techniques for nulliparous prolapse, paravaginal repairs, abdominal or laparoscopic sacrocolpopexy and hysteropexy (10-15). The laparoscopic sacropexy is considered to be the gold standard for correcting an apical prolapse.

Laparoscopic pectopexy is the most recent alternative surgical technique for POP, first described by Banerjee and Noé (3) in obese patients. There are only a few studies published in the literature that compared laparoscopic pectopexy with the standard laparoscopic sacropexy (9,16-18).

To the best of our knowledge, this is the first study to compare the duration of mesh fixation. We documented a significantly shorter duration of mesh fixation with laparoscopic pectopexy than laparoscopic sacropexy. No other study has described this parameter and thus comparison with the literature is impossible. The total operating time was also comparatively shorter using laparoscopic pectopexy (p=0.053). Similarly, Noé et al. (9) found a significantly shorter mean operating time using laparoscopic pectopexy (43.1 minutes; n=42) compared to laparoscopic sacropexy (52.1 minutes; n=41) (p=0.0002). Chuang et al. (16) also reported significantly shorter operative time with laparoscopic pectopexy [182.9±27.2 minutes; (n=18)] than sacropexy [256.2±45.5 minutes; (n=21)] (p<0.001). However, Obut et al. (17) reported no difference (88.44±15.42 vs. 88.33±14.22; p=0.978).

Table 4. Comparison of P-QOL domains

Prolapse quality of life domain scores	Group	Pre-operative (mean ± SD)	Post-operative (mean ± SD)	p^1	p^2	p^3
GHP	Group A	3.53±0.52	1.73±0.70	<0.001	0.479	0.785
	Group B	3.67±1.67	1.67±0.72			
PI	Group A	3.47±0.52	1.47±0.52	<0.001	0.487	0.479
	Group B	3.60±0.51	1.33±0.49			
RL	Group A	3.27±0.42	1.67±0.52	<0.001	0.690	0.618
	Group B	3.33±0.45	1.57±0.50			
PL	Group A	3.20±0.46	1.53±0.55	<0.001	0.485	0.619
	Group B	3.03±0.61	1.63±0.55			
SL	Group A	3.37±0.48	1.70±0.56	<0.001	0.457	0.742
	Group B	3.50±0.42	1.63±0.55			
PR	Group A	3.80±0.61	2.04±0.61	<0.001	0.439	0.450
	Group B	3.97±0.60	1.87±0.58			
EM	Group A	3.13±0.53	1.84±0.57	<0.001	0.572	0.438
	Group B	3.25±0.55	1.67±0.63			
SE	Group A	2.90±0.71	1.70±0.56	<0.001	0.455	0.748
	Group B	3.10±0.69	1.63±0.58			
SM	Group A	3.24±0.64	1.94±0.55	<0.001	0.555	0.479
	Group B	3.33±0.68	1.80±0.49			

GHP: General health perceptions, PI: Prolapse impact, RL: Role limitations, PL: Physical limitations, SL: Social limitations, PR: Personal relationships, EM: Emotions, SE: Sleep/energy, SM: Severity measures, SD: Standard deviation, p^1: Compare pre-operative and post-operative value in the same group, p^2: Compare two groups according to pre-operative value, p^3: Compare two groups according to post-operative value

Tahaoglu et al. (19), Karslı et al. (20), and Salman et al. (21) conducted observational, non-comparative studies in laparoscopic pectopexy and reported mean operating times of 86.8±17.7, 33.8±14.6, and 48±9.8 minutes, respectively.

In the present study, the intra-operative blood loss was minimal in both groups, but comparatively less with laparoscopic pectopexy. Likewise, Noé et al. (9) reported significantly less blood loss with laparoscopic pectopexy than with sacropexy. Other studies by Chuang et al. (16) and Obut et al. (17) documented similar blood loss in both groups.

The overall rate of major surgical complications was low (3.33%) in the present study. Similarly, the complication rate was also low in other studies. Noé et al. (9) reported 5 (1%) patients with severe complications [haemorrhage (n=1), bladder injury (n=3), ureter injury (n=1)]. Tahaoglu et al. (19) reported that one patient (4.5%) had urinary tract infection (Clavien-Dindo complication classification-grade 2) as an early complication and was treated with antibiotics. Biler et al. (22) reported haemorrhage in 1/16 patients (3.6%) during pectopexy but did not require blood transfusion. No patient required blood transfusion in the present study.

Tahaoglu et al. (19) reported that one patient (4.5%) of those (n=22) undergoing laparoscopic pectopexy converted to laparotomy due to adhesions and bleeding. In the present study, conversion to laparotomy was not required in any of the cases. Chuang et al. (16) and Obut et al. (17) did not find any major complications, including bladder, ureteral, or bowel injury, or uncontrolled bleeding in either group.

In the present study, no patient was lost to follow-up, and the follow-up period to six months after surgery was similar in both groups. We did not find any post-operative complications, such as wound-related complications, bladder, and bowel dysfunction, dyspareunia, mesh erosion, de novo occurrence of the anterior and lateral defect, cystocele, de novo urgency, and urinary incontinence, de novo constipation and rectocele in either groups.

A comparatively longer follow-up of 21.8 months for patients undergoing pectopexy and 19.5 months for sacropexy was described by Noé et al. (18), who reported that no patient had de novo defecation disorder in the pectopexy group while 19.5% patients developed it in the sacropexy group. The occurrence of rectoceles (9.5% vs. 9.8%) and de novo SUI (4.8% vs. 4.9%) was similar in both groups. No patient had de novo lateral defect and cystocele following pectopexy, whereas these affected 12.5% of the sacropexy group. Obut et al. (17), during a follow-up period of 12 months, noted that exacerbation of existing cystocele was greater after sacropexy than after the pectopexy procedure (6.3% vs. 10%; p=0.469). De novo urgency was similar in both groups (6.7% vs. 6.3%; p=0.669) while exacerbation of the existing rectocele was more marked in the pectopexy group (9.9% vs. 0%; p=0.131). Chuang et al. (16) used a post-operative mean follow-up period of 7.2 months in the pectopexy group and 16.2 months in the sacropexy group. They reported that occurrence of low back pain (0% vs. 19%; p=0.11) and low abdominal pain (11.1% vs. 19%; p=0.667) was greater after sacropexy than pectopexy while post-operative SUI affected more patients in the pectopexy group (33.3%) than the sacropexy group (9.5%) (p=0.112). In the present study, no patient had such complaints during follow-up periods. Tahaoglu et al. (19) noted the rate of cystocele, rectocele, de novo SUI, and de novo urgency UI was 4.5%, 9.0%, 4.5%, and 4.5%, respectively, during six months follow-up period of laparoscopic pectopexy.

Defecation disorders, including constipation, are often neglected. This can be attributed to injury to the hypogastric nerve during the sacropexy procedure. Noé et al. (18) documented significantly fewer de novo defecation problems after pectopexy than sacropexy (0% vs. 19.5%; p=0.002). Similarly, Chuang et al. (16) noted no defecation symptoms with pectopexy compared with sacropexy (0% vs. 19%; p=0.11). Obut et al. (17) also reported that more constipation occurred in sacropexy (20%) than pectopexy (3.2%) (p=0.036). Tahaoglu et al. (19) did not report any de novo defecation problem after the pectopexy procedure. In the present study there were no de novo defecation problems in either group over the follow-up period.

Data on surgical failures and recurrence rates after the pectopexy procedure are limited. In the present study, we noticed a relapse of apical prolapse in 1/15 (6.7%) patients in each group, for which repeat corrective surgery was performed. Likewise, an apical prolapse relapse rate of 2.3% in the pectopexy group and 9.8% in the sacropexy group (p=0.36) was reported by Noé et al. (18). Obut et al. (17) reported apical prolapse relapse in 3.3% cases after pectopexy and no relapse after sacropexy. On the contrary, Chuang et al. (16), Tahaoglu et al. (19), and Biler et al. (22) did not report recurrence of apical prolapse.

QoL, sexual function, and overall improvement in health was investigated in the present study using P-QOL, PISQ-12, and PGI-I questionnaires. P-QOL and PISQ-12 scores improved significantly from pre-operative to post-operative status and PGI-I scores improved significantly from first follow-up visit to the six-month follow-up visit. However, there was no difference between the two groups, suggesting that both corrective techniques were equally effective. Similar to our study, Karslı et al. (20) performed laparoscopic pectopexy [(n=31); of which pectouteropexy (n=10), pectocolpopexy (n=21)] and compared P-QOL and PISQ-12 questionnaires pre-operatively and six months post-operatively, documented significant improvement after surgery (p<0.05). Salman et al. (21)

performed laparoscopic pectohysteropexy in 36 women and reported significant improvement in POP-Q, and PISQ-12 scores after a follow-up of 12 months, ($p<0.05$).

Likewise, Tahaoglu et al. (19) [(n=22); hystero-pectopexy (n=21) and cervicopectopexy (n=1)] reported significant improvement in QOL and sexual score after pectopexy surgery ($p=0.0001$). Obut et al. (17) demonstrated that the quality of female sexual functions (FSFI) and P-QOL were significantly improved after both procedures ($p<0.01$). However, there was no difference between groups in terms of FSFI and P-QOL scores.

The above data suggest that the newer technique - pectopexy - bears no new intra-operative risks and has less post-operative complications, such as de novo defecation problems and constipation when compared to the standard technique, sacropexy. During pectopexy, the risk of injury to hypogastric nerves, ureter, sigmoid colon, and presacral veins is negligible. Therefore, laparoscopic pectopexy seems to be a novel and promising alternative corrective surgery for apical prolapse.

Study Limitations

This is probably the first study from India which compared laparoscopic pectopexy with laparoscopic sacropexy. The small number of cases, making the study underpowered, and relatively short follow-up period are the major limitations. Nevertheless, the results are reproducible due to the prospective nature of the study. Additionally, we evaluated the QoL, sexual function, and global impression of improvement through specific questionnaires, enhancing the value of the findings.

Conclusion

Laparoscopic pectopexy appears to be a safe, feasible, and comfortable alternative procedure to the standard sacropexy for apical prolapse. There was a significantly shorter mesh fixation time, shorter operating time, and less blood loss with laparoscopic pectopexy than with laparoscopic sacropexy, whereas the post-operative parameters were comparable in both techniques. Both corrective techniques for prolapse improved the PGI-I, P-QOL, and PISQ-12 scores from pre-operative to six-month follow-up points. Unfortunately, this study was underpowered and so future studies with appropriately large sample sizes and including longer follow-up periods, are required to produce more robust, reliable results.

Ethics Committee Approval: *Prior permission for data analysis was obtained from the All India Institute of Medical Sciences (AIIMS) Institutional ethical board (AIIMS/IEC/20/823, date: 21.11.2020).*

Informed Consent: *Informed written consent was taken for surgery.*

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References

1. Hendrix SL, Clark A, Nygaard I, Aragaki A, Barnabei V, McTiernan A. Pelvic organ prolapse in the Women's Health Initiative: gravity and gravidity. Am J Obstet Gynecol 2002; 186: 1160-6.
2. Ross JW, Preston M. Laparoscopic sacrocolpopexy for severe vaginal vault prolapse: five-year outcome. J Minim Invasive Gynecol 2005; 12: 221-6.
3. Banerjee C, Noé KG. Laparoscopic pectopexy: a new technique of prolapse surgery for obese patients. Arch Gynecol Obstet 2011; 284: 631-5.
4. Shiozawa T, Huebner M, Hirt B, Wallwiener D, Reisenauer C. Nerve-preserving sacrocolpopexy: anatomical study and surgical approach. Eur J Obstet Gynecol Reprod Biol 2010; 152: 103-7.
5. Banerjee C, Leufgen H, Hatzmann W, Noé G. Complications, re-prolapse rates and functional results after laparoscopic sacropexy: a cohort study. Geburtshilfe Frauenheilkd 2010; 70: 379-84.
6. Cosson M, Boukerrou M, Lacaze S, Lambaudie E, Fasel J, Mesdagh H, et al. A study of pelvic ligament strength. Eur J Obstet Gynecol Reprod Biol 2003; 109: 80-7.
7. Cam C, Sakalli M, Ay P, Aran T, Cam M, Karateke A. Validation of the prolapse quality of life questionnaire (P-QOL) in a Turkish population. Eur J Obstet Gynecol Reprod Biol 2007; 135: 132-5.
8. Cam C, Sancak P, Karahan N, Sancak A, Celik C, Karateke A. Validation of the short form of the Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12) in a Turkish population. Eur J Obstet Gynecol Reprod Biol 2009; 146: 104-7.
9. Noé KG, Spüntrup C, Anapolski M. Laparoscopic pectopexy: a randomised comparative clinical trial of standard laparoscopic sacral colpo-cervicopexy to the new laparoscopic pectopexy. Short-term postoperative results. Arch Gynecol Obstet 2013; 287: 275-80.
10. Nygaard IE, McCreery R, Brubaker L, Connolly A, Cundiff G, Weber AM, et al. Abdominal sacrocolpopexy: a comprehensive review. Obstet Gynecol 2004; 104: 805-23.
11. David-Montefiore E, Barranger E, Dubernard G, Nizard V, Antoine JM, Daraï E. Functional results and quality-of-life after bilateral sacrospinous ligament fixation for genital prolapse. Eur J Obstet Gynecol Reprod Biol 2007; 132: 209-13.
12. Maher C, Feiner B, Baessler K, Schmid C. Surgical management of pelvic organ prolapse in women. Cochrane Database Syst Rev

2013: CD004014. Update in: Cochrane Database Syst Rev 2016; 11: CD004014.

13. Beer M, Kuhn A. Surgical techniques for vault prolapse: a review of the literature. Eur J Obstet Gynecol Reprod Biol 2005; 119: 144-55.
14. Rivoire C, Botchorishvili R, Canis M, Jardon K, Rabischong B, Wattiez A, et al. Complete laparoscopic treatment of genital prolapse with meshes including vaginal promontofixation and anterior repair: a series of 138 patients. J Minim Invasive Gynecol 2007; 14: 712-8.
15. North CE, Ali-Ross NS, Smith AR, Reid FM. A prospective study of laparoscopic sacrocolpopexy for the management of pelvic organ prolapse. BJOG 2009; 116: 1251-7.
16. Chuang FC, Chou YM, Wu LY, Yang TH, Chen WH, Huang KH. Laparoscopic pectopexy: the learning curve and comparison with laparoscopic sacrocolpopexy. Int Urogynecol J 2022; 33: 1949-56.
17. Obut M, Oğlak SC, Akgöl S. Comparison of the Quality of Life and Female Sexual Function Following Laparoscopic Pectopexy and Laparoscopic Sacrohysteropexy in Apical Prolapse Patients. Gynecol Minim Invasive Ther 2021; 10: 96-103.
18. Noé KG, Schiermeier S, Alkatout I, Anapolski M. Laparoscopic pectopexy: a prospective, randomized, comparative clinical trial of standard laparoscopic sacral colpocervicopexy with the new laparoscopic pectopexy-postoperative results and intermediate-term follow-up in a pilot study. J Endourol 2015; 29: 210-5.
19. Tahaoglu AE, Bakir MS, Peker N, Bagli İ, Tayyar AT. Modified laparoscopic pectopexy: short-term follow-up and its effects on sexual function and quality of life. Int Urogynecol J 2018; 29: 1155-60.
20. Karslı A, Karslı O, Kale A. Laparoscopic Pectopexy: An Effective Procedure for Pelvic Organ Prolapse with an Evident Improvement on Quality of Life. Prague Med Rep 2021; 122: 25-33.
21. Salman S, Kumbasar S, Yeniocak AS. Uterine preserving technique in the treatment of pelvic organ prolapse: Laparoscopic pectopexy. J Obstet Gynaecol Res 2022; 48: 850-6.
22. Biler A, Ertas IE, Tosun G, Hortu I, Turkay U, Gultekin OE, et al. Perioperative complications and short-term outcomes of abdominal sacrocolpopexy, laparoscopic sacrocolpopexy, and laparoscopic pectopexy for apical prolapse. Int Braz J Urol 2018; 44: 996-1004.

The effectiveness of non-invasive prenatal test technology and the prenatal screening algorithm based on various methods for determining foetal aneuploidy

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Abstract

Objective: The purpose was to evaluate the effectiveness of a non-invasive prenatal test (NIPT) using mass parallel sequencing (MPS) to detect trisomy 13, 18, 21 and fetal sex chromosome abnormalities in maternal blood samples by isolating freely circulating foetal extracellular DNA (eDNA), and to develop an algorithm for prenatal screening.

Material and Methods: The research methods used included blood sampling from patients, isolation of eDNA, determination of DNA concentration and quality, library preparation for sequencing, MPS using an Illumina HiSeq2000, positive and negative control samples, monitoring, and analysis of results using the distributed algorithms platform based on calculations of z-value and the average absolute deviation. Pregnant women were divided into two groups based on gestational age at sampling, group 1; 9-14 weeks and group 2; 15-27 weeks.

Results: A total of 377 pregnant women were included with a mean (range) age of 33 (23-44) years. The mean gestational age at the time of blood sampling in group 1 was 11 (9-14) weeks, and in group 2 was 21 (15-27) weeks. In the first group, three cases of trisomy 18 chromosomes were detected in patients aged 43 years old, and female children were subsequently born with Edwards syndrome. In the second group, one case of trisomy 21 was detected in a patient aged 36 years and the pregnancy was terminated at 25 weeks.

Conclusion: The analysis of freely circulating foetal eDNA was a sensitive method for detecting chromosomal abnormalities. The study has a practical significance, since the NIPT for frequent aneuploidy considerably exceeds the effectiveness of traditional screening methods and allows identifying chromosomal disorders starting from the 9th week of the gestation period. (J Turk Ger Gynecol Assoc 2023; 24: 152-8)

Keywords: Pregnancy, chromosomal abnormalities, non-invasive prenatal test, extracellular deoxyribonucleic acid, mass parallel sequencing

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Introduction

Chromosomal abnormalities in the form of aneuploidy on chromosomes 21 and 18 are more common than other pathologies, and accordingly, more often lead to perinatal mortality and disability in highly developed countries. The outcome of pregnancy with such a pathology is a miscarriage, premature birth, or the birth of a child with the corresponding syndrome. It was found that about 60% of cases with

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aneuploidy end in miscarriage (1). When identifying patients at risk for chromosomal abnormalities in the first trimester of pregnancy, invasive interventions are recommended, which can lead to complications, such as fetal death, miscarriage, bleeding, and chorioamnionitis. Therefore, some pregnant women refuse invasive procedures, and in some cases, they are contraindicated. Therefore, it is essential to assess the condition of the fetus and diagnose aneuploidy at the initial stages of pregnancy. It is also important to consider the relationship/heredity of the parents, for example the degree of consanguinity, if any.

Today, there are increasingly more likely to be women over 40 years old who are planning a pregnancy. This age poses a higher risk of developing aneuploidy in the fetus. To screen pregnant women for chromosomal abnormalities, ultrasound examination may be performed, and biochemical markers may be measured. Biochemical screening is based on the determination of pregnancy associated plasma protein A (PAPP-A) and the free β-unit of human chorionic gonadotropin (β-hCG) using the maximum number of probability ratios (population differences, weight, ethnicity of the mother, smoking, presence of diabetes mellitus, multiple pregnancy, the use of assisted reproductive technologies). For example, to screen for trisomy of chromosome 21, the clinical characteristics of the mother and the foetal thickness of the collar space, measured by ultrasound, are evaluated. In addition, maternal blood biomarkers, including β-hCG and PAPP-A at 11-13.6 weeks of gestation, are considered. Using this approach, the rate of false positive results is reported to be 5.0% (2).

There is curently a non-invasive screening method for determining chromosomal abnormalities, known as the non-invasive prenatal test (NIPT). This method is based on the analysis of free genomic DNA of foetal origin from a blood sample taken from the pregnant woman, available from the 10^{th} week of gestation (3). This method has become more widely known as a result of many studies over the past 15 years, which have confirmed its utility in practice. Thus, NIPT has been widely adopted in clinical medicine in patients at high and medium risk of carrying a foetus with a chromosomal abnormality. However, it can be used for screening and risk determination in all pregnant women, regardless of age before the final diagnosis, as an independent method or in addition to the methods available. At the same time, the use of NIPT raises certain ethical questions (4). Despite its non-invasiveness, this technology is aimed at detecting chromosomal aneuploidy in the fetus, the treatment of which is currently impossible. Therefore, NIPT shares many of the ethical problems inherent in prenatal diagnosis in general, since the only way to prevent the birth of a sick child is to terminate the pregnancy. The proposed technology is potentially intended to replace the existing biochemical screening. Given the higher cost of this study, it is necessary to carry out special measures to ensure the availability of the proposed screening of foetal extracellular DNA (eDNA) for all social groups.

The risk of having a child with a chromosomal anomaly is 5% even in perfectly healthy young parents. Therefore, it is important to identify possible disorders, including aneuploidy, causing hereditary syndromes in early pregnancy. Conducting a combined screening study allows identifying pregnant risk groups. However, the most precise test is the NIPT, which allows detecting aneuploidy in the fetus, including trisomy, monosomy, numerical anomalies of sex chromosomes. NIPT detects chromosomal abnormalities with high reliability since it is based on a special signal processing algorithm. This allows comparing and detecting differences between maternal DNA present in plasma/leucoma and fetal DNA found only in plasma. Based on this test, the accuracy of the study reaches up to 99%. Thanks to this test, it is possible to exclude the presence of such diseases as Down syndrome, Edwards, Patau, Turner, etc. in the unborn child. A sample of 15 mL of venous blood of the expectant mother is sufficient for conducting NIPT to determine the risk of aneuploidy in the fetus. This avoids the use of invasive methods of prenatal diagnosis, leading to possible complications of pregnancy.

The total sensitivity of genomic DNA screening is 100.00%, with this indicator varying from 88.43 to 100%. The overall effectiveness of the test for the analysis of aneuploidy in chromosomes 21, 18, 13, X and Y, defined as the proportion of true results among all studies conducted was 98.97% (5). Thus, NIPT has been shown to be one of the most effective methods for detecting chromosomal abnormalities in the fetus from early pregnancy, which should be recommended to all women in the form of a universal prenatal test. First, the NIPT avoids invasive manipulations due to its high sensitivity to the exclusion of an elevated risk of fetal aneuploidy and in the case of complications of pregnancy (miscarriage, age risk, extragenital pathology).

The purpose of this study was to evaluate the effectiveness of NIPT for detecting trisomy 21, 18, 13 and fetal sex chromosome abnormalities in maternal blood samples and to develop an algorithm for prenatal screening based on the use of various methods for determining fetal aneuploidy.

Material and Methods

Blood sampling was carried out in 232 patients aged 23-44 years with a singleton pregnancy in the period from 9 to 14 weeks and in a further 145 pregnant women of the same age in the period from 15 to 27 weeks of gestation at the University Medical Centre, Astana, Republic of Kazakhstan. Blood sampling was carried out in specific tubes for eDNA analysis

(Streck Innovations), which were sent to the laboratory of LifeCodexx AG, Konstanz, Germany, by cold chain. Subject information, sent with the samples included patient's code, patient's age, pregnancy period, obstetric history, ultrasound results, biochemical screening, and other data.

NIPT was performed, based on mass parallel sequencing (MPS), according to the previously described method (6). The NIPT included the following stages: isolation of eDNA and determination of its concentration and quality; preparation of libraries for sequencing; conducting the MPS using "Illumina HiSeq2000", using positive and negative control samples; monitoring and analysis of the results obtained using the distributed algorithms platform algorithm based on calculations of the z-value and the average absolute deviation (median absolute deviation).

The study was approved by UMC University Medical Center National Ethics Commission of the Ministry of Health of the Republic of Kazakhstan (approval number: 1033-A, date: 25.06.2022). The authors informed the participants about the anonymous and voluntary participation, and the participants provided their consent.

Results

The mean (range) age of pregnant women included in this study was 33 (23-44) years, and the mean (range) gestational age at the time of blood sampling in group 1 was 11 (9-14) weeks, and in group 2 was 21 (15-27) weeks. The results of fetal DNA analysis in a blood sample were obtained for a total of 377 pregnant women, 232 in group 1 and 145 in group 2. In group 1, three cases of trisomy 18 were detected in patients all aged 43 years, pregnancy was continued at the request of the patients, and three female children were born with Edwards syndrome. In group 2, one case of trisomy 21 was detected in a patient, aged 36 years, and the pregnancy was terminated at 25 weeks. Women in this study underwent NIPT as they had an increased risk of carrying a foetus with chromosomal abnormality, either because of older age during pregnancy or because of the results of biochemical screening (β-hCG and PAPP-A). Based on the generally accepted examination scheme, these pregnant women would have undergone an invasive prenatal diagnosis. The average estimated risk of trisomy 21, 18, or 13 according to combined screening in the examined pregnant women was 1:23,512 (range; 1:160-1:46945). In the present study of fetal eDNA using single nucleotide polymorphism (SNP), three cases of trisomy 18 and one case of trisomy 21 were identified, which is an incidence of 1:94.25 cases but these women were pre-selected for being at high risk. The sex of the fetus in the cases considered was identified correctly. Five cases with an increased risk of trisomy 18 in 8 patients in group 1 and five possible cases of trisomy 21 in 39 patients in group 2 were not confirmed, since cytogenetic examination of amniotic fluid cells revealed no abnormalities in the chromosome set of the foetus. Out of 377 pregnancies the following pregnancy outcomes occurred: in one case (out of four), with revealed foetal chromosomal pathology and confirmed prenatal karyotyping, the pregnancy was terminated and all remaining 376 cases the pregnancy ended in childbirth. At birth, three newborns were confirmed to have Edwards syndrome from three patients aged 43 years.

Thus, the sensitivity of NIPT for Edwards syndrome in this study was 98.6%, with a false positive level of 1.4%. Due to the insufficient number of blood samples with trisomy 21, sensitivity for this pathology was not calculated. The results of this study demonstrated that NIPT for frequent aneuploidy considerably exceeds the effectiveness of traditional screening methods. The elevated risk of trisomy 18 is present in older reproductive-age mothers. Based on the results of ultrasound and biochemical analyses, a decision was made to perform invasive manipulations to determine the karyotype of the foetus. In the present study, 373 pregnant women were spared invasive prenatal diagnosis and the associated risk of complications for the mother and foetus. Although in most countries aneuploidy screening essentially focuses on screening for trisomy 21, invasive manipulations in the group with positive screening results lead to the detection of many other clinically significant aneuploidies. Analysis of free foetal eDNA allowed the avoidance of invasive interventions, complications, and the risk of abortion. NIPT is actively used as a second test after combined screening, if the results of ultrasound and biochemical studies raise suspicion of aneuploidy. Low-risk pregnant women were also included in this study. Therefore, NIPT can be used as a universal method for detecting chromosomal abnormalities in the fetus.

Discussion

In recent decades, there has been an unprecedented steady increase in the prevalence of both congenital malformations, the frequency of which ranges from 2.7% to 16.3% in different populations, and hereditary diseases themselves (monogenic and chromosomal), the total proportion of which is 1.5% (7). According to the European Registry of Congenital Anomalies, 5000 children with developmental defects and chromosomal aberrations are born in Europe every year (8). In the causes of infant death, congenital malformations are the second most common cause (19.1%). Although the infant mortality rate has shown a steady downward trend in recent years (7.9% in 2013, 7.0% in 2014, 5.7% in 2015 and 4.8% in 2016), the improvement of prenatal diagnostic methods and the introduction of modern perinatal technologies are two of the major factors reducing infant mortality in the long term (9). Screening programmes

will play a crucial role in preventing the birth of children with developmental abnormalities as these allow identifying a high-risk group for the occurrence of chromosomal aberrations, followed by invasive procedures in this group to determine the karyotype of the fetus and optimal pregnancy management tactics to prevent the birth of children with severe disabling diseases.

Clinical studies of the use of NIPT, conducted in the period 2014-2016, confirm the high incidence of trisomy 21, 18, and 13, namely 99.7%, 98.2%, and 99%, respectively, but false positive data were 0.13% (10). Based on these results, the world medicine approved the introduction of NIPT technology into clinical practice. This method is acceptable, since it is non-invasive, allowing the detection of the most frequent types of trisomy, that is trisomy 21, 18 and 13, quickly and with high sensitivity. NIPT defines a high-risk group for the development of chromosomal abnormalities. However, the fetus may have a normal karyotype (11).

Several criteria have been proposed for the use of NIPT. Firstly, NIPT is used as a first-line screening test before ultrasound examination in the first trimester of pregnancy. Secondly, it is used with ultrasound, determining the free eDNA of the fetus at 11-13+6 weeks of pregnancy. Then, based on the results obtained, high, medium, and low-risk groups may be identified (12). However, it is advisable to implement the first provision only if the cost of NIPT is considerably reduced. Currently, pregnant women who have entered the high-risk group prefer this test as a safer method compared to an invasive one. From an economic standpoint, the NIPT will reduce the budget of the regional health system to $726975.72. The use of NIPT for first-line testing is more appropriate from the standpoint of detecting cases of chromosomal abnormalities in early pregnancy, in contrast to the current prenatal screening in the first and second trimesters of pregnancy. The costs associated with the use of NIPT are lower as a result of eliminating the need for invasive interventions and iatrogenic pregnancy losses.

The use of NIPT with other methods, including ultrasound and blood sampling, will reveal chromosomal abnormalities and prevent unwanted abortions of pregnancies (13). It has been reported that 35.24% of obstetrician-gynaecologists believe that NIPT should be used as a universal screening test for all pregnant women, while 40.95% of physicians state that free fetal eDNA should be determined in all patients who fall into the medium risk group (over 1:1000) (14). However 21.90% of experts favor excluding its use in pregnant women of average risk, if anatomical structural abnormalities in the development of the fetus are detected during ultrasound, since they are subject to invasive interventions to determine the karyotype of the fetus. Only 1.91% of doctors are against any use of NIPT in clinical practice (14).

Currently, the majority of medical professionals believe that NIPT can be used as a universal method for screening of aneuploidy, based on the analysis of free fetal eDNA in the mother's blood, and also report that NIPT is the most sensitive and promising among methods being researched for prenatal screeing (15). Therefore, these professionals suggest that NIPT should be incorporated into the existing clinical practice.

Screening programs used during pregnancy should be aimed primarily at substantial reduction of the frequency of births of children with severe disabling or fatal diseases, and reducing infant and perinatal mortality rates (16). However, not all patients trust the screening methods used. Combined screening of the first trimester of gestation allows clinicians only to identify high-risk pregnant women. Pregnant women who are subsequently diagnosed with a normal karyotype in the fetus or newborn may be considered at high risk of aneuploidy in the foetus (17,18). Of note, the results of biochemical and ultrasound tests are only used as primary methods of identifying the at-risk group for chromosomal abnormalities. To exclude aneuploidy in the foetus, invasive interventions are necessary to determine the karyotype of the fetus, which can lead to the development of complications for both the mother and the foetus (19,20). Thus, many patients refuse invasive interventions and the outcome of pregnancy is the birth of a sick child. It was found that, in general, about 50% of pregnant women with an elevated risk of trisomy 21 in the fetus refuse invasive manipulation (21-24). However, this diagnostic method may have false positive results.

NIPT gives high positive predictive value and negative predictive value for trisomy 21, 18, and 13, but false negative results can also be obtained. It should be noted that NIPT is dependent on the available concentration of free foetal DNA in the mother's blood sample. A fetal DNA content of less than 4-5% is considered too low to obtain a high-quality test, and the concentration should be at least 10-11%. If the proportion of foetal DNA fraction is low, the test should not be repeated. In these cases, an invasive diagnosis is recommended. Screening by NIPT will be affected by the phenomenon of mosaicism, when fractions of both normal and abnormal fetal DNA are found in the mother's blood sample (25-27). This is because foetal eDNA obtained from the mother's blood originates from the cytotrophoblast (28,29). Therefore, it is recommended to confirm the positive result of NIPT by conducting an invasive prenatal diagnosis. In this case, amniocentesis is the preferred method of diagnosis since the amniotic fluid contains cells of the foetus itself.

The use of amniocentesis to verify a positive result of NIPT is recommended by the European Society of Human Genetics and the American Society for Human Genetic Information (30-33). Amniocentesis is performed only after 15 weeks of pregnancy.

This means a long waiting time for the final result for expectant parents, whereas NIPT can be performed starting from nine weeks of pregnancy. Thus, studies have been conducted to evaluate the effectiveness of chorionic villus biopsy (CVS) as an invasive method of confirming the NIPT result. CVS allows for cytogenetic diagnostics of the fetus in the first trimester, at 11-12 weeks. However, due to the phenomenon of confined placental mosaicism, the result of the study may be inconclusive, which requires a secondary invasive intervention (34,35).

Study Limitations

There are limitations of NIPT which include chromosomal pathologies in parents; the presence of balanced rearrangements; disappearing twin syndrome as DNA of the deceased fetus can circulate in the mother's blood; the presence of mosaicism; multiple pregnancy (more than two foetuses); malignant neoplasms in the mother; or if there is a maternal history of organ transplantation or blood transfusion. In these cases, invasive diagnostic methods are recommended (36,37).

Thus, at present the high value of NIPT seems evident regarding screening for the most common foetal abnormalities: Down, Edwards, and Patau syndromes and aneuploidy of sex chromosomes (38-40). The outcomes of pregnancy when trisomy 21 or 18 is present is miscarriage, or the premature or term birth of a child with the corresponding syndrome, causing perinatal mortality and disability in highly developed countries (41). The selection of patients at risk for chromosomal abnormalities among all pregnant women is an indication for invasive diagnostic methods that can lead to fetal death, miscarriage, bleeding, or chorioamnionitis. In addition, in some cases, performing invasive manipulations is contraindicated.

This study found that NIPT is an effective screening method for studying chromosomal abnormalities in the fetus from early pregnancy. We suggest that NIPT should be recommended to all women in the form of a universal prenatal test. This technology is now part of everyday clinical practice and use of NIPT has promise in the field of prenatal medicine. First, the high sensitivity of the NIPT to the most common chromosomal abnormalities in the fetus was determined, which meant unnecessary invasive manipulations were avoided and it was usable in cases of complications of pregnancy, such as high risk of miscarriage, older age mothers and extragenital pathology.

Conclusion

This study showed that the analysis of freely circulating fetal eDNA in the mother's blood using targeted sequencing of SNPs on chromosomes 13, 18, 21, X, and Y and the use of the Next-generation Aneuploidy Testing Using SNPs algorithm was a sensitive method for detecting autosomal aneuploidy, sex chromosome abnormalities and triploidy in the foetus. This technology can be recommended during pregnancy as effective prenatal screening, especially in high-risk pregancies. Then, it is recommended to confirm chromosomal abnormalities in the foetus.

NIPT technology demonstrates good sensitivity for identifying pregnancies with a high probability of developing one of these conditions and is usable as early as the 9th week of pregnancy. In this role, this test is more reliable compared to the use of combined screening. Using NIPT may avoid the need for unjustified invasive procedures. Considering the positive experience of introducing the innovative NIPT method into clinical practice, the authors of this study recommend including it in the algorithm of prenatal screening for determining fetal aneuploidy, which will reduce the birth rate of children with chromosomal abnormalities.

Ethics Committee Approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by UMC University Medical Center National Ethics Commission of the Ministry of Health of the Republic of Kazakhstan (approval number: 1033-A, date: 25.06.2022).*

Informed Consent: *The authors informed the participants about the anonymous and voluntary participation, and the participants provided their consent.*

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References

1. Khoshnood B, Loane M, de Walle H, Arriola L, Addor MC, Barisic I, et al. Long term trends in prevalence of neural tube defects in Europe: population based study. BMJ 2015; 351: h5949.
2. Gil MM, Revello R, Poon LC, Akolekar R, Nicolaides KH. Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. Ultrasound Obstet Gynecol 2016; 47: 45-52.

3. Zhang B, Lu BY, Yu B, Zheng FX, Zhou Q, Chen YP, et al. Noninvasive prenatal screening for fetal common sex chromosome aneuploidies from maternal blood. J Int Med Res 2017; 45: 621-30.
4. Kurmangali ZHK, Ddamanaeva KB, Bilyal RA, Olkhovik YUM, Kozhamzharov KK, Useeva MS, et al. Prenatal diagnosis of diaphragmatic hernia: Clinical observations. Astana Medicine Journals 2015; 4: 113-20.
5. Kozyulina PYU, Vashukova ES, Glotov AS, Baranov VS, Gladkikh NA. Patent No. RU 2018123457. Method of non-invasive prenatal screening of fetal aneuploidies. Moscow: Rospatent; 2019. https://i.moscow/patents/RU2018123457A_20191230
6. Stumm M, Entezami M, Haug K, Blank C, Wüstemann M, Schulze B, et al. Diagnostic accuracy of random massively parallel sequencing for non-invasive prenatal detection of common autosomal aneuploidies: a collaborative study in Europe. Prenat Diagn 2014; 34: 185-91.
7. Gray KJ, Wilkins-Haug LE. Have we done our last amniocentesis? Updates on cell-free DNA for Down syndrome screening. Pediatr Radiol 2018; 48: 461-70.
8. Hill M, Johnson JA, Langlois S, Lee H, Winsor S, Dineley B, et al. Preferences for prenatal tests for Down syndrome: an international comparison of the views of pregnant women and health professionals. Eur J Hum Genet 2016; 24: 968-75.
9. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017; 50: 302-14.
10. Norton ME, Jacobsson B, Swamy GK, Laurent LC, Ranzini AC, Brar H, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med 2015; 372: 1589-97.
11. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol 2017; 217: 691.e1-6.
12. Dahl F, Ericsson O, Karlberg O, Karlsson F, Howell M, Persson F, et al. Imaging single DNA molecules for high precision NIPT. Sci Rep 2018; 8: 4549.
13. Baranova EE, Belenikin MS, Zhuchenko LA, Izhevskaya VL. Non-invasive prenatal tests: European and American recommendations. Medical Genetics 2017; 16: 3-10.
14. Van Opstal D, Srebniak MI. Cytogenetic confirmation of a positive NIPT result: evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration. Expert Rev Mol Diagn 2016; 16: 513-20.
15. Kater-Kuipers A, Bunnik EM, de Beaufort ID, Galjaard RJH. Limits to the scope of non-invasive prenatal testing (NIPT): an analysis of the international ethical framework for prenatal screening and an interview study with Dutch professionals. BMC Pregnancy Childbirth 2018; 18: 409.
16. Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, et al. First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening. Cochrane Database Syst Rev 2017; 3: CD012600.
17. Society for Maternal-Fetal Medicine (SMFM). Electronic address: pubs@smfm.org; Norton ME, Biggio JR, Kuller JA, Blackwell SC. The role of ultrasound in women who undergo cell-free DNA screening. Am J Obstet Gynecol 2017; 216: B2-7.
18. Rezaie Keikhaie K, Moshfeghi M, Rezaie Kahkhaie L, Eftekhari M, Ajami S, Forghani F, et al. Evaluation of The Relationship between Cell-Free DNA Fetal Fraction of The Circulatory System and Fetal and Maternal Pregnancy Prognosis: A Prospective Study. Int J Fertil Steril 2023; 17: 115-9.
19. Karim JN, Roberts NW, Salomon LJ, Papageorghiou AT. Systematic review of first-trimester ultrasound screening for detection of fetal structural anomalies and factors that affect screening performance. Ultrasound Obstet Gynecol 2017; 50: 429-41.
20. Wei X, Yang Y, Zhou J, Zhou X, Xiong S, Chen J, et al. An Investigation of the Etiologies of Non-Immune Hydrops Fetalis in the Era of Next-Generation Sequence-A Single Center Experience. Genes (Basel) 2022; 13: 2231.
21. Reimers RM, Mason-Suares H, Little SE, Bromley B, Reiff ES, Dobson LJ, et al. When ultrasound anomalies are present: An estimation of the frequency of chromosome abnormalities not detected by cell-free DNA aneuploidy screens. Prenat Diagn 2018; 38: 250-7.
22. Pariza PC, Dima V, Ducu I, Cioca A, Mihai B, Bacalbasa N, et al. Early diagnosis of fetal abnormalities – the importance of ultrasonographic examination. Rom Med J 2021; 68: 113-8.
23. Griffin DK, Brezina PR, Tobler K, Zhao Y, Silvestri G, Mccoy RC, et al. The human embryonic genome is karyotypically complex, with chromosomally abnormal cells preferentially located away from the developing fetus. Hum Reprod 2023; 38: 180-8.
24. Kuzmina TE, Timokhina EV, Ignatko IV, Lebedev VA. Prenatal diagnostics in the I trimester of pregnancy: the current state of the problem. Arch Gynecol Obstet 2019; 6: 178-84.
25. Zhumalina AK, Tusupkaliev BT, Zame YA, Voloshina LV, Darzhanova KB. Clinical and immunological aspects of newborn adaptation born from mothers with intrauterine infection. Period Tche Quim 2020; 17: 656-66.
26. Tussupkaliev B, Zhumalina A, Zhekeyeva B, Sergazina T. The Content Of Some Essential Micronutrients In Infants With Low Birth Weight With Intrauterine Herpes Infection. Georgian Med News 2016: 67-72.
27. Toichuev RM, Zhilova LV, Paizildaev TR, Khametova MS, Rakhmatillaev A, Sakibaev KS, et al. Organochlorine pesticides in placenta in Kyrgyzstan and the effect on pregnancy, childbirth, and newborn health. Environ Sci Pollut Res Int 2018; 25: 31885-94.
28. Sainz JA, Gutierrez L, García-Mejido J, Ramos Z, Bonomi MJ, Fernández-Palacín A, et al. Early fetal morphological evaluation (11-13 + 6 weeks) accomplished exclusively by transabdominal imaging and following routine midtrimester fetal ultrasound scan recommendations. Since when can it be performed? J Matern Fetal Neonatal Med 2020; 33: 1140-50.
29. Dobrovanov O, Králinský K. The role of prenatal diagnostics in the identification congenital malformations of urogenital system in Slovakia. Lek Obz 2019; 68: 59-62.
30. Kucherov YuI, Stygar AM, Zhirkova YV, Borisova NI. Prenatal consultation with fetal malformations. Detskaya Khirurgiya 2016; 20: 211-5.
31. Sun X, Lu J, Ma X. An efficient method for noninvasive prenatal diagnosis of fetal trisomy 13, trisomy 18, and trisomy 21. PLoS One 2019; 14: e0215368.
32. Serniak YP, Sagalevych AI, Frolov OS, Serniak PY, Kryvopustov MS. extraperitoneoscopic radical prostatectomy after pelvic sugery procedures. Wiad Lek 2020; 73: 1093-6.
33. Dobrovanov O, Králinský K. Comparison of effectiveness and sensitivity of prenatal and postnatal sonografic diagnostics of anomalies the uropoetic system in Slovakia. Lek Obz 2020; 69: 130-3.
34. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011; 31: 7-15.
35. Aringazina R, Zharmakhanova G, Kurmanalina G, Bekkuzhin A, Kurmanalin B. Diagnostic Relevance of Ferrokinetic Laboratory Markers in Anemic Pregnant Women. Gynecol Obstet Invest 2020; 85: 420-7.

36. Fedorchenko V, Polyanskaya V, Zachepilo S, Bobrova N, Loban G. Comprehensive study of anti-fungal effect of evgenol emulsion in polysorbat-80 on reference strain of candida albicans atcc 885-653. Georg Med News 2018: 166-70.
37. Baranov VS, Kuznetsova TV, Kashcheeva TK, Ivaschenko TE. Prenatal diagnosis of hereditary diseases: state and prospects. Saint Petersburg: Eco Vector; 2017.
38. Olenev AS, Baranova EE, Sagaidak OV, Galaktionova AM, Belenikin MS, Gnetetskaya VA, et al. Regulatory regulation of antenatal screening using a non-invasive prenatal test. Issues of Gynecology, Obstetrics and Perinatology 2020; 19: 124-32.
39. Zharmakhanova G, Syrlybayeva L, Kononets V, Nurbaulina E, Baikadamova L. Molecular-Genetic Aspects of Methylmalonic Aciduria Development (review). Georgian Med News 2021: 118-24.
40. Svyatova GS, Berezina GM, Murtazaliyeva AV. Association of polymorphisms of cardiovascular system genes with idiopathic recurrent pregnancy loss of Kazakh populations. Rev Latinoam Hiperten 2019; 14: 319-25.
41. Svyatova G, Mirzakhmetova D, Berezina G, Murtazaliyeva A. Immunogenetic aspects of idiopathic recurrent miscarriage in the Kazakh population. J Med Life 2021; 14: 676-82.

Assessment of the ovarian reserve in patients with beta-thalassemia major: a prospective longitudinal study

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Abstract

Objective: Repeated blood transfusions in women with beta-thalassemia major (BTM) may lead to iron overload and increase oxidative stress, consequently resulting in ovarian damage. The aim was to evaluate alterations in ovarian reserve in transfusion-dependent BTM patients over a time period of one year and to compare levels of anti-Mullerian hormone (AMH) in women with BTM and their healthy peers.

Material and Methods: This longitudinal prospective study was conducted in women with transfusion-dependent BTM at a tertiary level hospital. The hospital database was interogated for women diagnosed with BTM between 1996 and 2021. AMH levels were assessed at baseline and one year later.

Results: Forty-one women with BTM were identified, of whom 25 (60.9%) had amenorrhea and 16 (39.1%) had normal cycles. The mean AMH level of all women was 2.7±1.8 ng/mL at baseline, significantly lower than the age-matched nomogram value of 4.0±0.4 ng/mL for a healthy population (p=0.001). The baseline AMH level of patients with amenorrhea were significantly lower than patients with normal menstrual cycles (2.1±1.8 vs. 3.6±1.5 ng/mL, p=0.009). After one-year follow-up, there was a trend towards a decrease in the AMH levels of patients with normal menstrual cycles.

Conclusion: Serum AMH values are decreased in patients with transfusion-dependent BTM. BTM patients should be educated about the possible effects of repeated blood transfusions on fertility. (J Turk Ger Gynecol Assoc 2023; 24: 159-64)

Keywords: Ovarian reserve, beta thalassemia, anti-mullerian hormone

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Introduction

Beta-thalassemia is the most common major monogenic hemoglobin disorder worldwide (1). Deficiency of β-globin chain synthesis is a condition that causes decreased production of red blood cells and hemoglobin, resulting in severe anemia (1,2). This disease, which was previously life-threatening, is now treated with repeated blood transfusions and iron chelation therapy, providing a decrease in morbidity and mortality. However, repeated blood transfusions may lead to an increase in reactive oxygen species over time due to excess production of free radicals and pro-oxidant/antioxidant imbalance with iron overload in multi-transfusion patients (3,4).

The accumulation of hemosiderosis in the hypothalamus and pituitary gland, also as a result of repeated blood transfusions, may cause hypogonadotropic hypogonadism (HH) in women

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(2,5) and is the most common endocrinopathy in thalassemia patients, affecting between 40-91%, and leads to sexual dysfunction and subfertility (6-8). Hypothalamo-pituitary damage is usually irreversible (2) and HH still appears to be common, despite advances in chelation regimens (6,9).

Anti-Mullerian hormone (AMH), secreted by granulosa cells of preantral and antral follicles, is a member of the transforming growth factor-β family (5,10) and its levels do not change throughout the menstrual period. It is the most reliable biochemical marker showing ovarian reserve. AMH levels are widely used in studies to show the extent of ovarian damage after the use of gonadotoxic agents in women, particularly in women who received chemotherapy after cancer diagnosis.

It is still unclear whether ovarian reserve is intact in patients with beta-thalassemia major (BTM), despite chronic blood transfusion and HH secondary to hemosiderosis. In this prospectively designed study, the aim was to evaluate the alterations in the ovarian reserve of BTM patients over a time period of one year and to compare the AMH levels between women with BTM and a healthy population. In addition, patients with BTM were divided into two groups, those with normal menstrual cycles and those who were amenorrheic, and the aim was to compare the ovarian reserve between these groups.

Material and Methods

Patient population

This longitudinal prospective study was conducted in women with transfusion-dependent BTM at a tertiary level hospital. The hospital database was interogated for women diagnosed with BTM between 1996 and 2021. In women with BTM, the clinical aim of the transfusion regimen was to maintain hemoglobin levels between 9.5 and 14.0 g/dL. Women were transfused at 14-28 day intervals. Chelation was initiated after two years of transfusion or when the serum ferritin level was consistently higher than 1000 mg/L. Clinical management aims included reducing ferritin levels below 1000 mg/L. All patients received chelation therapy. Deferasirox (maximum: 40 mg/kg/d) and deferiprone (40 mg/kg/d) were administered orally, alone or in combination. Deferoxamine was administered at a dose of 25-40 mg/kg over 5-7 days in combination with deferasirox or deferiprone. After verbal and written information about the study, all eligible and voluntary participants gave informed consent. Exclusion criteria were the presence of any comorbid systemic condition, prior chemotherapy, hormonal therapy or immunotherapy and previous ovarian surgery. This study was approved by the İzmir Katip Çelebi University Local Ethics Committee (IRB: 0044, date: 24.02.2021) and conducted according to the principles of the Declaration of Helsinki.

Serum iron and ferritin levels were tested. AMH levels were measured at admission to the study and one year later. The volume of the ovaries and the number of antral follicles were evaluated using ultrasound by a single gynecologist. Antral follicle count measurements were performed using high resolution transvaginal ultrasonography during the early follicular phase. The results of follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) hormone, used to assess the pituitary and gonadal functions of the patients were noted. HH was defined as FSH and LH levels <2 IU/L with accompanying E_2 levels <20 pg/mL (11). Menstrual regularity was assessed by self-report. Blood samples taken for pre-transfusion biochemistry monitoring of all cases were collected from the biochemistry laboratory after the examination, and two blood serum samples collected from female patients at the baseline and 12 months were stored at -80 °C until evaluation.

AMH measurement

Serum AMH levels were analyzed using the Elecsys AMH Plus test with AMH assay on the Cobas-E electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). Units for serum AMH were ng/mL and the assay range was 0.01-23 ng/mL. The intra and inter-assay coefficients of variation were <8% and <12%, respectively.

Outcome measures

The primary outcome was to assess AMH levels after a one-year follow-up and to compare AMH levels of patients with BTM to a healthy population. The secondary aim was to compare the characteristics and AMH values of the patients based on menstrual status. Although pregnancy outcomes were not an aim of the study, information about patients who continued to be followed up and who became pregnant over time or who received assisted reproductive therapy were also included in the study.

Statistical analysis

Mean and standard deviation values of measurable variables were calculated. The normality of data distribution in data sets were evaluated using Smirnov-Kolmogorov analysis. Subsequently, a paired t-test was used to compare normally distributed data while the Wilcoxon test was used for non-normally distributed data. Categorical variables were compared using the χ^2 test. A one-sample t-test was used to determine whether AMH levels of women with BTM differs from the AMH levels of a healthy Turkish population. The correlation between AMH and ferritin levels were calculated using Pearson's correlation analysis. All statistical analyses were performed

with SPSS, version 18.0 (IBM Inc., Armonk, NY, USA). A p-value of <0.05 was accepted as statistically significant.

Results

Forty-one patients with BTM were included, with a mean age of 23.0±5.7 years. Twenty-five (60.9%) had amenorrhea while 16 (39.1%) had normal cycles. The mean age (24.4±5.9 years vs. 20.8±4.9 years; p=0.061) and body mass index (21.8±1.8 vs. 21.9±1.9 kg/m^2; p=0.781) were similar in the amenorrheic and normal cycle groups, respectively. The clinical characteristics of all patients included in the study are given in Table 1.

The mean baseline AMH value of all women was 2.7±1.8 ng/mL. This was significantly lower than the expected healthy population mean of 4.0±0.4 ng/mL, published elsewhere (p=0.001) (12). The mean AMH levels of all women was evaluated after one year to find out whether there was a significant decline in AMH levels over this short time period. The mean AMH levels tended to be lower after one year compared to baseline but this was not significant (baseline 2.7±1.8 ng/mL vs. 2.5±1.8 ng/mL at one year; p=0.207).

Ovarian reserve of women with BTM was evaluated in subgroups, stratified by menstrual status. The mean number of antral follicles in the amenorrhea group was 8.6±5.7 while this was 12.0±2.3 in patients with normal cycles (p=0.048). Mean ovarian volume was 5.5±4.4 cm^3 and 10.9±5.7 cm^3 in patients with amenorrhea and normal cycle, respectively (p=0.003). Baseline AMH values in patients with amenorrhea were significantly lower than in patients with normal menstrual cycles (2.1±1.8 vs. 3.6±1.5 ng/mL, respectively, p=0.009). FSH and LH values of amenorrheic patients were also lower than in patients with normal cycles (Table 2). After a one-year follow-up, there was a trend towards a decrease in AMH levels in the normal cyle group, while a significant difference persisted between patients with amenorrhea and normal cycle (2.1±1.8 vs. 3.3±1.5, respectively; p=0.031).

In the correlation analysis, a significantly inverse correlation was found between AMH levels and ferritin levels, both at baseline (r=-0.486, p=0.004, Figure 1A) and one year later (r=-0.488, p=0.003, Figure 1B). Ferritin levels were higher in amenorrheic patients than in patients with normal menstrual cycle (p=0.041).

More than half of the cohort (22/41, 53.7%) was married. Twenty were married to healthy partners, whereas two were married with partners with BTM. Of these 22, 10 women were amenorrheic and 12 had normal menstrual cycle. Six of the 12 patients with normal menstrual cycle (50%) gave birth to seven healthy babies without receiving any treatment. Out of 10 women with amenorrhea, four underwent in vitro fertilization procedure. Only one (25%) achieved pregnancy and had a live birth. The remaining six women did not wish to become pregnant at the time of the study and had not started any infertility treatments.

Table 1. The demographic and clinical characteristics of all patients with beta-thalassemia major

	Patients (n=41)
Age, years	23.0±5.7
BMI, kg/m^2	21.8±1.8
FSH, mIU/mL	3.1±2.4
LH, mIU/mL	2.9±2.9
E_2, pg/mL	58.9±129.4
Initial AMH levels, ng/mL	2.7±1.8
AMH levels at 12 month, ng/mL	2.5±1.8
AFC	9.9±4.9
Mean ovarian volume, mm^3	7.6±5.5
Mean uterine volume, cm^3	29.2±23.5
Initial ferritin levels, ng/mL	2,208±1,811
Ferritin levels after one year, mL/ng	2,280±2,040

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, AMH: Anti-Mullerian hormone, AFC: Antral follicle count

Table 2. Comparison between the clinical features of patients with amenorrhea and normal cycle

	Amenorrhea, (n=25)	Normal cycle, (n=16)	p-value
Age, years	24.4±5.9	20.8±4.9	0.061
Years receiving transfusions	20±5	18±5	0.005
BMI, kg/m^2	21.8±1.8	21.9±1.9	0.781
FSH, mIU/mL	2.5±2.3	4.2±2.1	0.040
LH, mIU/mL	1.7±1.5	4.7±3.6	0.001
E_2, pg/mL	16.4±14.2	125.7±121.5	0.011
Initial AMH levels, ng/mL	2.1±1.8	3.6±1.5	0.009
AMH levels at 12 month, ng/mL	2.1±1.8	3.3±1.5	0.031
AFC	8.6±5.7	12.0±2.3	0.048
Mean ovarian volume, mm^3	5.5±4.4	10.9±5.7	0.003
Mean uterine volume, cm^3	19.1±19.0	45.0±21.5	0.001
Initial ferritin levels, mL/ng	2,742±2,019	1,475±1,155	0.041
Ferritin levels after one year, mL/ng	2,862±2,285	1,400±1,066	0.032

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, AMH: Anti-Mullerian hormone, AFC: Antral follicle count

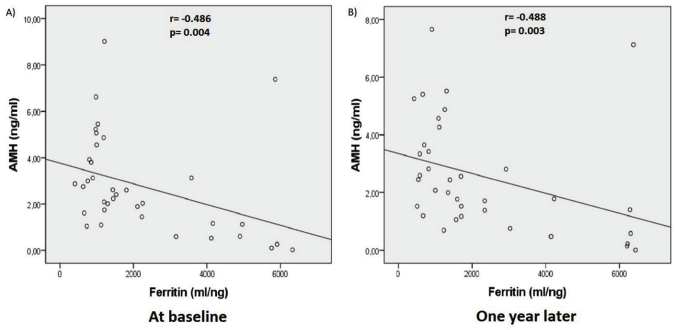

Figure 1. A) Correlation curve of initial anti-Mullerian hormone (AMH) and ferritin values at baseline, B) correlation curve of AMH and ferritin values one year later

Discussion

In this prospectively designed study, the effects of repeated blood transfusions on AMH levels at baseline and after a one-year follow-up in women with BTM were investigated and it was found that women with BTM had lower AMH levels compared to age-matched population norms. Furthermore, there was a trend towards a decrease in AMH levels even over such a short follow-up period. The presence of amenorrhea seemed to be mainly associated with hypothalamic dysfunction, rather than ovarian reserve. Hovewer, it appears that longer duration of blood transfusions may lead to hypogonadotrophic hypogonadism, resulting in lower AMH levels and decreased ovarian volume compared to women with BTM and with regular cycles. To the best of our knowledge, this is the first prospective longitudinal study demonstrating an alteration in ovarian reserve of women with BTM after only a one-year follow-up.

HH (70-80%) and amenorrhea due to hemosiderosis of the hypothalamus and pituitary gland are common in BTM patients (2,11,13). However, the effect of iron load on the ovaries is unclear (14-16). There are only limited data available in concerning ovarian reserve in patients with BTM (2,5,9,10), In a study conducted with 17 BTM patients (9 amenorrheic, 8 with normal menstrual cycles), the mean age of the patients was 33.8±5.6 years (2). AMH values of all 17 BTM patients were significantly lower than the control group and AMH levels in the amenorrheic group were lower than in patients with normal menstrual cycles. In our cohort, the frequency of amenorrhea was 60.9% in BTM and the same pattern was seen when comparing AMH levels between amenorrheic group and women with normal cycles. In another study, 29 BTM patients with a mean age of 21.4±5.8 years and 29 control patients with a mean age of 21.5±6.2 years were evaluated in terms of ovarian reserve. Although AMH values were lower in the BTM group compared to the control group, the result was not significant (5). Singer et al. (9) evaluated 26 patients with BTM and compared AMH levels between amenorrheic women (29±5 years) and women with normal menstrual cycles (27±8 years) and they concluded that there was no significant difference between the two groups in terms of AMH values and antral follicle numbers. All these studies were retrospective and study populations were small (2,5,9). Uysal et al. (10) compared AMH values between BTM patients (n=43) and healthy controls (n=44) in a larger, prospectively designed, case-control study. The mean age of patients with BTM was 23.4±5.1 years. When they compared the BTM group with the control group, the median AMH values (1.7 vs. 3.5 ng/mL; p=0.002, respectively) and median antral follicle numbers (3 vs. 11; p<0.001, respectively) were found to be lower in the BTM group. When subgroup analysis was performed among BTM patients, amenorrheic patients had lower AMH and antral follicle counts than patients with normal menstrual cycles. Since women with normal menstrual cycles were younger than the amenorrheic group and they had a shorter duration of transfusion therapy, it seems reasonable that they would have better ovarian reserves (10). Similar to the study by Uysal et al. (10), in the present study the amenorrheic group had lower AMH levels, antral follicle numbers and mean ovarian volumes. Given that there are studies reporting that AMH was not influenced by hypothalamic amenorrhea (17,18), significantly decreased AMH levels in women with amenorrhea may be due to the direct gonadotoxic effects of iron overload.

In a study that investigated patients with BTM who received chelation therapy for 5 years, high serum ferritin level, poor compliance with chelation therapy and early-onset transfusion therapy were found to be the main risk factors associated with endocrine complications (8). The same study also reported that serum ferritin levels of 2000 ng/mL were related with hypogonadism. Chang et al. (5) reported ferritin levels to be significantly higher in patients with HH than in those without HH. In addition, a negative correlation was found between ferritin and AMH levels (5,10). These authors concluded that high ferritin levels may lead to ovarian damage as well as the dysfunction in hypothalamo-pituitary axis. Although women with HH may have lower AMH levels due to long-term ovarian suppression (11,17,18), AMH level is still one of the best predictors of controlled ovarian stimulation in these women (17,18). In the present study, a significant inverse correlation was found between AMH and ferritin levels, tested both at baseline and one-year follow-up. Consistent with other studies (10), ferritin levels were demonstrated to be higher in amenorrheic patients than patients with normal menstrual cycles.

It is a matter of debate whether pregnancy and fetal outcomes are comparable between the healthy population and patients with BTM. Cesarean section is frequently preferred in women with beta-thalassemia, and infant weight at birth is usually

lower than the general population, despite term delivery (19). Spontaneous abortion, fetal loss, preterm delivery, fetal growth restriction and low birth weight are more common in pregnant women with BTM compared to healthy women (20). In the present study, we reported a small number of pregnancy outcomes. All went to term without any fetal or obstetric complications.

Study Limitations

One of the limitations of this study was the lack of control group. However, the availability of an age-specific AMH nomogram for the Turkish population was used to compare AMH levels. Furthermore, when we limited the study to the patients with regular menstruation, there was no difference in AMH levels with the healthy population. This may have been due to the inclusion of a relatively younger population and shorter follow-up period. Therefore, further research with a longer follow-up period is warranted to draw more definite conclusions regarding the possible time period in which iron accumulation initiates ovarian damage and to distinguish HH-related low AMH levels from low AMH levels due solely to direct ovarian damage.

Conclusion

The AMH values of transfusion-dependent BTM patients decreased significantly over time and the frequency of amenorrhea increased with advance in age. It would be beneficial to inform all patients regarding possible effects of repeated blood transfusions on fertility. Further research is required to understand the extent of ovarian damage in older BTM women with HH.

Ethics Committee Approval: *This study was approved by the İzmir Katip Çelebi University Local Ethics Committee (IRB: 0044, date: 24.02.2021) and conducted according to the principles of the Declaration of Helsinki.*

Informed Consent: *After verbal and written information about the study, all eligible and voluntary participants gave informed consent.*

Peer-review: *Externally peer-reviewed.*

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References

1. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet 2012; 379: 373-83.
2. Talaulikar VS, Bajoria R, Ehidiamhen AJ, Mujawar E, Chatterjee R. A 10-year longitudinal study of evaluation of ovarian reserve in women with transfusion-dependent beta thalassaemia major. Eur J Obstet Gynecol Reprod Biol 2019; 238: 38-43.
3. Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, et al. Cellular and molecular aspects of ovarian follicle ageing. Hum Reprod Update 2008; 14: 131-42.
4. Roussou P, Tsagarakis NJ, Kountouras D, Livadas S, Diamanti-Kandarakis E. Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. Anemia 2013; 2013: 617204.
5. Chang HH, Chen MJ, Lu MY, Chern JP, Lu CY, Yang YL, et al. Iron overload is associated with low anti-müllerian hormone in women with transfusion-dependent β-thalassaemia. BJOG 2011; 118: 825-31.
6. Singer ST, Sweeters N, Vega O, Higa A, Vichinsky E, Cedars M. Fertility potential in thalassemia major women: current findings and future diagnostic tools. Ann N Y Acad Sci 2010; 1202: 226-30.
7. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004; 89: 1187-93.
8. Poggi M, Sorrentino F, Pugliese P, Smacchia MP, Daniele C, Equitani F, et al. Longitudinal changes of endocrine and bone disease in adults with β-thalassemia major receiving different iron chelators over 5 years. Ann Hematol 2016; 95: 757-63.
9. Singer ST, Vichinsky EP, Gildengorin G, van Disseldorp J, Rosen M, Cedars MI. Reproductive capacity in iron overloaded women with thalassemia major. Blood 2011; 118: 2878-81.
10. Uysal A, Alkan G, Kurtoğlu A, Erol O, Kurtoğlu E. Diminished ovarian reserve in women with transfusion-dependent beta-thalassemia major: Is iron gonadotoxic? Eur J Obstet Gynecol Reprod Biol 2017; 216: 69-73.
11. Chan C, Liu K. Clinical pregnancy in a woman with idiopathic hypogonadotropic hypogonadism and low AMH: utility of ovarian reserve markers in IHH. J Assist Reprod Genet 2014; 31: 1317-21.
12. Anckaert E, Öktem M, Thies A, Cohen-Bacrie M, Daan NM, Schiettecatte J, et al. Multicenter analytical performance evaluation of a fully automated anti-Müllerian hormone assay and reference interval determination. Clin Biochem 2016; 49:260-7. Erratum in: Clin Biochem 2020; 76: 47-8.
13. Castaldi MA, Cobellis L. Thalassemia and infertility. Hum Fertil (Camb) 2016; 19: 90-6.
14. Bedrick BS, Kohn TP, Pecker LH, Christianson MS. Fertility preservation for pediatric patients with hemoglobinopathies: Multidisciplinary counseling needed to optimize outcomes. Front Endocrinol (Lausanne) 2022; 13: 985525.
15. Bajoria R, Chatterjee R. Hypogonadotrophic hypogonadism and diminished gonadal reserve accounts for dysfunctional gametogenesis in thalassaemia patients with iron overload presenting with infertility. Hemoglobin 2011; 35: 636-42.
16. Mamsen LS, Kristensen SG, Pors SE, Bøtkjær JA, Ernst E, Macklon KT, et al. Consequences of β-Thalassemia or Sickle Cell Disease for Ovarian Follicle Number and Morphology in Girls Who Had

Ovarian Tissue Cryopreserved. Front Endocrinol (Lausanne) 2021; 11: 593718.

17. Luisi S, Ciani V, Podfigurna-Stopa A, Lazzeri L, De Pascalis F, Meczekalski B, et al. Serum anti-Müllerian hormone, inhibin B, and total inhibin levels in women with hypothalamic amenorrhea and anorexia nervosa. Gynecol Endocrinol 2012; 28: 34-8.
18. Sönmezer M, Özmen B, Atabekoglu CS, Papuccu EG, Ozkavukcu S, Berker B, et al. Serum anti-Mullerian hormone levels correlate with ovarian response in idiopathic hypogonadotropic hypogonadism. J Assist Reprod Genet 2012; 29: 597-602.
19. Nourollahpour Shiadeh M, Cassinerio E, Modarres M, Zareiyan A, Hamzehgardeshi Z, Behboodi Moghadam Z. Reproductive health issues in female patients with beta-thalassaemia major: a narrative literature review. J Obstet Gynaecol 2020; 40: 902-11.
20. Charoenboon C, Jatavan P, Traisrisilp K, Tongsong T. Pregnancy outcomes among women with beta-thalassemia trait. Arch Gynecol Obstet 2016; 293: 771-4.

Chronic endometritis diagnosed using a cut-off of ≥5 CD138 plasma cells significantly affects the reproductive outcomes of frozen embryo transfer: a case-control study

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Abstract

Objective: To investigate the clinical significance of a diagnosis of chronic endometritis (CE) made using a diagnostic cut-off of ≥1 or ≥5 CD138 plasma cells per high power field (HPF) in asymptomatic patients undergoing in vitro fertilization (IVF) with frozen embryo transfer (FET).

Material and Methods: In this retrospective case-control study, 1,865 patients underwent freeze-all-IVF treatment between January and December 2019, with 419 undergoing endometrial biopsies at oocyte retrieval. Of the 419 biopsy-patients, 301 have since undergone first FET. The processed endometrial biopsies of the 301 patients underwent immunohistochemical (IHC) examination with anti-CD138 to count CD138+ plasma cells per HPF. CE diagnosis was defined as 0 CD138 plasma cells (control-group), ≥1 CD138 plasma cells ($CE^{control}$-group) or ≥5 CD138 plasma cells ($CE^{disease}$-group) per HPF.

Results: Twenty-six (8.6%) patients were retrospectively diagnosed having ≥1 CD138 plasma cells, and five patients (1.7%) having ≥5 CD138 plasma cells ($CE^{disease}$-group) per HPF. The live birth and pregnancy loss rates of the three groups were 52.7% and 27.9%, 53.8% and 26.3% and 20.0% and 66.7%, respectively. The antral follicle count (AFC) of the three groups were 15.0 (9.0-22.0), 10.5 (7.75-15.25), and 6.0 (5.0-14.0), respectively.

Conclusion: Asymptomatic patients diagnosed with CE with ≥5 CD138 plasma cells per HPF, had the lowest live birth and highest pregnancy loss rates, with these patients also having significantly reduced AFC. (J Turk Ger Gynecol Assoc 2023; 24: 165-71)

Keywords: Chronic endometritis, CD138, frozen embryo transfer, asymptomatic, live birth

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Introduction

Fertility work-ups performed before patients commence in vitro fertilization (IVF) treatment should include diagnostic tests that identify all factors with the potential to affect implantation. Implantation remains a perplexing issue, despite recent innovations in assisted reproductive technologies. Of clinical importance is the identification of all intrauterine anomalies, as these may affect endometrial receptivity. Chronic endometritis (CE) is a poorly understood and often underdiagnosed endometrial inflammatory disease, with microbial infection believed to result in the persistent inflammatory condition (1,2). CE, therefore, is often first diagnosed at the patient's fertility workup if there are indications to suggest the need for diagnostic hysteroscopy. Hysteroscopy may reveal anomalies characteristic of $CE^{disease}$, such as focal or diffuse micropolyps, stromal oedema, focal hyperemia, strawberry aspect, and endometrial haemorrhagic spots, or there may be other

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endometrial features indicating the possibility of endometrial dysfunction, which suggest the need for endometrial biopsy and histological assessment (3).

Implantation is a complex process, including the immunological regulation of blastocyst and endometrial interactions, with synchronized interactive dialogue mediated by steroid hormones, including estrogen and progesterone, cytokines, growth factors, adhesion molecules, prostaglandins, matrix-degrading enzymes and inhibitors (4). The CE hypothesis suggests that the presence of microorganisms and subsequent inflammation results in CE, which then affects implantation by altering local immunological regulation and time-dependent endometrial changes critical to successful reproduction (2,5). Modified immunological conditions may inhibit the ability of the endometrium to develop the critical inflammatory and immune-tolerant responses required for early pregnancy processes, including implantation, placentation and fetal protection (6). CE-related conditions may also affect the ability of the endometrium to respond to sex steroid hormones, impairing hormone-regulated morphological changes, such as endometrial cell proliferation, decidualization, and vascularization, as well as intrauterine contractility (7,8).

There is still no consensus regarding the clinical significance of CE, with no definitive evidence to support the hypothesis that CE is always associated with adverse reproductive outcomes (9,10). The higher prevalence of CE in patients with recurrent implantation failure (RIF), recurrent pregnancy loss (RPL), and preterm delivery, however, does support the belief that CE may adversely affect some reproductive outcomes in IVF (11). The controversy surrounding the significance of CE arises mainly because of the various criteria used in the diagnosis of CE, as reported from the analysis of surveys completed by pathologists (12). Moreover, basic histological assessment of hematoxylin & eosin (H&E) stained endometrial biopsies is generally accepted to be the gold standard for CE diagnosis, when coupled with immunohistochemical (IHC) staining using anti-Syndecan-1 (CD138) that increases the accuracy of plasma cell identification (13). Histopathologically, CE is described as the infiltration of the endometrial stroma by plasma cells, with some pathologists considering a single plasma cell per unit measure a diagnosis of CE (12). Moreover, emerging evidence suggests that ≥5 plasma cells per high-power field (HPF) may better define a diagnosis of CE with significant clinical implications (14,15).

At the IVF centre where the present study was conducted, freeze-all-IVF is performed routinely, which provides the opportunity to safely perform diagnostic endometrial biopsies at the time of the oocyte retrieval procedure (16). In the present study, the clinical significance of CE diagnosed using the criteria of either ≥1 or ≥5 plasma cells per HPF was investigated in asymptomatic patients undergoing freeze-all-IVF with frozen embryo transfer (FET).

Materials and Methods

Patients

This retrospective, case-control study was performed at a single IVF centre, with cycles of patients who had undergone endometrial biopsies at oocyte retrieval for freeze-all-IVF treatments performed between January and December 2019 being investigated. The original endometrial biopsies were performed to benefit from the rejuvenating effect of endometrial scratching (17). In the present study, the previously processed endometrial biopsy tissues were stained with CD138 IHC, to perform plasma cell counts, after patients had undergone their first FET. Routine fertility workup included 2-D transvaginal ultrasound scans, with saline-infused sonography, hysterosalpingography, and hysteroscopy performed if intrauterine anomalies were suspected. Importantly, freeze-all-IVF treatments provide the opportunity to perform therapeutic or diagnostic intrauterine procedures, including endometrial biopsy, at oocyte retrieval without adversely affecting the pregnancy prognosis of patients (16). Cycles were included according to the following criteria: if an endometrial biopsy was performed at oocyte retrieval; female age was ≤42 years; and female body mass index (BMI) was ≤35 kg/m^2. Exclusion criteria were: intrauterine or tubal anomalies not corrected; RIF or RPL diagnosed; corticosteroid treatment; or if the female patient had autoimmune disease, antiphospholipid syndrome, or thrombophilia. However, cycles in which hysteroscopic surgery for intrauterine or tubal anomalies were performed at the time of the oocyte retrieval and endometrial biopsy were included, with the FET of these patients delayed according to the type of surgery (16).

This work was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (approval number: KAEK-970, date: 22.12.2021).

Procedures

Endometrial biopsy

All endometrial biopsies were taken on ovarian cycle days 8-16, with all endometrial biopsies taken using 3.0 mm endometrial suction curettes (gynaecological sampler, Medbar, İzmir, Turkey) immediately following the oocyte retrieval. The endometrial biopsies were washed in phosphate buffered-saline and fixed overnight in vials containing fixative (10% phosphate-buffered formaldehyde).

Endometrial tissue assessment

The endometrial biopsies were transferred to an independent histopathology laboratory, where tissue preparation and assessment was performed by experienced gynecological histopathologists. Previously processed endometrial tissue sections were stained with anti-CD138 (EP201, cell-marque, Merck KGaA, Darmstadt, Germany) using Dako Autostainer Link48 (Dako Colorado Inc. Ft Collins, CO, USA), with tissue sections examined by light microscopy (400x magnification). The mean number of CD138 plasma cells counted per HPF (in 10 HPF) were recorded. CE diagnosis was reported according to the mean number of plasma cells counted per HPF, with CE diagnoses grouped as follows: 0 CD138 plasma cells (control-group); ≥1 CD138 plasma cells ($CE^{control}$-group); and ≥5 CD138 plasma cells ($CE^{disease}$-group).

Antibiotic prophylaxis

Antibiotic prophylaxis therapy was administered twice during treatment. Firstly, a single dose of azithromycin (2x500 mg, Zitrotek, Pfizer İlaç Ltd. Şti. İstanbul, Turkey) was administered for empirical antibiotic prophylaxis for unsuspected sexual transmitted disease to the couple (both male and female patients) before proceeding with ovarian stimulation (OS). The second antibiotic treatment consisted of 200 mg of doxycycline (Tetradox, Actavis, İstanbul, Turkey) administered for four days after oocyte retrieval. The doxycycline prophylaxis was a precautionary measure against any possible procedure-related infections (18), with the total antibiotic prophylaxis therapy administered in the attempt to normalize patient semen and vaginal microbiomes (19).

Ovarian stimulation, embryo culture, and blastocyst vitrification

OS was performed using standard protocols, as previously described (20). In vitro oocyte collection and embryo cultures were performed using standard protocols, as previously described (20). Blastocysts were scored and selected for cryopreservation on days 4-6 of in vitro embryo culture (21), with all viable blastocysts cryopreserved using vitrification protocols and technologies (Cryotop, Kitazato BioPharma Co. Ltd, Fuji-city, Japan).

Artificial cycle frozen embryo transfer

Artificial cycle FET (AC-FET) was performed, as previously described (22), with the daily administration of vaginal progesterone (90 mg TID, Crinone® 8%, Merck Serono, İstanbul, Turkey) started on day-15 and continued in conjunction with oral estrogen (2 mg TID) until the day of the pregnancy tests. In addition, patients were given weekly intramuscular (IM) progesterone (Proluton® depot 500 mg/2 mL, Bayer Türk, İstanbul, Turkey), with the first IM progesterone administered on the third day of vaginal progesterone. Pregnant patients continued with all luteal phase support drugs until the 10th week of gestation.

Blastocyst transfer

The start date (day-15) of progesterone administration and the day of blastocyst cryopreservation were used to schedule FET. Blastocysts cryopreserved on days 5 and 6 were transferred on the sixth day of progesterone and blastocysts cryopreserved on day 4 on the fifth day of progesterone (21). All blastocysts to be transferred were rescored approximately 2 hours before the transfer, with the day of cryopreservation and blastocyst scores (including, expansion, inner cell mass, and trophectoderm scores) used to define a poor, fair, or good quality blastocyst. Blastocysts with inner mass and trophectoderm scores of AC, CA, BC, CB, and CC were defined as poor quality, irrespective of expansion grade. A maximum of two blastocysts were transferred.

Outcomes and statistical analysis

The primary outcome measure was live birth (LB) from the first FET of freeze-all-IVF cycles, with LB defined as the delivery of a live infant at ≥22 weeks of gestation. The secondary outcome measure was the mean number of CD138 plasma cells per HPF. RIF was defined as the failure of more than three previously performed fresh or FET to result in a clinical implantation, with female age and embryo quality taken into consideration. RPL was defined as the loss of more than three pregnancies before 22 weeks of gestation.

Statistical analysis

SPSS, version 11.5 (IBM Inc., Armonk, NY, USA) was used in all statistical analyses, with variables analysed as means ± standard deviation, medians (plus interquartile ranges 25% and 75%; interquartile range), or as rates (percentages). In univariate comparative analyses, Mann-Whitney Rank Sum tests and chi-square tests were performed, with significant difference indicated by a $p<0.05$.

Results

In total 1865 patients aged 18-42 years (mean age: 33.2±6.01 years) underwent oocyte retrievals as part of freeze-all-IVF treatments in 2019, with 419 (22.6%) undergoing endometrial biopsies at oocyte retrievals (Table 1). Endometrial biopsies were done on ovarian cycle-day 12.3±2.14. One hundred and eighteen patients were excluded from the study, with the 301 patients who had undergone their first FET included. Forty-seven patients had delayed FET because of hysteroscopic surgery performed at oocyte retrieval, 43 (15.6%) were in the

control-group and 4 (15.4%) in the CE-group. The processed endometrial tissues of these patients were retrospectively stained with CD138 IHC to count the number of plasma cell present. Of the 301 patients, 26 (8.6%) were identified as having ≥1 CD138 plasma cells per HPF ($CE^{control}$-group) and 5 (1.7%) having ≥5 CD138 plasma cells per HPF ($CE^{disease}$-group).

In Table 2, the demographics of patients with 0 CD138 plasma cells (control-group) are compared with those of patients with ≥1 CD138 plasma cells ($CE^{control}$-group). Only the median AFCs of the two groups were significantly different, with patients in the $CE^{control}$-group having fewer AFCs. The majority of patients were diagnosed as having primary infertility, with unexplained and male infertility being the most prevalent of the infertility etiologies.

The overall LB rate from first FET of freeze-all-IVF treatments performed in 2019 was 47.9% (627/1308). The reproductive outcomes of the first FET in the different study groups are presented in Table 3. The pregnancy, clinical pregnancy, LB and pregnancy loss rates were similar in the control- and $CE^{control}$-groups. The pregnancy, clinical pregnancy, and LB rates were lower in the $CE^{disease}$-group, with a pregnancy loss rate of 66.7%. The patients in the $CE^{disease}$-group tended to have fewer AFC than patients with <5 plasma cell counts with a median of 6.0 (5.0-14.0) vs. 11.0 (8.5-17.0), respectively (p=0.161). The blastocyst quality of patients diagnosed with CE (either ≥1 or ≥5 plasma cells) was unaffected, with 96.2% of the primary blastocysts transferred defined as being of good quality. One patient in the $CE^{disease}$-group underwent a second FET treatment, which resulted in a clinical pregnancy loss.

Table 1. Patient inclusion and exclusion

n (endometrial biopsy cycles)	**419**
Excluded cycles	
Female age >42 years	17
Female BMI >35 kg/m²	9
RIF	3
RPL	3
Cycle outcome exclusions	
No oocytes retrieved	3
No mature oocytes retrieved	5
No fertilization	6
Embryo development arrest	63
No first FET	8
Unknown pregnancy outcome	1
n (cycles with first FET)	**301**

BMI: Body mass index, RIF: Recurrent implantation failure, RPL: Recurrent pregnancy loss, FET: Frozen embryo transfer, No FET: No first FET following oocyte retrieval

Discussion

While the cause-and-effect relationship between CE and adverse reproductive outcomes in IVF is clinically plausible, the impact of CE on the reproductive outcomes of IVF patients remain controversial (5). Patient selection in previous studies may have contributed to this controversy, with some previous studies investigating the effect of CE in patients

Table 2. Patient demographic comparisons

	Control-group (n=275)	**CE-group (n=26)**	**p**
Female age (years)	31.6 (27.8-36.6)	33.7 (27.8-36.8)	0.592
Female BMI (kg/m²)	25.0 (22.0-27.0)	24.0 (21.0-28.0)	0.517
AFC (n)	15.0 (9.0-22.0)	10.5 (7.75-15.25)	0.022
Infertility duration (years)	3.5 (2.0-6.0)	4.0 (1.50-5.25)	0.569
Primary infertility (%)	84.7 (233)	76.9 (20)	0.448
Patients with previous embryo transfers (%)	32.7 (90)	30.8 (8)	0.988
LB rate from previous embryo transfers (%)	8.3 (12/145)	7.1 (1/14)	0.717
Primary infertility etiologies (%)			
Unexplained	50.9 (140)	50.0 (13)	0.907
Male	27.3 (75)	30.8 (8)	0.879
DOR	7.6 (21)	11.5 (3)	
PCOS	7.3 (20)	3.8 (1)	
Tubal	6.2 (17)	3.8 (1)	
Endometrioma	0.7 (2)	0.0 (0)	

Data presented as median (interquartile range) or percentage (number), with statistical significance of p<0.05 in Mann-Whitney Rank Sum and chi-square tests. BMI: Body mass index, CE: Chronic endometritis, AFC: Antral follicle count, LB: Live birth, PCOS: Polycystic ovary syndrome, DOR: Diminished ovarian reserve (defined as total AFC of ≤5)

Table 3. The reproductive outcomes from first FET of CE patients

		Control-group (0)[a]	CE^{control}-group (≥1)[b]	p-value[a vs. b]	CE^{disease}-group (≥5)
First FET (n)		275	26		5
Number transferred	n	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.281	1.0 (1.0-1.5)
Blastocyst quality poor	% (n)	2.5 (7)	0.0 (0)		
Blastocyst quality fair	% (n)	8.0 (22)	3.8 (1)		
Blastocyst quality good	% (n)	89.5 (246)	96.2 (25)	0.455	80.0 (4)
Pregnancy rate (%)	% (n)	73.1 (201)	73.1 (19)	0.818	60.0 (3)
Clinical pregnancy rate (%)	% (n)	61.5 (169)	61.5 (16)	0.840	40.0 (2)
Live birth rate (%)	% (n)	52.7 (145)	53.8 (14)	0.923	20.0 (1)
Total pregnancy loss rate (%)	% (n)	27.9 (56)	26.3 (5)	0.901	66.7 (2)

Data presented as median (interquartile range) or percentage (number), with statistical significance of $p<0.05$ in Mann-Whitney Rank Sum and chi-square tests. CE: Chronic endometritis, FET: Frozen embryo transfer, Number transferred: The number of blastocysts transferred, NB for blastocyst quality: Poor includes all blastocysts with inner mass and trophectoderm scores of AC, CA, BC, CB, and CC, with only the score of the primary blastocysts transferred analysed. Pregnancy rate, defined as a β-HCG level of >5 mIU/mL measured 9 days after blastocyst transfer. Clinical pregnancy rate, defined as a pregnancy with normal fetal heart activity confirmed on ultrasound after 5 weeks of gestation. Early pregnancy loss, defined as a pregnancy lost before normal fetal heart activity confirmation and miscarriage, defined as a clinical pregnancy lost before 22 weeks of gestation. Total pregnancy loss includes all pregnancy losses

having RIF or RPL. RIF and RPL are complex, multifactorial infertility etiologies, which may complicate cause-and-effect investigations (23,24). Moreover, the factor that has contributed most to the controversy is the absence of universally accepted criteria for defining CE. In the present study, the reproductive outcomes in asymptomatic patients who had undergone their first FET following blastocyst freeze-all-IVF were investigated, with endometrial biopsies taken at oocyte retrievals retrospectively stained with CD138 IHC for the diagnosis of CE, and sub-grouped as either ≥1 or ≥5 CD138 plasma cells per HPF.

In the present study, endometrial biopsies were taken mid-cycle (day-12.3), with less than 10% having ≥1 CD138 plasma cells and fewer than 2% having ≥5 CD138 plasma cells per HPF. Asymptomatic patients in the CE^{control}-group had reproductive outcomes not dissimilar to those with zero CD138 plasma cells who constituted the non-CE^{control}-group in the present study. These reproductive outcomes supported the assertion made in the study of Kasius et al. (25) that "CE diagnosed in asymptomatic patients had minimal clinical implications in IVF treatments". In the same study, CE diagnoses were qualitative, that is the CE group had "evident CE". In a more recent study, no significant differences between pregnancy outcomes in patients with 0 CD138+/HPF and those with 1-4 CD138+/HPF were reported (15). In the present study, patients in the CE-group were associated with lower AFC but this reduction in AFC did not have a negative effect on pregnancy outcomes in the CE^{control}-group, with similar rates of good quality (primary) blastocysts transferred.

Historically, plasma cell identification in endometrial tissue has been a difficult challenge, even for the most experienced histopathologists when conventional HE staining was used, with the main difficulty being the ability to accurately distinguish plasma cells from other morphologically similar endometrial and immunological cells (26). Moreover, diagnostic accuracy is not only dependent on the experience of the pathologist, but also on staining protocol, as well as the timing, method, size, and location of endometrial biopsy (26). In the present study, all endometrial biopsies were obtained mid-cycle (day-12.3), with endometrial tissues retrospectively stained with CD138 IHC. Even though CD138 IHC has markedly increased the accuracy of plasma cell counts, there is still no consensus on the criteria defining CE that has clinical consequences (5,12,13). The study of Liu et al. (13), suggested that plasma cell density quantified per unit area improved the accuracy of CE diagnosis. Hirata et al. (27) found that the sensitivity and accuracy were optimal if CE was diagnosed as ≥1 plasma cells in 10 HPF. The emerging evidence, however, suggests that criteria that include plasma cell counts of ≥5 may more accurately define clinical CE (14,15).

Patients diagnosed having CE, with the diagnosis defined as ≥5 plasma cells, were reported to have significantly reduced LB rates (14,15). Xiong et al. (15) found no differences in pregnancy outcomes between patients with 0 CD138+ cells/HPF and those with 1-4 CD138+ cells/HPF. In the present study, the majority (81%) of patients in the CE-group had plasma cell counts of <5. Interestingly, patients diagnosed having ≥1 plasma cell had reduced AFC, with patients diagnosed having ≥5 plasma cells having an even lower, but not significantly lower AFC. This decrease in AFC may be the clinical consequence of progressive CE^{disease}, with those diagnosed having ≥5 CD138 plasma cells particularly affected. Patients with ovarian endometriosis or endometrioma have also been reported to have significantly reduced AFCs (28). In the present study, asymptomatic patients

diagnosed with ≥5 CD138 plasma cells had the lowest LB rate and highest pregnancy loss rate.

While the origins of the microorganisms associated with CE are a matter for speculation, pharmacological therapies have been reported to be effective (70-90%) in the treatment of CE (26). These therapies have included broad-spectrum, microbe-specific, and multi-course antibiotic therapies (11,15,29). Patients cured of CE, as evidenced by restored endometrial health, using antibiotic therapy were reported to have reproductive outcomes similar to those of patients without CE (11,15), with the authors suggesting that $CE^{disease}$ resolution confirmation procedures should be performed routinely before patients were allowed to commence with IVF treatment (11,15). In the present study, patients were administered azithromycin and doxycycline prophylactically during the course of their treatment, with doxycycline a common antibiotic included in CE antibiotic therapies (15,23,30). Moreover, whereas the standard prescribed duration of doxycycline was fourteen days for $CE^{disease}$, doxycycline was only administered for four days in the present study. It is uncertain whether the low-intensity antibiotic prophylaxis therapy administered by patients had an effect on $CE^{disease}$ because follow-up endometrial biopsies were not performed in the present study. However, while the $CE^{control}$-group were observed to have normal pregnancy prognoses, the $CE^{disease}$-group continued to have poor pregnancy prognoses, with the latter indicating the possible persistence of high level $CE^{disease}$.

Study Limitations

The present study confirms the importance of having universally accepted criteria defining clinical CE, with the study adding to the growing evidence to support ≥5 plasma cells per unit measure as a defining criterion. In addition, because the greatest improvement in reproductive outcomes has been reported for patient groups with restored endometrial health, it might be advisable to routinely perform follow-up endometrial biopsies for all patients with a diagnosis of CE (15). Limitations such as the retrospective cycle analyses, the low number of patients diagnosed having CE, the routine use of precautionary antibiotic prophylaxis, and the uncertain status of $CE^{disease}$ at FET weakened the strength of evidence of the present study.

Conclusion

Asymptomatic patients diagnosed with CE, defined as a mean of ≥5 CD138 plasma cells per HPF, had the lowest LB and highest pregnancy loss rates, with these patients also having significantly reduced AFC compared to patients with zero CD138 plasma cells identified.

Ethics Committee Approval: *This work was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (approval number: KAEK-970, date: 22.12.2021).*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

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References

1. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. Obstet Gynecol 1981; 58: 176-84.
2. Puente E, Alonso L, Laganà AS, Ghezzi F, Casarin J, Carugno J. Chronic Endometritis: Old Problem, Novel Insights and Future Challenges. Int J Fertil Steril 2020; 13: 250-6.
3. Mazur MT, Kurman RJ. Diagnosis of endometrial biopsies and curettings. A practical approach. New York: Springer Verlag; 1995. p. 260.
4. Kim SM, Kim JS. A Review of Mechanisms of Implantation. Dev Reprod 2017; 21: 351-9.
5. Buzzaccarini G, Vitagliano A, Andrisani A, Santarsiero CM, Cicinelli R, Nardelli C, et al. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. J Assist Reprod Genet 2020; 37: 2897-911.
6. Kushnir VA, Solouki S, Sarig-Meth T, Vega MG, Albertini DF, Darmon SK, et al. Systemic Inflammation and Autoimmunity in Women with Chronic Endometritis. Am J Reprod Immunol 2016; 75: 672-7.
7. Di Pietro C, Cicinelli E, Guglielmino MR, Ragusa M, Farina M, Palumbo MA, et al. Altered transcriptional regulation of cytokines, growth factors, and apoptotic proteins in the endometrium of infertile women with chronic endometritis. Am J Reprod Immunol 2013; 69: 509-17.
8. Wu D, Kimura F, Zheng L, Ishida M, Niwa Y, Hirata K, et al. Chronic endometritis modifies decidualization in human endometrial stromal cells. Reprod Biol Endocrinol 2017; 15: 16.
9. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, et al. Review: Chronic endometritis and its effect on reproduction. J Obstet Gynaecol Res 2019; 45: 951-60.
10. Margulies SL, Flores V, Parkash V, Pal L. Chronic endometritis: A prevalent yet poorly understood entity. Int J Gynaecol Obstet 2022; 158: 194-200.
11. Vitagliano A, Saccardi C, Noventa M, Di Spiezio Sardo A, Saccone G, Cicinelli E, et al. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation

failure: a systematic review and meta-analysis. Fertil Steril 2018; 110: 103-12.

12. Margulies SL, Dhingra I, Flores V, Hecht JL, Fadare O, Pal L, et al. The Diagnostic Criteria for Chronic Endometritis: A Survey of Pathologists. Int J Gynecol Pathol 2021; 40: 556-62.
13. Liu Y, Chen X, Huang J, Wang CC, Yu MY, Laird S, et al. Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure. Fertil Steril 2018; 109: 832-9.
14. Li Y, Xu S, Yu S, Huang C, Lin S, Chen W, et al. Diagnosis of chronic endometritis: How many CD138+ cells/HPF in endometrial stroma affect pregnancy outcome of infertile women? Am J Reprod Immunol 2021; 85: e13369.
15. Xiong Y, Chen Q, Chen C, Tan J, Wang Z, Gu F, et al. Impact of oral antibiotic treatment for chronic endometritis on pregnancy outcomes in the following frozen-thawed embryo transfer cycles of infertile women: a cohort study of 640 embryo transfer cycles. Fertil Steril 2021; 116: 413-21.
16. Ozgur K, Bulut H, Berkkanoglu M, Humaidan P, Coetzee K. Concurrent oocyte retrieval and hysteroscopy: a novel approach in assisted reproduction freeze-all cycles. Reprod Biomed Online 2016; 33: 206-13.
17. Olesen MS, Hauge B, Ohrt L, Olesen TN, Roskær J, Bæk V, et al. Therapeutic endometrial scratching and implantation after in vitro fertilization: a multicenter randomized controlled trial. Fertil Steril 2019; 112: 1015-21.
18. Pereira N, Hutchinson AP, Lekovich JP, Hobeika E, Elias RT. Antibiotic Prophylaxis for Gynecologic Procedures prior to and during the Utilization of Assisted Reproductive Technologies: A Systematic Review. J Pathog 2016; 2016: 4698314.
19. Eskew AM, Stout MJ, Bedrick BS, Riley JK, Herter BN, Gula H, et al. Association of vaginal bacterial communities and reproductive outcomes with prophylactic antibiotic exposure in a subfertile population undergoing in vitro fertilization: a prospective exploratory study. F S Sci 2021; 2: 71-9.
20. Ozgur K, Bulut H, Berkkanoglu M, Donmez L, Coetzee K. Prediction of live birth and cumulative live birth rates in freeze-all-IVF treatment of a general population. J Assist Reprod Genet 2019; 36: 685-96.
21. Ozgur K, Bulut H, Berkkanoglu M, Humaidan P, Coetzee K. Artificial cryopreserved embryo transfer cycle success depends on blastocyst developmental rate and progesterone timing. Reprod Biomed Online 2018; 36: 269-76.
22. Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Agonist depot versus OCP programming of frozen embryo transfer: a retrospective analysis of freeze-all cycles. J Assist Reprod Genet 2016; 33: 207-14.
23. Demirdag E, Guler I, Cevher Akdulum MF, Sahin E, Erdem O, Erdem A, et al. Subsequent IVF outcomes following antibiotic therapy for chronic endometritis in patients with recurrent implantation failure. J Obstet Gynaecol Res 2021; 47: 4350-6.
24. Morimune A, Kimura F, Nakamura A, Kitazawa J, Takashima A, Amano T, et al. The effects of chronic endometritis on the pregnancy outcomes. Am J Reprod Immunol 2021; 85: e13357.
25. Kasius JC, Fatemi HM, Bourgain C, Sie-Go DM, Eijkemans RJ, Fauser BC, et al. The impact of chronic endometritis on reproductive outcome. Fertil Steril 2011; 96: 1451-6.
26. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. Fertil Steril 2018; 110: 344-50.
27. Hirata K, Kimura F, Nakamura A, Kitazawa J, Morimune A, Hanada T, et al. Histological diagnostic criterion for chronic endometritis based on the clinical outcome. BMC Womens Health 2021; 21: 94.
28. Tian Z, Zhang Y, Zhang C, Wang Y, Zhu HL. Antral follicle count is reduced in the presence of endometriosis: a systematic review and meta-analysis. Reprod Biomed Online 2021; 42: 237-47.
29. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod 2015; 30: 323-30.
30. Gay C, Hamdaoui N, Pauly V, Rojat Habib MC, Djemli A, Carmassi M, et al. Impact of antibiotic treatment for chronic endometritis on unexplained recurrent pregnancy loss. J Gynecol Obstet Hum Reprod 2021; 50: 102034.

The oncologic outcomes of endometrial cancer metastasizing to the adrenal gland and kidney: from case to analysis

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Abstract

Objective: To evaluate the oncologic outcomes of endometrial cancer metastasis to the adrenal gland and kidney, based on a case study and review of the literature.

Material and Methods: A systematic review of the medical literature was performed to identify articles about metastatic endometrial cancer to the adrenal gland and kidney from 1975 until 2021.

Results: A 55-year-old female patient was admitted to our center. On pelvic examination, a mass protruding out of the cervix was observed, which was shown to be endometrioid carcinoma on biopsy. Disease stage was IVB, based on radiological and pathological results and the International Federation of Gynecology and Obstetrics 2018 staging. Neo-adjuvant chemotherapy was given. After therapy, the patient underwent type 2 hysterectomy, bilateral salpingo-oophorectomy, total omentectomy and lymph node dissection. Left nephrectomy, left adrenalectomy and left hemicolectomy were also performed because the conglomerate tumor invaded the left kidney, left adrenal gland, and left colon mesentery. Pathological findings were consistent with metastasis of endometrioid carcinoma in the left adrenal gland, left kidney parenchyma and hilum.

Conclusion: Metastasis of endometrial cancer to the adrenal gland and kidney is extremely rare and metastasis to the kidney has been reported in only two previous cases. When there is an intraperitoneal spread of endometrial cancer, as well as ovarian cancer, cytoreductive surgery without leaving a residual tumor should be undertaken andshould include adrenalectomy and nephrectomy, if necessary. (J Turk Ger Gynecol Assoc 2023; 24: 172-6)

Keywords: Adrenal gland, case report, endometrial cancer, kidney, metastasis

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Introduction

Endometrial cancer is the most common gynecological cancer in developed countries (1). Epithelial malignant neoplasms are the most frequent malignant neoplasms of the endometrium (approximately 80%). Although most cases are detected at an early stage, 3-13% of patients are diagnosed with stage IV disease (2). The estimated 5-year survival rate for stage IVB endometrial cancer according to the International Federation of Gynecology and Obstetrics (FIGO) is around 15% (3).

Aggressive histological subtypes also have a worse prognosis with serous and clear cell types having a worse prognosis than the endometrioid type (4). In a study in which the SEER database results were analysed, distant organ metastases were reported in 39.1% of patients with FIGO stage IVB endometrial cancer (5). Non-lymphatic distant metastases are most common in the lungs, and spread to the adrenal gland and kidney is extremely rare. To the best of our knowledge, to date, there have been only two reports of endometrial cancer spreading to the kidney. In this case report, we describe a patient with metastasis

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of endometrial cancer to the adrenal gland and kidney. In addition, the limited literature is reviewed.

Material and Methods

A systematic review of the medical literature was performed to identify articles about metastatic endometrial cancer to the adrenal gland and kidney. The electronic database search was conducted between the years 1975 and 2021 using PubMed/MEDLINE for English language abstracts. The search included metastatic endometrial cancer, adrenal metastasis from endometrial cancer, renal metastasis from endometrial cancer, renal metastasis, and metastatic tumours of the adrenal glands under medical subject headings or keywords. The study protocol was reviewed and approved by University of Health Sciences Turkey, Ankara City Hospital Ethics Committee (approval: E2-22-2358, date: 07.09.2022).

At the end of the search, 14 articles were eligible for further analysis. In accordance with these cases, and in conjunction with our case, 16 cases were evaluated for this study.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows, version 22.0 (IBM Inc., Armonk, NY, USA). Descriptive values are expressed as arithmetic mean ± standard deviation, median and percent.

Results

Case Presentation

A 55-year-old female patient was admitted to our center with the complaint of vaginal bleeding. On pelvic examination, a mass protruding out of the cervix was observed. The physical examination was unremarkable. The results of complete blood count, kidney and liver function tests, and coagulation parameters were all normal. Among the tumor markers, CA125 107.4 IU/mL (normal <30), CA15-3 34.6 IU/mL (normal <32), and CA19-9 65.1 IU/mL (normal <30) were all high. Other tumor markers were normal. The biopsy of the mass protruding from the cervix was compatible with endometrioid carcinoma. F-18 fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) imaging revealed a 70x68x101 mm mass in the left adnexal area with indistinguishable borders from the uterus with a standardized uptake value (SUV_{max}) of 8.55. Involved lymph nodes were also observed in the left supraclavicular region (10x8 mm, SUV_{max} 4.63), in the paratracheal region (25x21x34 mm, SUV_{max} 10.96), and in the left paraaortic region (52x47 mm, SUV_{max} 12.33). The mass in the left paraaortic region invaded the left adrenal gland, left renal pelvis, and renal parenchyma (Figure 1). Fine needle aspiration biopsy of the left supraclavicular lymph node was consistent with endometrial carcinoma metastasis. The disease stage was IVB, based on radiological and pathological results and FIGO 2018 staging. Neo-adjuvant chemotherapy was administered to the patient. Supraclavicular and paratracheal lesions were not observed in the comparative analysis of FDG PET/CT performed after three cycles of carboplatin-paclitaxel chemotherapy, but the lesion in the left paraaortic region persisted. The decision to perform surgery was made by the gynecological-oncology team.

Under general anesthesia, the abdomen was entered through a midline incision from the xiphoid process to the pubis. In the intraoperative observation, peritoneal implants, bulky pelvic and paraaortic lymph nodes, and a 20 cm conglomerate mass including the uterus, left adnexa, and sigmoid colon were observed. Furthermore, a 10 cm mass was observed invading the inferior mesenteric artery and vein on the left side of the aorta, surrounding the renal vessels, and invading the left kidney and left adrenal gland. The patient underwent type 2 hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, bilateral pelvic and paraaortic lymph node dissection. Left nephrectomy, left adrenalectomy and left hemicolectomy were also performed because the conglomerate tumor invaded the left kidney, left adrenal gland, and left colon mesentery (Figure 2). The operation was terminated with maximal cytoreduction. Intraoperative blood loss was approximately 1500 cc, and the operation lasted for about seven hours. The patient was discharged after six days without any early postoperative complications. The postoperative pathology was reported as grade 3 endometrioid carcinoma of the endometrium. The tumor size was 8x4x4 cm. Deep myometrial invasion, lymphovascular space involvement and cervical spread were observed. Histopathological findings were consistent with metastasis of endometrioid carcinoma in the left ovary, left adrenal gland, left kidney parenchyma and hilum, and pelvic and paraaortic lymph nodes (Figure 3, 4). The disease progressed one month after surgery. Unfortunately, the patient died due to disease progression before adjuvant therapy at four months after surgery.

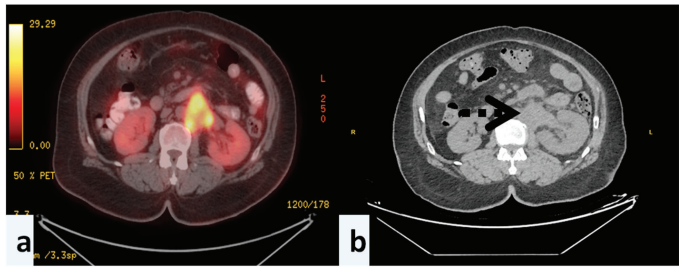

Figure 1. a) PET/CT scan of abdomen showing left adrenal mass in the left paraaortic region invaded the left adrenal gland, left renal pelvis and renal parenchyma; b) CT image of the same lesion

PET/CT: Positron emission tomography/computed tomography

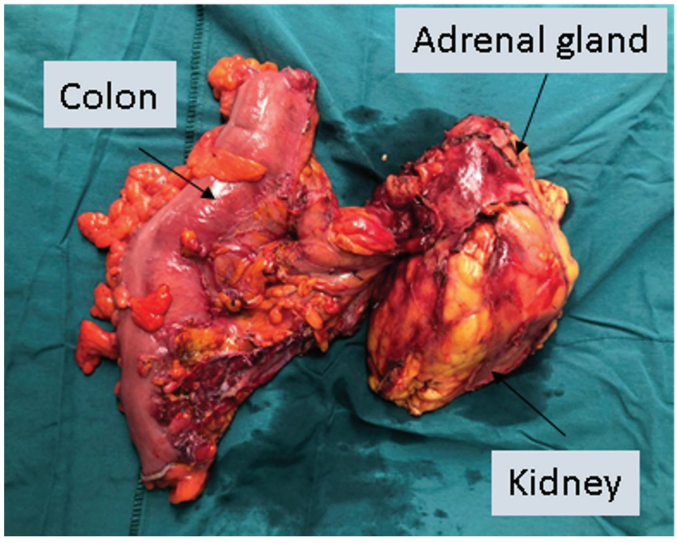

Figure 2. Surgical specimen of the left kidney, left adrenal gland, and left colon

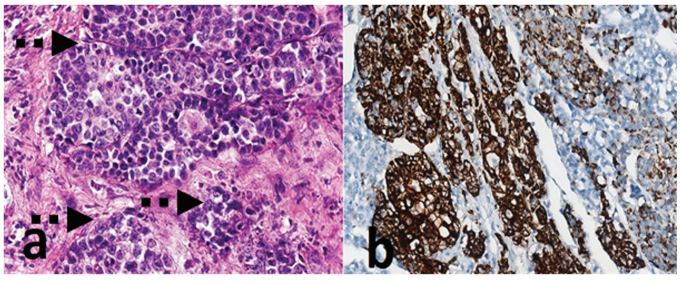

Figure 3. a) Tumoral areas in the adrenal (hematoxylin-eosin, x400); b) CK8-18 positivity in adrenal tumoral areas (x400) (tumor areas are marked with arrows)

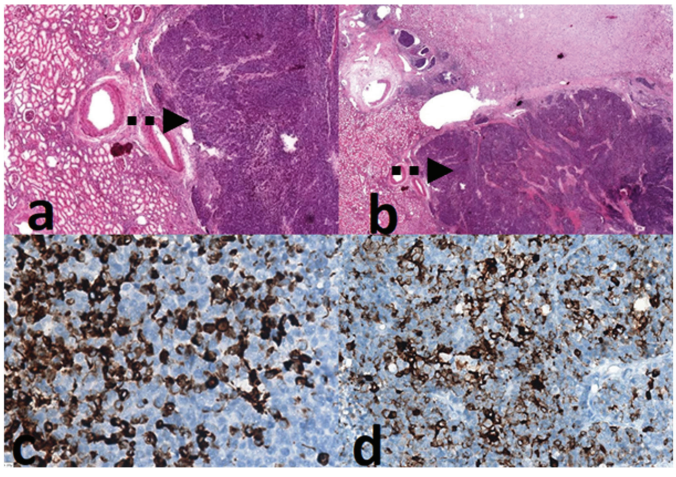

Figure 4. a) Tumoral areas in the kidney (hematoxylin & eosin, x400); b) Tumoral areas in the kidney with vascular tumor embolisms (hematoxylin & eosin, x100); c) CK8-18 positivity in renal tumoral areas (x400); d) Vimentin positivity in tumoral areas in the renal specimen (x400) (tumor areas are marked with arrows)

Discussion

In this case, a patient with endometrial cancer who had metastasis to the adrenal gland and kidney at the time of the diagnosis is described. Endometrial cancer spreads locally as well as through the lymphatic and vascular systemic circulation. Locally, it spreads mostly to the cervix, vagina, bladder and bowel. Rarely, hematogenous distant organ metastasis may also occur (6). The lung is the most common distant metastatic site. Having a distant metastatic site predicts overall survival (5). In the study of Blecharz et al. (7), in which 1,610 patients with hematogenous metastases were examined, 5-year survival rates were found to range from 0.6% to 7.2%. Survival rates also vary according to the location and the number of distant metastases (5).

Endometrial cancer that spreads to the adrenal gland is rare (8-11) and, to date, a total of 15 cases have been described (9,10,12) (Table 1). Twelve out of 15 cases presented with metachronous metastasis, while three had synchronous metastasis. Synchronous metastasis describes a concurrent tumor elsewhere, while metachronous metastasis describes a newly developing tumor after treatment. In the presented case, adrenal metastasis was also synchronous. When the tumor types of the patients in the literature were examined, endometrioid type was reported in nine patients, serous type in one patient, squamous type in one patient, dedifferentiated in one patient and mixed type in two patients (9,10,12). The patient in the presented case also had endometrioid type adenocarcinoma.

The described patient received three cycles of chemotherapy before surgery, and adjuvant therapy was planned after surgery but the disease progressed one month after surgery and the patient died before adjuvant therapy at four months after surgery. When the post-surgical management of the 15 patients in the literature was examined, the adjuvant treatment data was available for nine patients. Of these, seven were given chemotherapy as adjuvant therapy, and two were given external beam radiation therapy. Similar to our patient, adrenal metastases in patient 7, 14, and 15 (Table 1) were synchronous. However, there is insufficient data about survival in patients with these synchronous metastases. Similar to our patient, adrenalectomy was performed on patient 7 in primary cytoreduction but not performed in patient 14 or patient 15. Patient 15 underwent hysterectomy with bilateral salpingo-oophorectomy and adjuvant therapy was started after surgery. Due to the progression of the disease, patient 15 died in the sixth month after diagnosis before chemotherapy was completed. In the presented case, recurrence was observed in the first month, and the patient died in the fourth month.

Although the majority of adrenal metastases are asymptomatic, they may cause adrenal insufficiency depending on the size of the tumor and the extent to which it affects the adrenal gland (13). There were no signs of adrenal insufficiency in the present case despite the tumor being large. Diagnosis of adrenal metastases may be difficult with CT and magnetic resonance imaging examinations. Even though PET/CT isn't always recommended for endometrial cancer, it can help identify metastases in the adrenal glands (14).

Metastasis to the kidney was also present in the presented patient. Metastasis of endometrial carcinoma to the kidney is extremely rare and was described in only two previous case reports in the literature search (15,16). In one of these cases, a woman who underwent surgery for endometrial adenocarcinoma 24 years previously had recurrence in the kidney (15). After two-years of follow-up, it was highlighted that she remained alive. In the second case, a patient with uterine serous carcinoma and synchronous kidney metastases was reported (16).

In a study of 2,948 patients with stage IVB endometrial cancer, renal metastases were not identified (5). In another study examining kidney metastasis by Choyke et al. (17), the data of 27 patients was analyzed and no endometrial cancer was reported in these patients. Choyke et al. (17), reported, the most common types of cancer metastasis to the kidney were from lung and colon neoplasia, and the effect of renal metastasis on the survival of the patients was not reported.

Conclusion

Metastasis of endometrial cancer to the adrenal gland and kidney is extremely rare and metastasis to the kidney has been reported in only two earlier cases in the literature. These metastases to the adrenal gland and kidney may occur in patient management and care should be taken in terms of areas that may recur in patient follow-up. When there is an

Table 1. Patients with adrenal metastases from endometrial cancer on literature review

Author	Patient no	Age	Histology of primary	Adrenal metastasis	Stage	Adjuvant treatment	F-U (month)
Nakano and Schoene (18)	1	77	Mixed (clear cell + squamous)	Metachronous	NR	NR	28
Lam and Lo (19)	2	NR	NR	Metachronous	NR	NR	NR
Baron et al. (20)	3	76	Endometrioid	Metachronous	IVB	EBRT	24
	4	62	Endometrioid	Metachronous	NR	VBT + chemotherapy (adriamycin + cisplatin; 6 cycles)	110
Izaki et al. (21)	5	55	Endometrioid	Metachronous	IIIC	Chemotherapy (carboplatin + paclitaxel; 7 cycles)	82
Choi et al. (22)	6	62	Mixed (squamous + mucinous)	Metachronous	IIIC	Chemotherapy (cisplatin; 6 cycles)	45
Berretta et al. (11)	7	67	Mixed (anaplastic + endometrioid)	Synchronous	IVB	NR	NR
Zaidi et al. (8)	8	75	Endometrioid	Metachronous	IB	NR	9
Singh Lubana et al. (23)	9	60	Serous	Metachronous	II	EBRT + Chemotherapy (carboplatin + paclitaxel; 3 cycles)	90
Rekhi (24)	10	39	Endometrioid	Metachronous	II	VBT+EBRT	NR
Mouka et al. (25)	11	58	Endometrioid	Metachronous	IB	Chemotherapy (6 cycles)	NR
Da Dalt et al. (9)	12	53	Endometrioid	Metachronous	IIB	NR	45
Coward et al. (26)	13	62	Endometrioid	Metachronous	IB	NR	9
Ryan et al. (10)	14	68	Endometrioid	Synchronous	IVB	Chemotherapy (carboplatin + paclitaxel; 6 cycles)	6
Shiraishi et al. (12)	15	50	Dedifferentiated (undifferentiated + endometrioid)	Synchronus	IVB	Chemotherapy (carboplatin + paclitaxel; 4 cycles)	6
Present case	16	55	Endometrioid	Synchronous	IVB	no	4

NR: Not reported, EBRT: External beam radiation therapy, VBT: Vaginal brachytherapy, F-U: Follow-up

intraperitoneal spread of endometrial cancer, as in ovarian cancer, cytoreductive surgery without leaving residual tumor should be performed, and should include adrenalectomy and nephrectomy, if necessary.

Ethics Committee Approval: *The study protocol was reviewed and approved by University of Health Sciences Turkey, Ankara City Hospital Ethics Committee (approval: E2-22-2358, date: 07.09.2022).*

Informed Consent: *Due to the retrospective nature of the study, the ethic comittee did not request informal consent.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: O.A., SA., O.T., G.K.C., K.H.M., T.T.; Concept: SA., O.T., K.H.M., T.T.; Design: T.T.; Data Collection or Processing: O.A., F.K., B.E.; Analysis or Interpretation: O.A., B.E., M.Ü., G.K.C.; Literature Search: O.A., F.K., M.Ü.; Writing: O.A., G.K.C., T.T.*

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

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-49.
2. Ayhan A, Taskiran C, Celik C, Yuce K, Kucukali T. The influence of cytoreductive surgery on survival and morbidity in stage IVB endometrial cancer. Int J Gynecol Cancer 2002; 12: 448-53.
3. Patsavas K, Woessner J, Gielda B, Rotmensch J, Yordan E, Bitterman P, et al. Optimal surgical debulking in uterine papillary serous carcinoma affects survival. Gynecol Oncol 2011; 121: 581-5.
4. Park S, Bae GE, Kim J, Kim HS. Mesonephric-like Differentiation of Endometrial Endometrioid Carcinoma: Clinicopathological and Molecular Characteristics Distinct from Those of Uterine Mesonephric-like Adenocarcinoma. Diagnostics (Basel) 2021; 11: 1450.
5. Liu Y, Chi S, Zhou X, Zhao R, Xiao C, Wang H. Prognostic value of distant metastatic sites in stage IV endometrial cancer: A SEER database study of 2948 women. Int J Gynaecol Obstet 2020; 149: 16-23.
6. Fung-Kee-Fung M, Dodge J, Elit L, Lukka H, Chambers A, Oliver T, et al. Follow-up after primary therapy for endometrial cancer: a systematic review. Gynecol Oncol 2006; 101: 520-9.
7. Blecharz P, Urbański K, Mucha-Małecka A, Małecki K, Reinfuss M, Jakubowicz J, et al. Hematogenous metastases in patients with Stage I or II endometrial carcinoma. Strahlenther Onkol 2011; 187: 806-11.
8. Zaidi SS, Lakhani VT, Fadare O, Khabele D. Adrenal gland metastasis is an unusual manifestation of endometrial cancer. Case Rep Surg 2013; 2013: 428456.
9. Da Dalt G, Friziero A, Grego A, Serafini S, Fassina A, Blandamura S, et al. Adrenal metastasis from endometrial cancer: A case report. World J Clin Cases 2019; 7: 1844-9.
10. Ryan M, Laios A, Pathak D, Weston M, Hutson R. An Unusual Presentation of Endometrial Cancer with Bilateral Adrenal Metastases at the Time of Presentation and an Updated Descriptive Literature Review. Case Rep Obstet Gynecol 2019; 2019: 3515869.
11. Berretta R, Patrelli TS, Faioli R, Mautone D, Gizzo S, Mezzogiorno A, et al. Dedifferentiated endometrial cancer: an atypical case diagnosed from cerebellar and adrenal metastasis: case presentation and review of literature. Int J Clin Exp Pathol 2013; 6: 1652-7.
12. Shiraishi T, Kisu I, Nakamura K, Iijima M, Matsuda K, Hirao N. Bilateral adrenal metastases of endometrial cancer with adrenal insufficiency. J Obstet Gynaecol Res 2021; 47: 4106-9.
13. Bausewein C, Kühnbach R, Haberland B. Adrenal insufficiency caused by bilateral adrenal metastases -- a rare treatable cause for recurrent nausea and vomiting in metastatic breast cancer. Onkologie 2006; 29: 203-5.
14. Kobayashi E, Kawai A, Beppu Y, Seki K, Tateishi U. Bilateral adrenal gland metastasis from malignant fibrous histiocytoma: value of [F-18] FDG PET-CT for diagnosis of occult metastases. J Nucl Med 2006; 20: 695-8.
15. Gupta C, Wojno KJ, Schervish EW. Late renal metastasis of endometrial adenocarcinoma. J Urol 2003; 170: 519.
16. Cochrane E, Menzies A, Sweeney K, Burke W. Synchronous renal and para-aortic metastasis in a uterine serous carcinoma: A case review and clinical considerations. Gynecol Oncol Rep 2019; 28: 12-4.
17. Choyke PL, White EM, Zeman RK, Jaffe MH, Clark LR. Renal metastases: clinicopathologic and radiologic correlation. Radiology 1987; 162: 359-63.
18. Nakano KK, Schoene WC. Endometrial carcinoma with a predominant clear-cell pattern with metastases to the adrenal, posterior mediastinum, and brain. Am J Obstet Gynecol 1975; 122: 529-30.
19. Lam KY, Lo CY. Metastatic tumours of the adrenal glands: a 30-year experience in a teaching hospital. Clin Endocrinol (Oxf) 2002; 56: 95-101.
20. Baron M, Hamou L, Laberge S, Callonnec F, Tielmans A, Dessogne P. Metastatic spread of gynaecological neoplasms to the adrenal gland: case reports with a review of the literature. Eur J Gynaecol Oncol 2008; 29: 523-6.
21. Izaki H, Takahashi M, Shiirevnyamba A, Taue R, Furumoto H, Bando Y, et al. Long-term recurrence-free survivor after laparoscopic removal of solitary adrenal metastasis from endometrial adenocarcinoma. J Med Invest 2010; 57: 174-7.
22. Choi JJ, Buttrick S, Zakashansky K, Nezhat F, Chin EH. Laparoscopic adrenalectomy for isolated adrenal metastasis from cervical squamous cell carcinoma and endometrial adenocarcinoma. Gynecol Oncol 2011; 122: 684-5.
23. Singh Lubana S, Singh N, Tuli SS, Seligman B. Adrenal Metastasis from Uterine Papillary Serous Carcinoma. Am J Case Rep 2016; 17: 289-94.
24. Rekhi B. Mismatch repair protein deficient endometrioid adenocarcinomas, metastasizing to adrenal gland and lymph nodes: Unusual cases with diagnostic implications. Indian J Pathol Microbiol 2015; 58: 491-5.
25. Mouka V, Tsili AC, Messinis T, Papoudou-Bai A, Kamina S, Argyropoulou MI. Solitary adrenal metastasis from early-stage dedifferentiated endometrial carcinoma: CT findings and review of the literature. J Obstet Gynaecol 2016; 36: 881-2.
26. Coward J, Ladwa R, Crandon A. A case report of solitary adrenal metastasis from early stage endome-trial carcinoma. Gynecol Obstet Res Open J 2014; 1: 8-11.

Validity and reliability of a Turkish version of the human papillomavirus knowledge scale: a methodological study

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Abstract

Objective: The aim of the study was to test validity and reliability of the Human Papilloma Virus Knowledge Scale (HPV-KS) in Turkish.

Material and Methods: The methodological study was conducted with 920 participants at a training and research hospital in Ankara, Turkey, between February and May 2019. The data collection form consisted of descriptive characteristics of the participants and HPV-KS. Guidelines for the Process of Cross-Cultural Adaptation of Self-Reported Measures were followed for the language and cultural adaptation of the scale. Content validity, exploratory, and confirmatory factor analyses were performed to test the validity of the scale. The scale's reliability was assessed using the item-total correlation, Cronbach's alpha, and test-retest analysis.

Results: In line with the experts' suggestions, two item were excluded from the scale. The scale content validity index was found to be 0.96. The exploratory factor analysis determined the four subscales of the scale with 33-items. The explained variance was found to be 64.56%. In the confirmatory factor analysis, all the goodness of fit indexes had acceptable values. The item-total correlations determined that each item was positively correlated with the total scale ranging from 0.53 to 0.80. The Cronbach's alpha coefficient calculated for the overall scale was 0.96. It was found that there was a statistically significant positive relationship between test and retest ($r=0.166$; $p=0.05$).

Conclusion: The scale was shown to be a valid and reliable standard measurement that can be used to accurately evaluate the efficacy of health education provided by healthcare professionals. (J Turk Ger Gynecol Assoc 2023; 24: 177-86)

Keywords: HPV knowledge, HPV screening tests, HPV vaccine, validity, reliability

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Introduction

Human papillomavirus (HPV) is one of the major causes of sexually transmitted disease globally (1). HPV infections spread via direct skin-to-skin contact or through skin-to-mucous membrane contact, and the most common form of this infection is sexual contact (2). Many sexually active individuals are exposed to HPV at some point in their lives (3-5). HPV infections are strongly associated with development of cervical cancer (1,6). According to estimations made by the World Health Organizations, 604,000 new cervical cancer cases would be diagnosed and 342,000 deaths would be recorded in 2020 (7). Over the past five years in Turkey, it has been reported that the prevalence of cervical cancer is 7.163, cancer of the vulva is 862, anus cancer is 666, and penile cancer is 70 (8).

HPV vaccines are important for the prevention of HPV infections (9,10). The HPV vaccine is a safe and effective method for preventing most common HPV infections and HPV-related cancers (9,11). Clinical applications of the HPV vaccine demonstrated that it was highly effective against HPV infection in both sexes before the first sexual experience (12,13). In the past decade, HPV vaccines have been included in the national vaccination programs of 121 countries (13). However, the HPV vaccine is not yet included in the national vaccination program in Turkey (14).

The study was a master's thesis and was presented as an oral presentation at the 6th International 17th National Nursing Congress held on 19-21 December 2019.

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Studies have reported that social awareness and knowledge of HPV infection, modes of transmission, and vaccination and screening programs are not at the optimal level globally (15-21). As a result of an increase in HPV-related diseases, and use of HPV screening tests and HPV vaccines, Waller et al. (22) developed the Human Papilloma Virus Knowledge Scale (HPV-KS) to address the need for a scale that measures and evaluates HPV knowledge. The most important factor contributing to increasing the validity and reliability of a developed scale is testing it in different cultures (17,19,22).

In studies evaluating information, attitudes, and behaviors regarding HPV and vaccines among women, medical staff, and health sciences students in Turkey, it was reported that knowledge of HPV was not at the desired level (16,17,19). Validity and reliability measures information about current HPV screening programs and HPV vaccines in both genders, and has been tested in community-based studies. It was found that there is a need for a comprehensive HPV knowledge scale with confirmed Turkish validity and reliability. Therefore, the aim of this study was to adapt the HPV-KS to the Turkish language and culture and to test its validity and reliability.

Material and Methods

This was a methodological study conducted at the obstetrics and gynecology, urology, dermatology, and general surgery clinics of a tertiary training and research hospital in Ankara, Turkey, between February and May 2019. "Guidelines for the Process of Cross-Cultural Adaptation of Self-Reported Measures" were followed in the language and cultural adaptation of the HPV-KS (23).

The scientific research Ethics Committee of a University of Health Sciences Turkey, Gülhane Training and Research Hospital approved the study protocol (approval number: 41418926-19/20, date: 17.01.2019). All the participants were informed about the study methods, and verbal and written informed consent was obtained. Written permission was obtained from the original author, Joe Waller, via e-mail to use the HPV-KS.

Stage 1: translation

The first stage in adaptation is the forward translation from the original language to the target language (23). To produce the Turkish language validation of the HPV-KS, it was first translated from English to Turkish by three academician experts in their field.

Stage 2: synthesis of the translations

Then it is expected that the translations of each item are brought together and documented, and a synthesis of the common translations is created at the end of this stage (23). In this stage, three different academicians evaluated the compatibility of the translations with each other and Turkish culture.

Stage 3: back translation

In this stage, in order to provide validity of the translation, other translators who are blinded to the original text are expected to translate the text backward to the original language (23). In this stage, the scale was translated from Turkish back to English by three different academicians.

Stage 4: expert committee

The semantics, idiomatic, experiential, and conceptual equivalence in the back translation and the original scale were examined (23). For the content validity, 13 academicians experienced in their field scored the scale according to its suitability with Turkish language and culture by comparing each item's original English and Turkish translation (24,25).

Stage 5: test of the pre-final version

To pretest is the final stage of the adaptation process. Ideally, this field test of the new questionnaire should be tested in the pre-final version among 30 to 40 people from the target population (23). To evaluate the operability and comprehensibility of the data collection form, a pre-test was made with 20 women and 20 men. As a result of the pre-test, further corrections were made throughout the data collection form. The data obtained in the pre-test were excluded from the final analysis of the study.

Sample size

In scale validity and reliability studies, it is reported that the participant/item ratio should be at least 10/1 to satisfy factor analysis conditions when calculating sample sizes (26,27). Anticipating that some participants would drop out of the study, for this study 30% more participants were recruited than the recommended number. The present study sample size was calculated as 910, made up of 455 women and 455 men for the 35-item HPV-KS. The study sample consisted of 920 volunteers who visited the hospital's gynecology and obstetrics, urology, dermatology, and general surgery polyclinics for any reason. Inclusion criteria for the study were subjects between the ages of 18-49 years who were literate and with no written or oral communication barriers. For the scale retest, the first 140 participants were interviewed twice, with the second round of data collected two weeks after the first survey administration.

Data collection tools

Data collection forms were developed following a literature review conducted by a researcher (15,17,20,28,29). The data collection forms were organized into a female (58 questions) and male (54 questions) version. The forms consisted of three

parts. In the first part, there were 14 items for the participants' sociodemographic characteristics. The second part consisted of questions assessing their knowledge of sexually transmitted diseases (STDs), age of first sexual intercourse, history of disease/discomfort in the genitals, and other factors that were prepared differently for both sexes.

HPV Knowledge Scale: The HPV-KS was included in the third part of the data collection form. The HPV-KS was developed by Waller et al. (22) in 2013 to measure individuals' knowledge of HPV, HPV testing, and HPV vaccination. The HPV-KS has a total of 35 items. The first 29 items are examined in three subscales, and the remaining six items are evaluated as an independent subscale (22). This scale was chosen to be adapted into Turkish because it evaluates essential issues, such as knowledge of HPV, HPV-related cancers, HPV screening tests, and HPV vaccines. There is no short and comprehensive information scale to assess this issue in Turkey. It has been shown as an effective measurement tool for assessing these areas and it is easy to score. The first subscale of the HPV-KS is "General HPV knowledge", with 16 questions on the subjects' general knowledge of HPV. The second subscale, "HPV testing knowledge", has six items related to HPV screening tests. The third subscale, "HPV vaccine knowledge" consists of seven items regarding HPV vaccine information. "HPV vaccine availability" items, the independent HPV-KS subscale, is organized in three different ways for the HPV vaccine program, which is conducted in three countries (the UK, the US, and Australia) where the scale is applied. The independent subscale can be modified in accordance with the HPV vaccine policy of each country Waller et al. (22).

Participants mark each item of the HPV-KS as "Yes", "No", and "I don't know". In the evaluation phase, each correct answer is scored as "1", and wrong answers and "I don't know" statements are scored as "0". The total HPV-KS score may be between "0 and 35". Higher scores indicate a thorough understanding of HPV general knowledge, HPV screening tests, and the HPV vaccine.

Data collection

Data collection forms were administered face to face and under appropriate conditions where the privacy of the participants was ensured. After the briefing, the participants were allowed to answer the form individually. Participant questions during form completion were answered by the researcher without giving any information about HPV. Answering the data collection forms took an average of 25-35 minutes.

Statistical analysis

The data obtained in the study are presented as number and percentage for categorical variables and as mean ± standard deviation for continuous variables. The normality of the distribution was analyzed using the Kolmogorov-Smirnov test. The compatibility of the factor analysis of compatibility of data sets was examined by Kaiser-Meyer-Olkin (KMO) and Bartletts' tests. To test the construct validity of the HPV-KS, explanatory factor analysis (EFA) with varimax axis rotation and confirmatory factor analysis (CFA) were conducted. CFA based on polychoric correlation was applied to the data set and non-weighted least squares estimation method was chosen as a parameter estimation method. The scale's reliability was assessed using the item-total correlation, Cronbach's alpha, item analysis, and test-retest analysis. The average total and subscale scores were calculated. The Mann-Whitney U test and paired sample t-tests were used to analyze the relationships between dependent and independent variables. The HPV-KS scale total score average and subscale scale mean scores were calculated. IBM SPSS Statistics for Windows, version 22.0 (30) and IBM Statistics AMOS 21.0 (31) were used to analyze the data statistically. A p-value less than 0.05 was considered statistically significant.

Results

Characteristics of participants

In the current study, 50% (n=460) of the participants were female, 52.2% (n=480) were married, and 12.4% (n=114) had an education of eight years or less (Table 1). Overall, 8.0% (n=37) of the women in the study and 33.7% (n=155) of the men reported that they had their first sexual intercourse between the ages of 12 and 18. A total of 193 (42%) of the women participants and 201 (43.7%) of men stated that they needed health education about STDs. A total of 296 (64.3%) of the male participants reported that they used condoms during sexual intercourse. Only four (0.9%) of the participants [female: (n=4), male: (n=0)] had an HPV vaccine (Table 2).

Validity analyses

When testing the content validity of the Turkish language version of the HPV-KS, the Lawshe technique was used (25). In line with the experts' suggestions, the 32nd item in the independent subscale "The Vaccines for Children Program provides free HPV vaccines to children who are uninsured, underinsured, or on Medicaid" and the 35th item "The HPV vaccine is usually given to girls in school settings" were excluded because they were unsuitable for the Turkish national vaccination program. As a result of the language and content validity, 33 items were included in the Turkish version of the HPV-KS. In this study, the content validity index (CVI) score of the Turkish version of the HPV-KS was 0.96 (24,25).

Table 1. Sociodemographic characteristics of the participants (n=920)

Variables	n	%
Gender		
Female	460	50.0
Male	460	50.0
Marital status		
Single	419	45.5
Married	480	52.2
Others (divorced or engaged)	21	2.3
Educational status		
Primary education	114	12.4
High school education	290	31.5
Bachelor level education and equivalent	516	56.1
Employment status		
Not working	268	29.1
Public official	415	45.1
Private-sector employee	165	17.9
Unemployed	30	3.3
Retired	15	1.6
Student	27	2.9
Perception of monthly income status		
Upper	28	3.0
Good	293	31.8
Middle	477	51.8
Lower	112	13.2
Children		
No children	520	56.5
Have children	400	43.5

KMO and Bartlett's tests were used to measure the suitability of the sampling for factor analysis. The KMO measure of sampling adequacy of the data was 0.96, and Bartlett's test was highly significant (χ^2=3006.5, p<0.001). EFA conducted with varimax axis rotation yielded eigenvalues greater than 1, and four factors were identified that explained 64.56% of the total variance (Table 3). The EFA determined that the four subscales of the 33-item HPV-KS scale had factor loads varying between 0.54 and 0.80 (Table 3, Figure 1).

CFA was conducted to test the accuracy of the factor structure identified in the EFA within the scope of the HPV-KS construct validity to identify the relevance values. The CFA found that the χ^2/SD, Goodness of Fit Index, Adjusted Goodness of Fit Index, Comparative Fit Index, Root Mean Square Error of Approximation, and Normed Fit Indexes of the Turkish version had acceptable values (32) (Table 4). The first factor included items 1 to 16 and this factor was termed General HPV knowledge. The fourth factor included items 17 to 22 and this factor was termed HPV Testing Knowledge and consisted of

Table 2. The distribution of knowledge and experience regarding the participants' sexual health by sex (n=920)

Variables	Women (n=460)		Men (n=460)	
	n	%	n	%
Do you have information about STDs?				
Yes	374	81.3	387	84.1
No	86	18.7	73	15.9
Do you need health education about STDs?				
Yes	193	42.0	201	43.7
No idea	101	22.0	102	22.2
No	166	36.0	157	34.1
Have you ever had sexual intercourse?				
No	135	29.3	79	17.2
I don't want to answer	72	15.7	16	3.5
Yes	253	55.0	365	79.3
How old were you when you first had sexual intercourse?				
I don't have that experience	135	29.3	79	17.2
I don't want to answer	75	16.4	18	3.9
Between the ages of 12-18	37	8.0	155	33.7
Between the ages of 19-29	199	43.3	202	43.9
30 years or older	14	3.0	6	1.3
To date, have you had a gynecological disease?[1]				
Yes	154	33.5	-	-
No	306	66.5	-	-
To date, have you ever heard of cervical cancer?[1]				
Yes	444	96.5	-	-
No	16	3.5	-	-
Do you have information on cervical cancer?[1]				
Yes	304	66.1	-	-
No	156	33.9	-	-
Where did you get your information about cervical cancer?[1] (*n was folded)				
Websites	163	35.4	-	-
Social media, TV	139	30.2	-	-
TV or newspapers	137	29.8	-	-
Health personnel	131	28.5	-	-
In school	116	25.2	-	-
Friends or social environment	100	21.7	-	-
To date, have you ever had a Pap test?[1]				
No, I don't have ever sexual intercourse	135	29.3	-	-
Yes	206	44.8	-	-
No	119	25.9	-	-
To date, have you had an illness related to your genitals?[2]				
Yes	-	-	53	11.5
No	-	-	407	88.5

Table 2. Continued				
Have you used condoms when you suspect STDs?[2]				
I don't have ever sexual intercourse	-	-	79	17.2
Yes	-	-	296	64.3
No	-	-	85	18.5
To date, have you ever had HPV vaccines?				
Yes	4	0.9	-	-
No	456	99.1	460	100

*STDs: Sexually Transmitted Diseases, [1]Only women were asked, [2]Only men were asked, TV: Television, HPV: Human papillomavirus

the original scale's second subscale. The third factor included items 23 through 27 and this factor was termed HPV vaccine knowledge and was the original scale's third subscale. The 28th and 29th items in the HPV vaccine knowledge scale in the original HPV-KS were separated from this scale because of the factor analysis. The second factor included items 28 to 33 and this factor was named as HPV vaccine availability (Table 3, Figure 1).

Reliability analysis

In the current study, item analysis was conducted to determine the internal consistency of the HPV-KS. Item-total correlations were evaluated to analyze the contribution of the items to the total score. The item analysis determined that each item was positively correlated with the total scale in a range of 0.53 to 0.80 and that there was no need to remove any items from the scale. The scale's internal reliability coefficient was calculated to determine the internal consistency. Cronbach's alpha of the HPV-KS was 0.96 for the total scale (Table 5). When any item of the HPV-KS was deleted, there was no change in the scale's reliability coefficient.

In this study, Spearman's correlation analysis was used to assess the relationship between the HPV-KS total score of the 920 participants and the HPV-KS total retest score applied to 140 participants to determine the internal consistency of the HPV-KS. The analysis found a statistically significant positive relationship between the two applications (r=0.166; p=0.05).

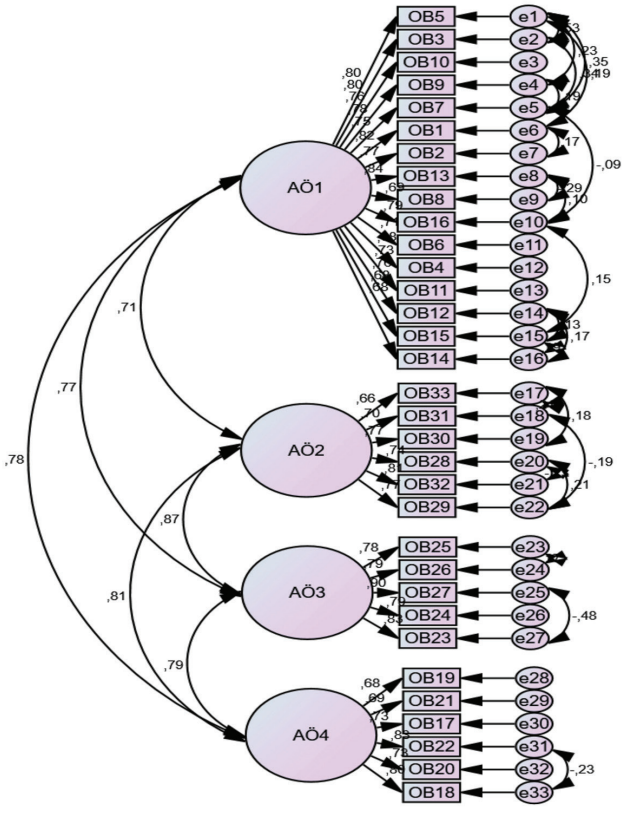

Figure 1. HPV-KS confirmatory factor analysis model
HPV-KS: Human Papilloma Virus Knowledge Scale

Relationship between the HPV-KS and some sociodemographic characteristics

A statistically significant difference was found between the female participants' Pap tests and the HPV-KS scale total and subscale mean scores (p<0.05). The total scale and subscale mean scores of the women who had Pap tests were higher than the mean scores of the women who did not (z=2.454, p=0.01). According to the analyses between gender, marital status, marriage duration, age, family type, economic income level, perception of income, longest-lived region, health history, and sexual health-related features and the HPV-KS total scale and subscale mean scores, there was no statistically significant relationship (p>0.05).

Discussion

This study investigated the validity and reliability of the Turkish version of the HPV-KS. The scale validity was evaluated for linguistic validity, content validity, and construct validity (26,32). First, language and cultural adaptation of the scale application used Guidelines for the Process of Cross-Cultural Adaptation of Self-Reported Measures by Beaton et al. (23). After the language validity, the scope was validated to evaluate the scale's suitability for the sociocultural characteristics of Turkish society. The Lawshe technique was used, and the CVI value was 0.96. The content validity of the Turkish version of the HPV-KS was quite high (24,25).

The construct validity of scales is assessed using factor analysis (26,32). First, the KMO value was calculated, and Bartlett's test was used to determine whether the study data were compatible with the construct validity. The KMO value was greater than 0.80, demonstrating that the sample size was sufficient for factor analysis. The findings showed that the sample size was ideal and the correlation between the items as appropriate for factor analysis (32).

Table 3. The factor structure of the HPV-KS according to EFA

Items	English	Turkish	Factor 1	Factor 2	Factor 3	Factor 4
Item 1	HPV can cause cervical cancer (T)	HPV, rahim ağzı kanserine neden olabilir (D)	0.71			
Item 2	A person could have HPV for many years without knowing it (T)	Bir kişi, kendisinde HPV olduğunu bilmeden, yıllarca yaşayabilir (D)	0.70			
Item 3	Having many sexual partners increases the risk of getting HPV (T)	Birden fazla cinsel eşe sahip olmak, HPV bulaşma riskini artırır (D)	0.80			
Item 4	HPV is very rare (F)	HPV çok nadir görülür (Y)	0.66			
Item 5	HPV can be passed on during sexual intercourse(T)	HPV cinsel ilişki sırasında bulaşabilir (D)	**0.82**			
Item 6	HPV always has visible signs or symptoms (F)	HPV'nin her zaman gözle görülür belirti ve bulguları vardır (Y)	0.67			
Item 7	Using condoms reduces the risk of getting HPV (T)	Prezervatif kullanmak HPV bulaşma riskini azaltır (D)	0.74			
Item 8	HPV can cause HIV/AIDS (F)	HPV, HIV/AIDS'ye neden olabilir (Y)	0.68			
Item 9	HPV can be passed on by genital skin-to-skin contact (T)	HPV cinsel bölgedeki deriden- deriye, temas ile bulaşabilir (D)	0.74			
Item 10	Men cannot get HPV (F)	HPV erkeklere bulaşmaz (Y)	0.75			
Item 11	Having sex at an early age increases the risk of getting HPV (T)	Erken yaşta cinsel ilişkiye girmek, HPV bulaşma riskini artırır (D)	0.63			
Item 12	There are many types of HPV (T)	HPV'nin birçok tipi vardır (D)	0.61			
Item 13	HPV can cause genital warts (T)	HPV cinsel bölgede siğillere neden olabilir (D)	0.69			
Item 14	HPV can be cured with antibiotics (F)	HPV antibiyotiklerle tedavi edilebilir (Y)	**0.54**			
Item 15	Most sexually active people will get HPV at some point in their lives (T)	Cinsel açıdan aktif olan kişilerin çoğuna, yaşamlarının bir döneminde HPV bulaşacaktır (D)	0.59			
Item 16	HPV usually doesn't need any treatment (T)	HPV'de genellikle herhangi bir tedaviye gerek yoktur (D)	0.67			
Item 17	If a woman tests positive for HPV, she will definitely get cervical cancer (F)	Eğer bir kadının HPV testi pozitifse kesinlikle rahim ağzı kanserine yakalanacaktır (Y)				0.66
Item 18	An HPV test can be done at the same time as a Pap test (T)	HPV testi, simir (Pap-smear) testi ile aynı anda yapılabilir (D)				**0.54**
Item 19	An HPV test can tell you how long you have had a HPV infection (F)	HPV testi size ne kadar zamandan beridir, HPV enfeksiyonunuz olduğunu söyler (Y)				0.69
Item 20	HPV testing is used to indicate if the HPV vaccine is needed (F)	HPV testi, HPV aşısının gerekli olup olmadığını belirlemek için kullanılır (Y)				0.62
Item 21	When you have an HPV test, you get there results the same day (F)	HPV testi yaptırdığınız zaman sonuçlarınızı aynı gün içinde alabilirsiniz (Y)				**0.69**
Item 22	If an HPV test shows that a woman does not have HPV, her risk of cervical cancer is low (T)	HPV testi bir kadında HPV olmadığını gösteriyorsa, o kadının rahim ağzı kanserine yakalanma riski düşüktür (D)				0.64
Item 23	Girls who have had an HPV vaccine do not need a Pap test when they are older (F)	HPV aşısı olan kızların ileri yaşlarında simir testi yaptırmasına gerek yoktur (Y)			**0.56**	
Item 24	One of the HPV vaccines offers protection against genital warts (T)	HPV aşılarından birisi cinsel bölgedeki siğillere karşı koruma sağlar (D)			0.61	
Item 25	The HPV vaccines offer protection against all sexually transmitted infections (F)	HPV aşıları cinsel yolla bulaşan tüm enfeksiyonlara karşı koruma sağlar (Y)			**0.73**	

Table 3. Continued

Item 26	Someone who has an HPV vaccine cannot develop cervical cancer (F)	HPV aşısı yapılmış olan bir kişi rahim ağzı kanserine yakalanmaz (Y)			0.71	
Item 27	HPV vaccines offer protection against most cervical cancers (T)	HPV aşıları, rahim ağzı kanser türlerinin birçoğundan korur (D)			0.71	
Item 28	The HPV vaccine requires three doses (T)	HPV aşısının üç doz yapılması gerekir (D)		0.60		
Item 29	The HPV vaccines are most effective if given to people who have never had sex (T)	HPV aşılarının en etkili olduğu bireyler hiç cinsel ilişkide bulunmamış olanlardır (D)		**0.53**		
Item 30	HPV vaccine is recommended for all females aged 11-26 years (T)	HPV aşısı 11-26 yaşlar arasındaki tüm kadınlara önerilir (D)		0.68		
Item 31	HPV vaccine is licensed for women aged 30-45 years (F)	HPV aşısı 30-45 yaşlarındaki kadınlar için lisanslıdır (ruhsatlıdır-izinlidir) (Y)		0.73		
Item 32	Both HPV vaccines that are available (Gardasil & Cervarix) protect against both genital warts and cervical cancer (F)	Mevcut olan her iki HPV aşısı da (Gardasil ve Cervarix) hem cinsel bölge siğillerine hem de rahim ağzı kanserine karşı koruma sağlar (Y)		0.58		
Item 33	HPV vaccine is permitted for males aged 11-26 years (T)	HPV aşısının 11-26 yaşlar arasındaki erkeklere yapılmasına izin verilmiştir (D)		**0.76**		
Eigenvalue and variance characteristics of HPV-KS						
Eigenvalues			8.95	4.53	4.06	3.76
Explained variance (%)			27.13	13.74	12.31	11.38
Explained total variance (%)			**64.56**	-	-	-

T: True, D: Doğru; F: False, Y: Yanlış, HPV: Human papillomavirus, AIDS: Acquired Immune Deficiency Syndrome, HPV-KS: Human Papilloma Virus Knowledge Scale

In the original HPV-KS study concurrently conducted in the UK, Australia, and the US by Waller et al. (22), the first 29 items were analyzed using EFA and three factors explained 20.13% of the total variance. In this study, the EFA determined that four factors explained 64.56% of the total variance (Table 3). The total variance above 60% in the scales was considered sufficient to define the investigated features (32,33). The total variance above 60% in this study was much higher than the original scale. This may have been because only Turkish society was included, and the language and content validity were assessed before the test implementation. In the original scale's factor analysis, the results of three different countries were analyzed simultaneously. Therefore, the independent subscale that was created in three different ways according to the national vaccination policies of the three countries was not included in the factor analysis in the original scale study (22). In the present study, considering Turkey's current HPV vaccine policy, the independent subscale was modified, and factor analysis was applied to the items included in the independent subscale together with other subscales.

In the present study, factor loads were examined according to the EFA of the HPV-KS results. Based on the factor loads result, no item was removed from the HPV-KS. In the original scale, although the factor load was below 0.33, it was reported that no item was removed according to the results of an advanced analysis (22). In the final stage of the EFA analysis, the factors obtained were identified based on the relationship of the meaning and the original scale. The original scale includes three main subscales (29 items) and one independent subscale (6 items).

According to the factor load distribution obtained in the present study, the General HPV knowledge scale and the HPV testing knowledge subscales had the same structure as the original scale, and the HPV vaccination knowledge subscale and the HPV vaccine availability items subscale were partially similar to the original scale. Two items (the 28th and 29th) in the HPV vaccination knowledge subscale of the original scale were collected under the HPV vaccine availability Items subscale (22). These items were thought to be displaced because of cultural differences.

Waller et al. (22) reported that in the validity and reliability study of the original HPV-KS, according to the CFA results, three factors were confirmed and the fit indexes were acceptable (22). In this study, the HPV-KS, which had a four-factor structure, and the CFA fit indexes had acceptable values (32) (Table 4). As a result of the CFA, we decided to preserve the scale's structure determined using the EFA.

A scale's reliability is determined by its invariance and internal consistency (26,32). For this study's internal consistency, the item analysis was based on the item-total correlation.

Table 4. Distribution of the compliance indices of the HPV-KS according to CFA

Compliance indexes	Reference values	Calculated values
χ^2/SD	<5	4.121
GFI	>0.85	0.886
AGFI	>0.85	0.863
CFI	>0.90	0.936
RMSEA	<0.08	0.058
SRMR	<0.08	0.023

χ^2/SD: Chi-squared Index, GFI: Goodness of Fit Index, AGFI: Adjusted Goodness of Fit Index, CFI: Comparative Fit Index, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized root mean square residual, HPV-KS: Human Papilloma Virus Knowledge Scale, CFA: Confirmatory factor analysis

Table 5. The distribution of mean scores of HPV-KS and subscales, and Cronbach's alpha values

HPV-KS	Items	Min-max.	Mean ± SD	Cronbach's alpha
General HPV knowledge	16	0-16	4.72±4.84	0.93
HPV testing knowledge	6	0-6	1.00±1.60	0.81
HPV vaccine knowledge	5	0-5	1.12±1.70	0.90
HPV vaccine availability	6	0-6	0.61±1.15	0.72
HPV-KS total	33	0-33	7.44±8.36	0.96

HPV-KS: Human Papilloma Virus Knowledge Scale, CFA: Confirmatory factor analysis, HPV: Human papillomavirus, Min.: Minimum, Max.: Maximum, SD: Standard deviation

The total score of the HPV-KS Turkish version and each scale item were positively correlated with a range of 0.53 to 0.80, so there was no need to remove items from the scale. In the original scale, no comparison was conducted because the item-total correlation was not examined.

The internal consistency analysis also calculates the reliability coefficient. For the 29 items in the original scale, Cronbach's alpha was 0.83, and the internal consistency was reported to be high (22). In this study, Cronbach's alpha of the 33-item HPV-KS Turkish version was 0.96, similar to the result of the original scale. This showed that the internal consistency of the Turkish version of the HPV-KS was quite high.

The test-retest method is another method for reliability analysis. In the present study, a positive and significant relationship between the HPV-KS total score and the retest score comparing the first application result and the retest result supported the scale's internal consistency. The t-test is another method used to examine the internal consistency of the scale with the test-retest method (33,34). In the present study, a comparison of the mean scores of the HPV-KS total and subscales obtained from the test and retest results determined that there was no significant difference between the total and subscale mean scores. This demonstrates that the scale's test and retest scale mean scores were similar, strengthening the internal consistency. The Turkish version of the 33-item HPV-KS was thus shown to be a valid and reliable measurement instrument. The total HPV-KS score may be between "0 and 33". Higher scores indicate a thorough understanding of HPV general knowledge, HPV screening tests, and the HPV vaccine. Studies have examined the knowledge, beliefs, attitudes, and behavior in many societies concerning HPV, HPV screening tests, and HPV vaccines (28,29,35). Studies have also examined whether sociodemographic characteristics, such as gender, age, educational status, marital status, monthly income level, and women who have had Pap tests have an impact on the results related to HPV. Studies conducted worldwide on HPV and HPV vaccines reported that women have more information on HPV and HPV vaccines than men (28,29,35). In this study, and in contrast to previous reports, no significant difference was found between the sexes in terms of comprehensive information about HPV. Thus, the public needs comprehensive information on this subject without separating the genders. The current study found that sociodemographic characteristics, such as age, educational status, marital status, and monthly income levels made no difference regarding knowledge of HPV and HPV vaccines. In the present study, women who had undergone a Pap test had significantly higher HPV knowledge than women who had not have Pap tests, in line with previous reports (16,29). Therefore, women participating in screening programs have more information about and awareness of HPV. In the present study, sociodemographic features, health history, and sexual health-related features made no difference in terms of HPV and HPV vaccine knowledge, whereas the greater knowledge level of women who underwent Pap tests supported the opinion that the HPV-KS was a consistent scale for measuring information.

Study Limitations

The fact that the study was conducted in a single center was considered as a limitation of the study.

Conclusion

Conducting validity and reliability analyses of a scale in different cultures contributes to the widespread use of that scale as a standard measurement instrument and provides intercultural comparison. The results of the validity and reliability analyses in this study determined that the 33-item Turkish version of the HPV-KS was a valid and reliable measurement instrument that can be used in women and men aged 18-49 in Turkish society. The HPV-KS can be used to determine the level of knowledge

of healthcare professionals responsible for providing health education and healthcare students regarding HPV infection HPV screening tests, and HPV vaccines. The HPV-KS is considered a valid and reliable standard measurement instrument that can be used to accurately evaluate the efficacy of health education provided by healthcare professionals.

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Ethics Committee Approval: *The scientific research Ethics Committee of a University of Health Sciences Turkey, Gülhane Training and Research Hospital approved the study protocol (approval number: 41418926-19/20, date: 17.01.2019).*

Informed Consent: *All the participants were informed about the study methods, and verbal and written informed consent was obtained. Written permission was obtained from the original author, Joe Waller, via email to use the HPV-KS.*

Peer-review: *Externally peer-reviewed.*

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References

1. Mavundza EJ, Iwu-Jaja CJ, Wiyeh AB, Gausi B, Abdullahi LH, Halle-Ekane G, et al. A Systematic Review of Interventions to Improve HPV Vaccination Coverage. Vaccines (Basel) 2021; 9: 687.
2. Cheng L, Wang Y, Du J. Human Papillomavirus Vaccines: An Updated Review. Vaccines (Basel) 2020; 8: 391.
3. Bretagne CH, Jooste V, Guenat D, Riethmuller D, Bouvier AM, Bedgedjian I, et al. Prevalence and distribution of HPV genotypes and cervical-associated lesions in sexually active young French women following HPV vaccine. J Gynecol Obstet Hum Reprod 2018; 47: 525-31.
4. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet 2013; 382: 889-99.
5. Priest HM, Knowlden AP. Systematic review of primary prevention Human Papillomavirus interventions targeting college students. Int J Sex Health 2015; 27: 125-44.
6. Centers for Disease Control and Prevention. Cancers associated with human papillomavirus. [Cited 20 December 2021]. United States— 2012–2016. U.S. Cancer Statistics data brief, no 10. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services. Retrieved from https://www.cdc.gov/cancer/uscs/pdf/USCS-DataBrief-No10-August2019-h.pdf
7. World Health Organization. Cervical cancer. [Cited January 24, 2022]. Retrieved from: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer#:~:text=HPV%20is%20the%20most%20common,populations%20eventually%20clear%20the%20infection.
8. GLOBOCAN. Global cancer statistics: Turkey. [Cited January 24, 2022]. Retrieved from http://gco.iarc.fr/today/data/factsheets/populations/792-turkey-fact-sheets.pdf
9. Apaydin KZ, Fontenot HB, Shtasel DL, Mayer KH, Keuroghlian AS. Primary Care Provider Practices and Perceptions Regarding HPV Vaccination and Anal Cancer Screening at a Boston Community Health Center. J Community Health 2018; 43: 792-801.
10. Kaur P, Mehrotra R, Rengaswamy S, Kaur T, Hariprasad R, Mehendale SM, et al. Human papillomavirus vaccine for cancer cervix prevention: Rationale & recommendations for implementation in India. Indian J Med Res 2017; 146: 153-7.
11. Donadiki EM, Jiménez-García R, Hernández-Barrera V, Sourtzi P, Carrasco-Garrido P, López de Andrés A, et al. Health Belief Model applied to non-compliance with HPV vaccine among female university students. Public Health 2014; 128: 268-73.
12. Chatterjee A. The next generation of HPV vaccines: nonavalent vaccine V503 on the horizon. Expert Rev Vaccines 2014; 13: 1279-90.
13. World Health Organization. Vaccine-preventable diseases: monitoring system. Global Summary. [Cited November 29, 2021]. Retrieved from: http://apps.who.int/immunization_monitoring/globalsummary/schedules
14. Selçuk I, Üstün Y. Future Feasibility of Nonavalent 9-valent HPV Vaccine in Turkey. The Journal of Gynecology - Obstetrics and Neonatology 2019; 16: 41-4.
15. Capogrosso P, Ventimiglia E, Matloob R, Colicchia M, Serino A, Castagna G, et al. Awareness and knowledge of human papillomavirus-related diseases are still dramatically insufficient in the era of high-coverage vaccination programs. World J Urol 2015; 33: 873-80.
16. Dönmez S, Öztürk R, Kısa S, Karaoz Weller B, Zeyneloğlu S. Knowledge and perception of female nursing students about human papillomavirus (HPV), cervical cancer, and attitudes toward HPV vaccination. J Am Coll Health 2019; 67: 410-7.
17. Guvenc G, Seven M, Akyuz A. Health Belief Model Scale for Human Papilloma Virus and its Vaccination: Adaptation and Psychometric Testing. J Pediatr Adolesc Gynecol 2016; 29: 252-8.
18. Kops NL, Hohenberger GF, Bessel M, Correia Horvath JD, Domingues C, Kalume Maranhão AG, et al. Knowledge about HPV and vaccination among young adult men and women: Results of a national survey. Papillomavirus Res 2019; 7: 123-8.
19. Özdemir E, Kısa S. Validation of the Turkish Cervical Cancer and Human Papilloma Virus Awareness Questionnaire. Int Nurs Rev 2016; 63: 465-72.
20. Turhan E, Cetin S, Cetin M, Abacigil F. Awareness and Knowledge Levels of 18-Year-Old and Older Individuals Regarding Human Papillomavirus (HPV) and HPV Vaccine in Hatay, Turkey. J Cancer Educ 2019; 34: 234-41.
21. Vaidakis D, Moustaki I, Zervas I, Barbouni A, Merakou K, Chrysi MS, et al. Knowledge of Greek adolescents on human papilloma virus (HPV) and vaccination: A national epidemiologic study. Medicine (Baltimore) 2017; 96: e5287.
22. Waller J, Ostini R, Marlow LA, McCaffery K, Zimet G. Validation of a measure of knowledge about human papillomavirus (HPV) using item response theory and classical test theory. Prev Med 2013; 56: 35-40.
23. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine (Phila Pa 1976) 2000; 25: 3186-91.

24. Ayre C, Scally, AJ. Critical values for Lawshe's content validity ratio: revisiting the original methods of calculation. Meas Eval Couns Dev 2014; 47: 79-86.
25. Lawshe CH. A quantitative approach to content validity. Pers Psychol 1975; 28: 563-75.
26. Erdoğan S, Nahcivan N, Esin MN. Hemşirelikte araştırma; süreç, uygulama ve kritik. Nobel Tıp Kitapevleri, Inc; 2017. p. 195-232.
27. Norman GR, Streiner DL. Biostatistics the bare essentials. Lewiston, BC Decker, 3.rd ed, Inc; 2008.
28. Dodd RH, McCaffery KJ, Marlow LA, Ostini R, Zimet GD, Waller J. Knowledge of human papillomavirus (HPV) testing in the USA, the UK and Australia: an international survey. Sex Transm Infect 2014; 90: 201-7.
29. Wheldon CW, Krakow M, Thompson EL, Moser RP. National Trends in Human Papillomavirus Awareness and Knowledge of Human Papillomavirus-Related Cancers. Am J Prev Med 2019; 56: e117-23.
30. IBM Corp. Released. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp; 2013.
31. Arbuckle JL. Amos (Version 23.0) [Computer Program]. Chicago: IBM SPSS; 2014.
32. Kartal M, Bardakçı S. SPSS ve AMOS Uygulamalı Örneklerle Güvenirlik ve Geçerlik Analizleri. Akademisyen Yayınevi, Inc; 2018.
33. Ercan I, Kan I. Reliability and validity in the scales. Journal of Uludag University Medicine Faculty 2004; 30: 211-6.
34. Karakoç A, Dönmez P. Basic Principles Of Scale Development. Tıp Eğitimi Dünyası 2014; 13: 39-49.
35. Radecki Breitkopf C, Finney Rutten LJ, Findley V, Jacobson DJ, Wilson PM, Albertie M, et al. Awareness and knowledge of Human Papillomavirus (HPV), HPV-related cancers, and HPV vaccines in an uninsured adult clinic population. Cancer Med 2016; 5: 3346-52.

ARID4B loss leads to activated STAT1-dependent interferon pathway in mouse embryonic stem cells and during meso/endodermal differentiation

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Abstract

Objective: Proper deactivation of the pluripotency network and activation of a lineage-specific gene expression program are critical for mouse embryonic stem cell (mESC) differentiation. This is achieved by the coordinated action of transcription and chromatin factors. Our previous work identified ARID4B as a critical chromatin factor for mesoderm and endoderm differentiation. As part of a histone deacetylase complex, ARID4B plays a role in transcriptional suppression of its direct targets. Here, we investigated the mechanism of ARID4B function in mESC differentiation by focusing on genes and pathways that are upregulated in its absence.

Material and Methods: We analyzed transcriptomic results of wild-type and arid4bΔ endoderm or mesoderm differentiated cells through integrative genomics viewer and ingenuity pathway analysis. We performed real-time quantitative polymerase chain reaction for selected genes. To understand pathway activation, we performed Western blot for candidate proteins during the time-course of differentiation. We also analyzed H3K4me3, H3K27me3 and H3K27Ac ChIP-seq results to understand changes in the chromatin environment.

Results: Interferon-related genes were activated in arid4bΔ mESCs and endoderm or mesoderm directed cells. Consistent with this, higher phosphorylated STAT1 levels were found in arid4bΔ mESCs while a related phosphorylated STAT3 was unchanged. Finally, we observed a significant increase in H3K4me3 around interferon-related distal gene regulatory regions with a combination of either upregulation of H3K27Ac level or downregulation of H3K27me3 level.

Conclusion: These results provide evidence that ARID4B is involved in the suppression of interferon-related genes in mESCs and during meso/endoderm differentiation through modulation, mainly of H3K4me3. This regulation might be important for successful mESC differentiation.

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Introduction

Embryonic stem cells (ESC) originate from the blastocyst stage of the developing embryo. They possess self-renewal ability and pluripotency, which make them invaluable in vitro models for studies focusing on early embryonic development. While self-renewal enables culturing ESCs almost indefinitely, through their pluripotent nature, ESCs can be directed to differentiate towards any lineage in vitro. Mimicking embryonic development, ESC commitment towards mesoderm, endoderm and neuroectoderm can be achieved with the carefully timed use of a combination of cytokines (1,2).

ESC stage is established and maintained by pluripotency transcription factors (TF), such as OCT4, SOX2, NANOG and KLF4 (3). During ESC differentiation, the pluripotency network achieved by these TFs need to be shut off while a lineage-specific gene expression program is established. Activation of lineage-specific genes poses a challenge, since their chromatin environment is not permissive of transcription at the ESC stage. Therefore, TFs that initiate differentiation need to gain

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access to their gene regulatory sequences at the promoter and enhancers and increase the accessibility of these regions to downstream transcription related machinery. The lineage-specific TFs that start this cascade of events are called pioneer TFs (4). Endoderm lineage originates from a mesodermal embryonic structure called the primitive streak and thus share an early common differentiation path (5,6). Neuroectoderm, on the other hand, is distinct from meso/endodermal lineages (7). BRACHYURY (Bry) was identified as a pioneer TF for mesodermal lineage and is used as a marker gene for meso/endodermal commitment. Similarly, FOXA2 is a pioneer TF for definitive endodermal lineage.

TFs instigate ESC differentiation by alteration of the target gene chromatin environment (8). Eukaryotic chromatin is made up of repeating units of conserved proteins called histones (9). Two copies of histones 2A, 2B, 3 and 4 form nucleosomes that wrap the genomic DNA. Modifications of histones at particular modifiable amino-acids, the number or the positioning of nucleosomes across the gene region, the use of histone variants in the nucleosome structure or methylation of DNA can change the chromatin environment and alter the frequency or efficiency of transcription.

Histones can be methylated at lysine or arginines, acetylated at lysines and phosphorylated at serine or threonines (9). Modification itself, as well as the exact residue that gets modified, can have profound effects on transcription. For example, histone acetylation leads to weakening of interaction between nucleosomes and DNA. High levels of histone acetylation, especially around promoter regions, help create an accessible environment for recruitment of factors critical for transcription initiation. Thus, histone acetylation generally correlates with active transcription, although acetylation in specific lysines can have unique outcomes. The effect of histone methylation depends on the level of methylation (mono, di or trimethylation) and which residue is methylated. While H3K4me3 is generally observed over transcriptionally active gene regions, H3K27me3 plays a suppressive role.

During differentiation, lineage-specific genes become more accessible and start harboring histone modifications that correlate with active transcription such as high level of histone acetylation including H3K27Ac and H3K4me3. Pluripotency genes are instead suppressed with repressive histone modifications, such as H3K27me3 or DNA methylation (10).

Our previous study focused on chromatin factors that regulate mouse embryonic stem cells (mESC) differentiation (11). We identified ARID4B as an important factor that is required for both mesoderm and endoderm commitment. In its absence, mESCs fail to express critical pioneer TFs for these lineages and cannot instigate differentiation. The failure of meso/endodermal differentiation was not due to improper pluripotency network shut-off. Instead, ARID4B loss resulted in accumulation of repressive H3K27me3 histone markers on lineage-specific genes.

As part of an RPD3 histone deacetylase complex, ARID4B regulates the overall histone acetylation landscape, primarily around promoter and enhancer regions (12). Histone acetylation levels around regulatory regions can impact transcriptional output by altering their accessibility to binding of other critical factors. ARID4B lacks any enzymatic domain but has a DNA and various protein-protein interaction domains. Particularly, the presence of TUDOR, PWWP and chromobarrel domains indicate interactions with modified histones and chromatin in general. DNA binding is mediated through its AT-rich interaction domain (ARID) in a sequence-independent manner. Recruitment of ARID4B along with the rest of the RPD3 complex generally leads to transcriptional suppression of target genes through histone deacetylation. In order to further delineate the mechanism of ARID4B function in mESC differentiation, we therefore decided to focus on pathways that are activated in the absence of ARID4B.

Material and Methods

mESC culture and differentiation

The study was done using already established mESC lines. Therefore, ethics committee approval form or institutional review board forms were not required. ARID4B knockout mESCs were prepared previously (11). Wild-type and arid4bΔ mESCs were cultured using standard serum containing medium on irradiated MEFs (13). mESC differentiation towards mesoderm and endoderm was performed using previously optimized protocols (11,14).

Transcriptomic analysis and RT-qPCR

RNA extraction, cDNA synthesis and quantitative polymerase chain reaction (qPCR) were done using previously published protocols (11). rRNA minus RNA-sequencing was previously performed (11). The transcriptomic data is available at NCBI GEO (GSE153633). Primers used for real-time quantitative polymerase chain reaction (RT-qPCR) are listed in Table 1.

Western blot

Cellular extract preparation and Western blot was done using previously published protocols (11). Antibodies used were: ARID4B (Bethyl Laboratories, A302-233A), ACTIN (Millipore, MAB1501), STAT1 (CST, 9172), pSTAT1 (CST, 9167), STAT3 (CST, 9139), pSTAT3 (CST, 9145).

Chromatin immunoprecipitation-sequencing and analysis

H3K4me3, H3K27Ac and H3K27me3 chromatin immunoprecipitation (ChIP)-seq experiments and subsequent

data analysis were previously performed in wild-type and arid4bΔ cells on day 5 of endoderm differentiation (11,15). Sequencing data is available at NCBI GEO (GSE153634).

Statistical analysis

Experiments were conducted with three independent biological replicates. RNA-seq and ChIP-seq experiments, the statistical analysis was done within the analysis package (11). Algorithms used within the study, such as Ingenuity Pathway Analysis (IPA), Gene Set Enrichment Analysis (GSEA) and Genomic Regions Enrichment of Annotations Tool (GREAT) have their own statistical analysis embedded within and were used as default. RT-qPCR results were graphed using GraphPad Prism Software and statistically analyzed using Student's t-test (*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$).

Results

Meso/endodermal differentiation defect observed in arid4bΔ mESCs might stem either from pathways that cannot be turned on or from those that are aberrantly upregulated. Our previous studies had focused on which pathways and functions were lost in arid4bΔ mESCs as they differentiate towards meso/endoderm lineages. In this paper, we decided to investigate abnormally upregulated functions.

We had previously optimized the embryoid body-based mesoderm and endoderm differentiation protocol and could observe upregulation of pioneer TFs for either lineage by day 5 of differentiation (14). arid4bΔ mESCs could not induce the expression of these TFs, leading to meso/endodermal commitment defect (11). RNA-seq analysis of day 5 samples from wild-type or arid4bΔ cells showed no apparent increase in meso/endoderm related signaling and gene expression pathways. Inappropriate pathway induction in arid4bΔ cells might hinder successful differentiation. We, therefore, looked for pathways that are enriched in the differentially upregulated genes in arid4bΔ cells over wild-type cells on day 5 of endoderm differentiation. IPA (Qiagen) revealed interferon signaling, activation of cytosolic pattern recognition receptors and the role of pattern recognition receptors in recognition of bacteria and viruses as the most enriched pathways (Figure 1a). Similarly, GSEA (the Broad Institute) showed cellular defense response and interferon α/β signaling as aberrantly upregulated in arid4bΔ cells (Figure 1b, c). Additionally, the IRF TF motif was found to be enriched in genes that were more expressed in arid4bΔ cells over wild-type (Figure 1d). The overrepresentation of innate immune response-specific genes (red dots) in arid4bΔ cells was visualized using the volcano-plot (Figure 1e, Table 2) and Integrative Genomics Viewer [(IGV), the Broad Institute] (Figure 1f-k).

We wondered whether ARID4B loss would lead to a similar upregulation of interferon signaling in mesoderm differentiation. To investigate this hypothesis, we directed wild-type and arid4bΔ mESCs towards mesodermal lineage and assessed differentiation success using the induction of mesodermal pioneer TF BRACHYURY. arid4bΔ mESCs were unable to express BRACHYURY, as well as its downstream target genes (11). IPA and GSEA similarly identified interferon signaling and activation of IRF by cytosolic pattern recognition receptors among top canonical pathways in arid4bΔ cells (Figure 2a-c). However, volcano plot representation showed that the interferon signaling related genes were not as highly expressed in mesoderm as they were in endoderm (Figure 2d, Table 3). Regardless, they showed apparent upregulation in arid4bΔ cells compared to wild-type (Figure 2e-j). These results collectively point to a role of ARID4B in suppression of interferon response during ESC differentiation towards meso/endodermal lineages.

The next step was to validate transcriptomic data and perform RT-qPCR for a list of highest differentially upregulated genes in arid4bΔ cells. Interferon induced transmembrane protein encoding *IFITM3* was the top of this list and showed clear induction upon ARID4B loss on day 5 of endoderm differentiation (Figure 3a). As the major interferon signaling mediator, *STAT1* was expressed markedly higher in arid4bΔ endodermal cells (Figure 3b). Cytosolic dsRNA sensor *DDX58* (RIG-I) and related antiviral protein encoding *DDX4* were similarly upregulated (Figure 3c, d). The expression of these genes was still significantly higher than wild-type in mesoderm commitment (Figure 3e-h) but their fold induction was more severe during endoderm differentiation. Therefore, we focused on endoderm differentiation hereafter.

To this point, results had been obtained at a specific time point (day 5) during mESC differentiation. In order to gain a

Table 1. Primers used in the study

Gene	Forward primer	Reverse primer
IFITM3	CCCCCAAACTACGAAAGAATCA	ACCATCTTCCGATCCCTAGAC
STAT1	GCTGCCTATGATGTCTCGTTT	TGCTTTTCCGTATGTTGTGCT
DDX58	ATTCAGGAAGAGCCAGAGTGTC	GTCTTCAATGATGTGCTGCAC
DDX4	GGTCCAAAAGTGACATATATACCC	TTGGTTGATCAGTTCTCGAGT
B-ACTIN	ATGAAGATCCTGACCGAGCG	TACTTGCGCTCAGGAGGAGC

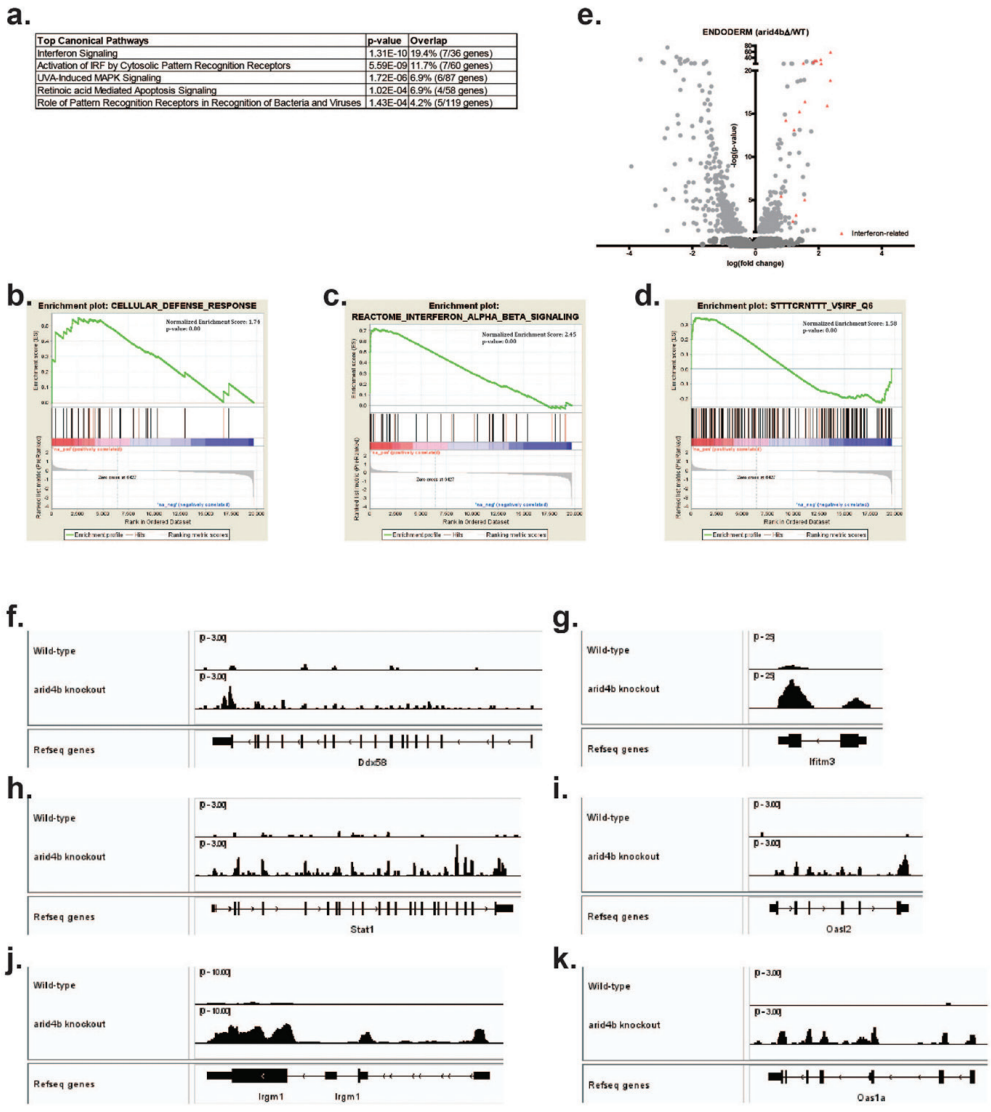

Figure 1. ARID4B loss leads to elevated expression of interferon-related genes during endoderm differentiation. Ingenuity Pathway Analysis (a) and Gene Set Enrichment Analysis (b-d) of differentially upregulated genes in arid4bΔ cells on day 5 of endoderm differentiation, (e) Volcano plot representation of RNA-seq data (day 5 of endoderm differentiation, arid4bΔ/wild-type). Red triangles show interferon-related genes., Integrative Genomics Viewer visualization of *DDX58* (f), *IFITM3* (g), *STAT1* (h), *OASL2* (i), *IRGM1* (j) and *OAS1A* (k) using RNA-seq data

more detailed understanding of aberrant induction kinetics of immune-related genes in arid4bΔ cells, RT-qPCR analysis was performed in wild-type and arid4bΔ mESCs through the time course of endoderm differentiation. At the mESC stage (depicted as day 0 of differentiation in graphs), *IFITM3* was already highly expressed in arid4bΔ cells, although the fold difference seemed to increase later during endoderm differentiation (after day 3) (Figure 3i). *STAT1* showed a highly variable but elevated expression in arid4bΔ mESCs and its expression remained elevated throughout our time-course (Figure 3j). *DDX58* (RIG-I) followed a similar pattern to *STAT1* (Figure 3k) while *DDX4* levels were similar between wild-type and arid4bΔ mESCs and became elevated in arid4bΔ cells only late in endoderm differentiation (Figure 3l). These results show that ARID4B loss leads to higher interferon-related gene expression at the mESC stage and this effect gets stronger through the endoderm differentiation time-course with variable kinetics and extent for each target gene.

Transcriptional upregulation might not necessarily lead to protein level alterations and pathway activation. To understand whether observed increased transcripts activate interferon signaling in arid4bΔ cells, we performed Western blot using total cell lysates of mESCs (day 0 of differentiation) and during the time course of endodermal differentiation. Cytosolic

Table 2. RNAseq results of interferon-related genes in endoderm differentiated cells

Rank in arid4bΔ/WT	EntrezID	Gene symbol	P-adjusted	Log fold change
1	66141	*IFITM3*	3.62612E-59	2.38
2	15944	*IRGM1*	1.35974E-19	2.37
3	20846	*STAT1*	1.17823E-16	2.27
4	24110	*USP18*	9.24286E-21	2.08
5	230073	*DDX58*	1.26967E-34	2.07
6	23962	*OASL2*	6.27663E-31	1.93
7	54396	*IRGM2*	3.01479E-31	1.88
9	246730	*OAS1A*	1.32575E-25	1.83
16	17858	*MX2*	4.03169E-17	1.57
17	15957	*IFIT1*	9.14703E-06	1.56
20	19106	*EIF2AK2*	2.58357E-22	1.52
26	23960	*OAS1G*	5.80493E-16	1.39
33	15959	*IFIT3*	0.000560908	1.29
36	234311	*DDX60*	7.47842E-14	1.22
38	19039	*LGALS3BP*	0.002821116	1.18
51	56417	*ADAR*	5.944E-15	0.96
68	16391	*IRF9*	3.55256E-06	0.81

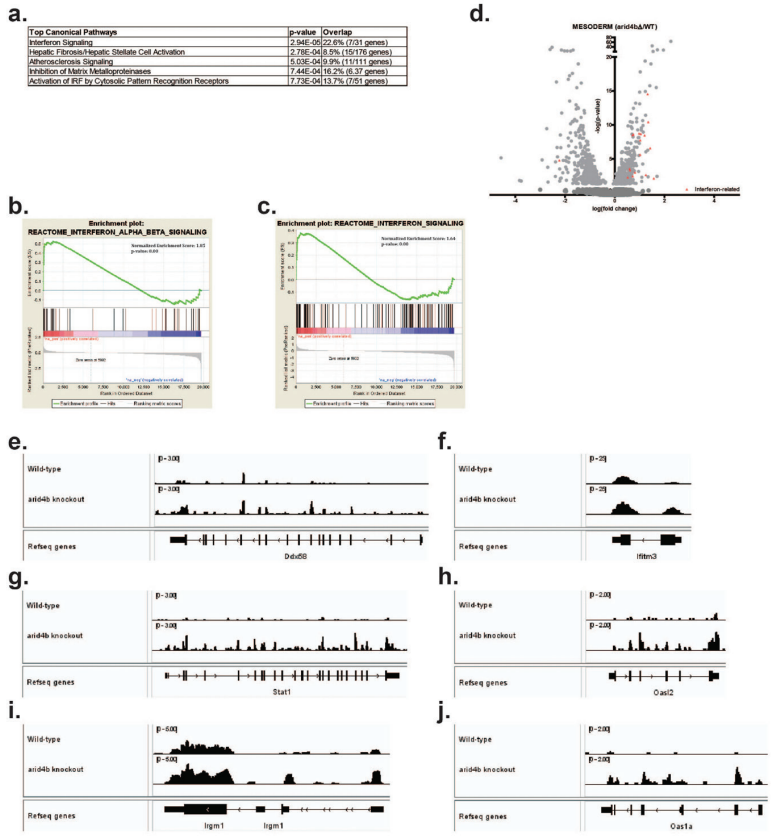

Figure 2. ARID4B loss leads to elevated expression of interferon-related genes during mesoderm differentiation. Ingenuity Pathway Analysis (a) and Gene Set Enrichment Analysis (b, c) of differentially upregulated genes in arid4bΔ cells on day 5 of mesoderm differentiation, (d) Volcano plot representation of RNA-seq data (day 5 of mesoderm differentiation, arid4bΔ/wild-type). Red triangles show interferon-related genes., Integrative Genomics Viewer visualization of *DDX58* (e), *IFITM3* (f), *STAT1* (g), *OASL2* (h), *IRGM1* (i) and *OAS1A* (j) using RNA-seq data

Table 3. RNAseq results of interferon-related genes in mesoderm differentiated cells

Rank in ARID4BΔ/WT	EntrezID	Gene symbol	P-adjusted	Log fold change
4	15959	*IFIT3*	0.007579062	1.57
7	20846	*STAT1*	2.6319E-07	1.43
10	24110	*USP18*	3.82944E-11	1.35
13	246730	*OAS1A*	2.92352E-15	1.32
14	15957	*IFIT1*	0.002376266	1.25
19	23962	*OASL2*	3.40716E-09	1.20
30	230073	*DDX58*	2.11369E-09	1.05
32	54396	*IRGM2*	1.7808E-08	1.02
33	234311	*DDX60*	2.49212E-06	1.02
43	23960	*OAS1G*	1.81248E-09	0.98
63	15958	*IFIT2*	0.001007765	0.81
80	56417	*ADAR*	2.45002E-09	0.75
88	100038882	*ISG15*	0.003604275	0.72
90	66141	*IFITM3*	3.13061E-09	0.72
143	16391	*IRF9*	0.000478808	0.62
154	19106	*EIF2AK2*	0.00028144	0.60
211	23961	*OAS1B*	0.005215998	0.52

double-stranded viral RNAs are recognized by DDX58 (RIG-I), which in turn activate expression and secretion of interferons including interferon α and interferon β (16). Interferons bind to their cognate receptors on neighboring cells and lead to activation of STAT1 and STAT2 through phosphorylation. Our western blot results showed elevated STAT1 level in arid4bΔ mESCs (day 0 of differentiation) (Figure 4a). Following exit from pluripotency, in both cell type, STAT1 levels declined although they still remained higher in arid4bΔ cells than in wild-type. Since interferon pathway activation correlated with the active phosphorylated STAT1 (pSTAT1), we compared its level in wild-type or arid4bΔ cells. At the mESC stage, there was a dramatic increase in pSTAT1 upon ARID4B loss. Mirroring total STAT1 level, the phosphorylated form was reduced upon endoderm differentiation. However, arid4bΔ cells had markedly more pSTAT1 than wild-type, even during differentiation.

Pluripotency of mESCs is regulated through a related STAT protein, STAT3 (17). We wanted to test whether the effect seen in arid4bΔ mESCs is specific to STAT1 or it also affects the STAT3 pathway. Unlike STAT1, total and phosphorylated forms of STAT3 were similar in wild-type and arid4bΔ cells at the mESC stage and through endoderm commitment (Figure 4b). These results show that the transcriptional upregulation of the pathway, originally observed through our RNA-seq results, is indicative of interferon-related STAT1 pathway activation in arid4bΔ cells.

We next wondered what the mechanism behind ARID4B-dependent regulation of interferon signaling was. To investigate this, changes in the chromatin environment of target genes were compared in endoderm committed cells (day 5). ChIP experiments were done for H3K4me3, H3K27Ac and H3K27me3 marks in wild-type or arid4bΔ endoderm cells (11). H3K4me3 and H3K27Ac generally correlates with active transcription while H3K27me3 plays a suppressive role (9). Our results suggest unique chromatin-based mechanisms lead to upregulation of target genes. For example, *OASL2* did not show a change in H3K4me3 or H3K27me3 level but had higher H3K27Ac in arid4bΔ cells, presumably leading to higher transcript levels (Figure 5a). The *STAT1* region had elevated H3K27Ac markers concomitant with decreased H3K27me3 (Figure 5b). In comparison, *DDX58* and *IRGM2* only had marked H3K4me3 increase in arid4bΔ cells (Figure 5c, d), while in *OAS1A* and *IFITM3*, H4K4me3 increase was accompanied with a similar increase in H3K27Ac (Figure 5e, f).

In order to understand which of these histone marks is more specifically associated with interferon pathway activation in arid4bΔ cells, we performed GREAT analysis (Stanford University) for ChIP-seq DNA sequences. This tool associates ChIP peaks with nearby genes and surveys a possible pathway enrichment. We performed GREAT for H3K4me3, H3K27me3 and H3K27Ac ChIP results in comparison with arid4bΔ over wild-type. We found that relevant pathways, such as defense response to virus, immune effector process and innate immune response, were only enriched in regions with higher H3K4me3 level in arid4bΔ cells over wild-type (Figure 5g). Although increase in H3K27Ac or loss of H3K27me3 markers were seen in individual target genes (Figure 5a, b, e, f), our results indicate a more generalized effect on H3K4me3 leads to interferon pathway activation upon ARID4B loss.

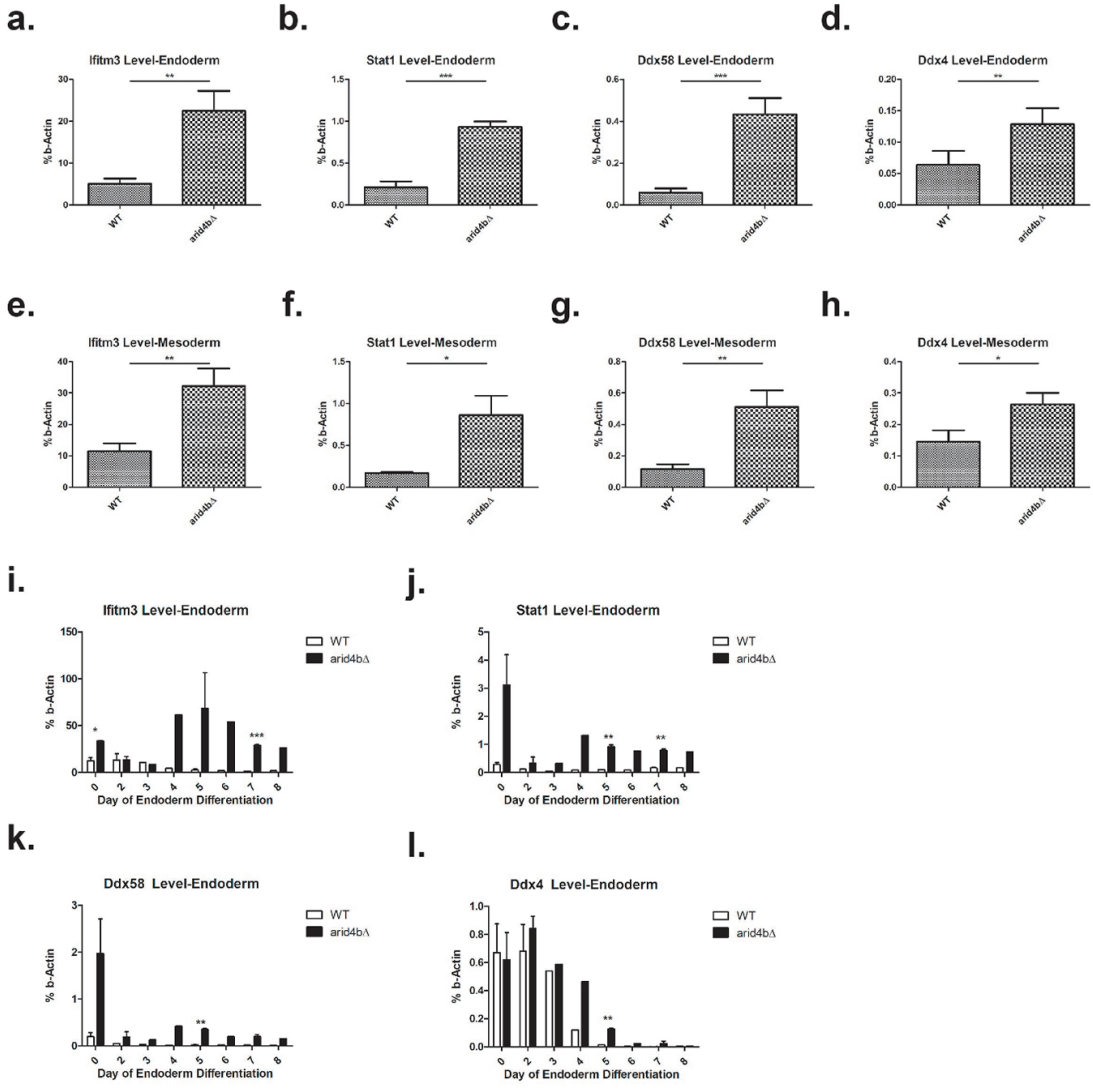

Figure 3. Validation of the expression of relevant differentially upregulated genes in arid4bΔ cells. RT-qPCR of *IFITM3*, *STAT1*, *DDX58* and *DDX4* in wild-type or arid4bΔ cells on day 5 of endoderm (a-d) or mesoderm (e-h) differentiation. RT-qPCR of *IFITM3* (i), *STAT1* (j), *DDX58* (k) and *DDX4* (l) during detailed endoderm differentiation time-course in wild-type or arid4bΔ cells. Statistical analyses (t-test) were done using GraphPad Prism software (*: p≤0.05, **: p≤0.01, ***: p≤0.001)
RT-qPCR: Real-time quantitative polymerase chain reaction

Discussion

Our previous results showed a clear mESC differentiation defect upon ARID4B loss. Derepression of lineage-specific meso/endoderm TF was not observed in arid4bΔ cells, despite cytokine-directed differentiation conditions.

We had previously investigated TF binding motifs enriched in our ChIP results (15). No particular TF motif was found enriched in H3K4me3 or H3K27me3 ChIP peaks. Interestingly, STAT1::STAT2 binding motif was enriched in regions with higher H3K27Ac level in arid4bΔ cells. When the ChIP peaks were separated based on their distance to transcription start site, it became clear that the H3K27Ac increases in arid4bΔ cells coincided with STAT1::STAT2 binding sites in promoter-distal regions that are presumably enhancers.

Viral RNA and DNA sensors in the cytoplasm initiate the antiviral response that leads to type I interferon (interferon α, β) expression (16). Secreted interferons bind to IFNAR 1-2 receptors through autocrine and paracrine mechanisms and activate JAK1-TYK2. This leads to phosphorylation of STAT1 and its nuclear translocation to transcribe interferon-stimulated genes.

mESCs naturally have limited antiviral response, at least partially due to the low levels of viral RNA sensors and inactive nuclear factor kappa B (18-20). Type 1 interferon pathway becomes mature coincident with mESC differentiation. Although intrinsic

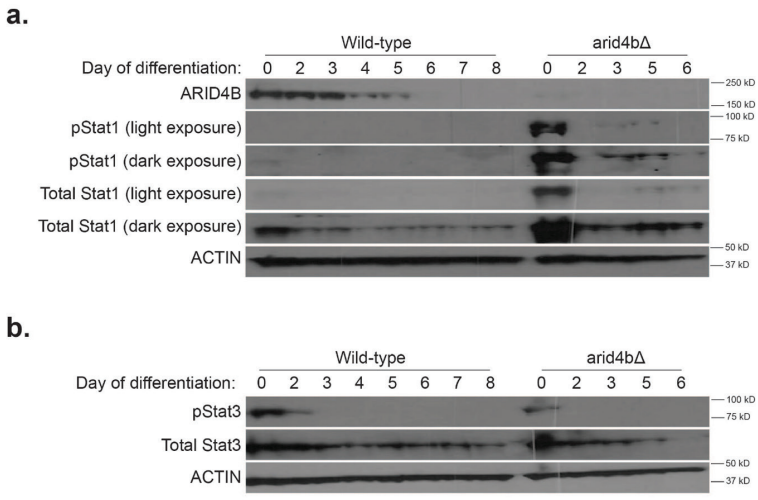

Figure 4. ARID4B loss leads to increased STAT1 level and activation at the mESC stage and during endoderm differentiation. (a) Western blot of ARID4B, STAT1, pSTAT1 (Tyr701) and loading control ACTIN using wild-type and arid4bΔ cell lysates during endoderm differentiation time-course, (b) Western blot of STAT3, pSTAT3 (Tyr705) and loading control ACTIN using wild-type and arid4bΔ cell lysates during endoderm differentiation time-course. Day 0 of differentiation represents the mESC stage

mESC: Mouse embryonic stem cell

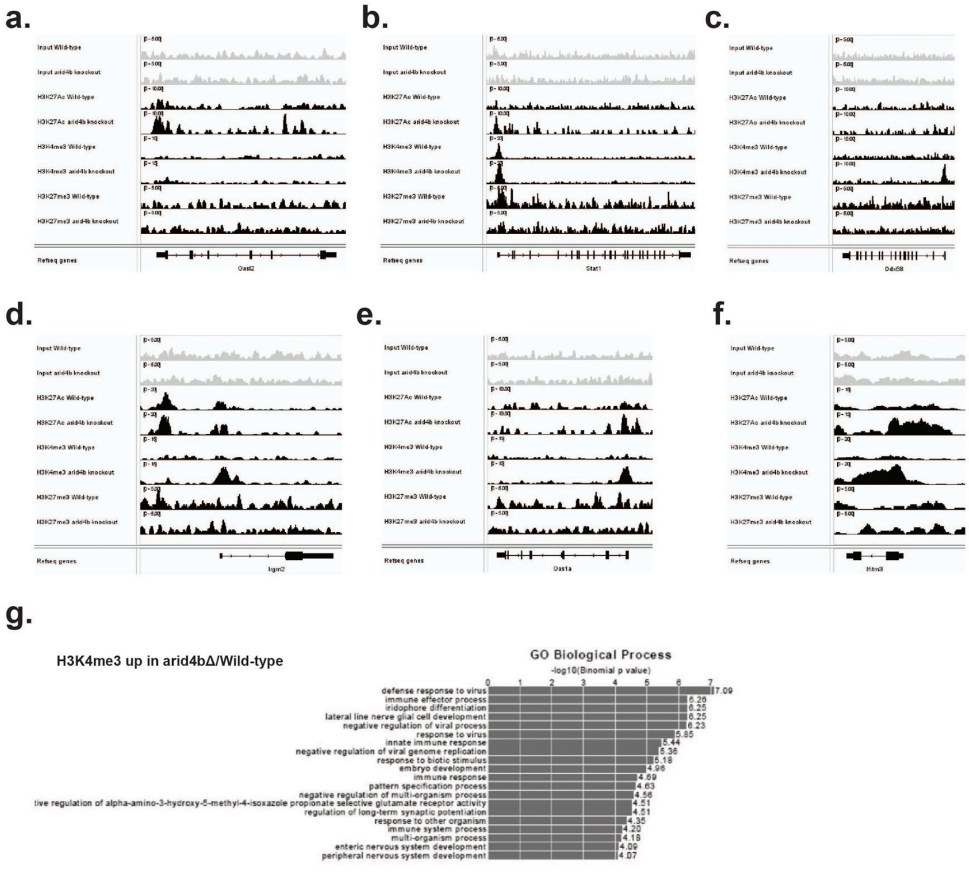

Figure 5. ARID4B loss results in changes in the chromatin landscape of relevant interferon-related genes. Integrative Genomics Viewer visualization of ChIP-seq tracks for selected interferon-related genes [*OASL2* (a), *STAT1* (b), *DDX58* (c), *IRGM2* (d), *OAS1A* (e), *IFITM3* (f)] in endoderm-directed WT and ARID4BΔ cells. *y* axes of WT and ARID4BΔ tracks are set to the same data range, (g) Genomic Regions Enrichment of Annotations Tool analysis of H3K4me3 ChIP-seq results upregulated in arid4bΔ cells over wild-type

low expression of interferon-stimulated genes independent of viral infection was recently reported in mESCs, this was shown to be dependent on the TF, IRF9 (21). We did not observe a change in *IRF9* transcript level in the absence of ARID4B, suggesting other mechanisms that include ARID4B in ISG regulation might be at play.

Our RNAseq results show no significant change in the level of sensor, interferon β or receptors. Instead, we observed a dramatic increase in STAT1 and interferon-stimulated gene transcript levels. This argues against an active viral response in arid4bΔ mESCs but suggests an aberrant transcriptional response due to high *STAT1* expression. Interestingly, in addition to *STAT1* mRNA levels, its protein, as well as active (phosphorylated) levels, are also elevated. Since JAK1 phosphorylates STAT1, this presumably points to the presence of other interferons that are secreted and can activate IFNAR-JAK receptor-kinase axis. Consistent with this, the less well studied interferon ϵ, interferon κ and interferon ω can also bind to IFNAR1/2 (22).

Our time-course RT-qPCR analysis of ARID4BΔ cells showed interferon-related genes have different expression kinetics through endoderm differentiation and in comparison to wild-type. Out of four genes tested, *STAT1* and *DDX58* had highest, though variable, arid4bΔ/wild-type expression difference in mESC stage. *IFITM3* and *DDX4*, on the other hand, reached highest arid4bΔ/wild-type expression difference later in endoderm differentiation. These differences might reflect the roles of the genes in early versus late interferon pathway activation. As one of the main TF of the interferon pathway, STAT1 is higher in the pathway hierarchy compared to the rest of the tested genes (16). Consistent with our data, it was reported that some interferon-related genes including *IFITM3* were expressed in mESCs without prior viral infection (19). Nevertheless, the expression of many interferon-related genes were altered during ESC differentiation (18,19). At the exit from pluripotency, IRF1 was shown to activate some interferon pathway genes including *DDX58* (21).

Alternatively, the differences might be due to the chromatin environment of these genes. The effect of ARID4B loss in the chromatin environment might be more or less permissive for efficient transcription. Consistently, we observed a decrease in H3K27me3 level that accompanied H3K4me3 increase in arid4bΔ cells in *STAT1* and *DDX58*. On the other hand, there was some residual, if not elevated, level of H3K27me3 in arid4bΔ cells in *IFITM3*, presumably dampening the transcriptional upregulation.

We found that H3K4me3 increase was associated strongly with interferon response and immune response pathway activations in arid4bΔ cells while H3K27me3 decrease or H3K27Ac increase did not lead to any related pathway enrichments. This result argues against a direct link between ARID4B and the altered chromatin environment of these pathway genes, since ARID4B is part of the RPD3L histone deacetylase complex. It is also possible that the heterogenous chromatin landscape of interferon-related genes masks direct effect of ARID4B. In fact, we found such differences in the IGV results. ARID4B-dependent histone acetylation changes might lead to a transcriptionally permissive environment that is conducive to H3K4me3 accumulation before or coincident with transcription activation. Since both histone acetylation and H3K4me3 is correlative with transcription (9), it is possible our results reflect a downstream rather than an immediate direct effect of ARID4B at these gene loci.

We observed higher antiviral response in endoderm committed arid4bΔ cells compared to mesoderm committed arid4bΔ cells. Although mesoderm and endoderm lineages originate from a common embryonic structure and thus share early progenitors, endoderm emergence occurs later during embryonic development. It is conceivable that the higher level of interferon-related gene expression in arid4bΔ cells in endoderm differentiation reflects the further maturation of an antiviral response through the differentiation timeline.

Previous reports identified the effect of histone deacetylation and STAT1 expression and transcription activity (23-25). Since ARID4B is part of the RPD3 histone deacetylase complex, our results are consistent with these findings and underline a possible link between STAT1 activity and mESC differentiation. Histone acetyltransferases and deacetylases can modify proteins other than histones (26). Another connection between acetylation and an interferon pathway comes from STAT1 post-translational modifications. Along with phosphorylation, STAT1 activity can be modulated by acetylation at its DNA-binding domain (27-29). Acetylation follows STAT1 phosphorylation and dampens its prolonged transcriptional activity. Our data is consistent with a model where the loss of ARID4B and its deacetylase complex might extend the duration of active STAT1 bound to its target genes, resulting in high level interferon-stimulated gene expression.

Study limitations

Our results show high expression of interferon related genes in arid4bΔ mESCs and differentiating cells. We found STAT1 protein level and its phosphorylated active form to be elevated in arid4bΔ cells. To gain a more comprehensive perspective on pathway activation, other proteins involved in STAT1 phosphorylation and downstream pathway activation would need to be verified through Western blot analysis.

Conclusion

Overall, we present evidence of transcript, protein and pathway level activation of interferon response, specifically through STAT1, in ARID4B-deficient mESCs through endoderm differentiation. Furthermore, our results show that the chromatin environment of interferon related genes is altered through changes, predominantly in H3K4me3 and H3K27Ac and to a lesser extent H3K27me3. Collectively, our data points to a role of ARID4B in the suppression of innate immune response via STAT1 through H3K4me3 and H3K27Ac regulation in mESCs and during endoderm differentiation.

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References

1. Irion S, Nostro MC, Kattman SJ, Keller GM. Directed differentiation of pluripotent stem cells: from developmental biology to therapeutic applications. Cold Spring Harb Symp Quant Biol 2008; 73: 101-10.
2. Kalkan T, Smith A. Mapping the route from naive pluripotency to lineage specification. Philos Trans R Soc Lond B Biol Sci 2014; 369: 20130540.
3. Orkin SH, Wang J, Kim J, Chu J, Rao S, Theunissen TW, Shen X, et al. The transcriptional network controlling pluripotency in ES cells. Cold Spring Harb Symp Quant Biol 2008; 73: 195-202.
4. Keller GM. In vitro differentiation of embryonic stem cells. Curr Opin Cell Biol 1995; 7: 862-9.
5. Lewis SL, Tam PP. Definitive endoderm of the mouse embryo: formation, cell fates, and morphogenetic function. Dev Dyn 2006; 235: 2315-29.
6. Gadue P, Huber TL, Paddison PJ, Keller GM. Wnt and TGF-beta signaling are required for the induction of an in vitro model of primitive streak formation using embryonic stem cells. Proc Natl Acad Sci U S A 2006; 103: 16806-11.
7. Ying QL, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nat Biotechnol 2003; 21: 183-6.
8. Orkin SH, Hochedlinger K. Chromatin connections to pluripotency and cellular reprogramming. Cell 2011; 145: 835-50.
9. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007; 128: 707-19.
10. Dixon JR, Jung I, Selvaraj S, Shen Y, Antosiewicz-Bourget JE, Lee AY, et al. Chromatin architecture reorganization during stem cell differentiation. Nature 2015; 518: 331-6.
11. Terzi Cizmecioglu N, Huang J, Keskin EG, Wang X, Esen I, Chen F, et al. ARID4B is critical for mouse embryonic stem cell differentiation towards mesoderm and endoderm, linking epigenetics to pluripotency exit. J Biol Chem 2020; 295: 17738-51.
12. Fleischer TC, Yun UJ, Ayer DE. Identification and characterization of three new components of the mSin3A corepressor complex. Mol Cell Biol 2003; 23: 3456-67.
13. Balbasi E, Guven G, Terzi Cizmecioglu N. Mouse Embryonic Stem Cell Culture in Serum-Containing or 2i Conditions. Methods Mol Biol 2022; 2520: 275-94.
14. Balbasi E, Sezginmert D, Alganatay C, Terzi Cizmecioglu N. Directed Differentiation of Mouse Embryonic Stem Cells to Mesoderm, Endoderm, and Neuroectoderm Lineages. Methods Mol Biol 2022; 2520: 295-307.
15. Keskin EG, Huang J, Terzi Çizmecioğlu N. Arid4b physically interacts with Tfap2c in mouse embryonic stem cells. Turk J Biol 2021; 45: 162-70.
16. Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001; 14: 778-809.
17. Zhang Y, Wang D, Xu J, Wang Y, Ma F, Li Z, et al. Stat3 activation is critical for pluripotency maintenance. J Cell Physiol 2019; 234: 1044-51.
18. D'Angelo W, Gurung C, Acharya D, Chen B, Ortolano N, Gama V, et al. The Molecular Basis for the Lack of Inflammatory Responses in Mouse Embryonic Stem Cells and Their Differentiated Cells. J Immunol 2017; 198: 2147-55.
19. Wu X, Dao Thi VL, Huang Y, Billerbeck E, Saha D, Hoffmann HH, et al. Intrinsic Immunity Shapes Viral Resistance of Stem Cells. Cell 2018; 172: 423-38.
20. Wang R, Wang J, Paul AM, Acharya D, Bai F, Huang F, et al. Mouse embryonic stem cells are deficient in type I interferon expression in response to viral infections and double-stranded RNA. J Biol Chem 2013; 288: 15926-36.
21. Romeike M, Spach S, Huber M, Feng S, Vainorius G, Elling U, et al. Transient upregulation of IRF1 during exit from naive pluripotency confers viral protection. EMBO Rep 2022; 23: e55375.
22. Harris BD, Schreiter J, Chevrier M, Jordan JL, Walter MR. Human interferon-ϵ and interferon-κ exhibit low potency and low affinity for cell-surface IFNAR and the poxvirus antagonist B18R. J Biol Chem 2018; 293: 16057-68.
23. Gonneaud A, Turgeon N, Jones C, Couture C, Lévesque D, Boisvert FM, et al. HDAC1 and HDAC2 independently regulate common and specific intrinsic responses in murine enteroids. Sci Rep 2019; 9: 5363.
24. Filgueiras LR, Brandt SL, Ramalho TR, Jancar S, Serezani CH. Imbalance between HDAC and HAT activities drives aberrant STAT1/MyD88 expression in macrophages from type 1 diabetic mice. J Diabetes Complications 2017; 31: 334-9.
25. Klampfer L, Huang J, Swaby LA, Augenlicht L. Requirement of histone deacetylase activity for signaling by STAT1. J Biol Chem 2004; 279: 30358-68.
26. Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. Gene 2005; 363: 15-23.
27. Tang X, Gao JS, Guan YJ, McLane KE, Yuan ZL, Ramratnam B, et al. Acetylation-dependent signal transduction for type I interferon receptor. Cell 2007; 131: 93-105.
28. Krämer OH, Baus D, Knauer SK, Stein S, Jäger E, Stauber RH, et al. Acetylation of STAT1 modulates NF-kappaB activity. Genes Dev 2006; 20: 473-85.
29. Kumar P, Gogulamudi VR, Periasamy R, Raghavaraju G, Subramanian U, Pandey KN. Inhibition of HDAC enhances STAT acetylation, blocks NF-κB, and suppresses the renal inflammation and fibrosis in Npr1 haplotype male mice. Am J Physiol Renal Physiol 2017; 313: F781-95.

This article will be credited by TMA within the scope of CME/CPD.

The importance of molecular classification of endometrial carcinomas in clinical practice: how to apply it and difficulties in application

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Abstract

Classification of endometrial carcinomas (EC) based solely on histological features is not sufficient for the prognostic and therapeutic guidance of patients. Furthermore, the existence of EC in which the histological type cannot be determined clearly and the poor reproducibility of histological typing have led to difficulties in clinical management. However, molecular classification of EC is very promising because of the high reproducibility and good correlation with clinical outcome. Within the scope of "the Cancer Genome Atlas Project", EC were divided into four different genomic subtypes, and molecular classification models for EC were developed based on these molecular subcategories. The prognostic differences between these molecular subgroups and the benefit for guidance for adjuvant therapy have been clearly demonstrated in studies. In this article, the importance of molecular classification for EC is discussed and its use in clinical practice is reviewed.

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Introduction

More than 50% of endometrial carcinomas (EC) present at an early stage, are in the low-risk group and can be treated with surgery alone, but there is a significant proportion of patients with an aggressive disease course. There is a need for an accurate and useable risk stratification model and for the identification of predictive biomarkers that will determine the extent of surgery, the need for post-operative adjuvant therapy and, if needed, the type of adjuvant therapy for optimal management of these agressive cases.

According to the traditional dualistic model, defined by Bokhman (1) in 1983, ECs have been categorized into two main groups, type 1 and type 2 carcinomas, in terms of clinical, endocrine and histopathological features. Based on this classification, endometrioid type ECs (EEC) constitute the majority of type 1, are associated with excess unopposed estrogenic stimulation of the endometrium and have a favourable prognosis. However, type 2 ECs, which include non-endometrioid histotypes, such as serous and clear cell carcinoma, have a worse prognosis and don't respond well to hormonal therapy. While some ECs will be prototypic examples of type 1 and type 2 ECs, many of them, particularly high grade [International Federation of Gynecology and Obstetrics-(FIGO) grade 3] ECs often do not fit into either category. Clear cell endometrial carcinomas (CCEC) generally fall into the type 2 category, but not all CCECs show the expected aggressive course of type 2 ECs. Histological type of the EC is an important factor in the selection of appropriate adjuvant therapy. However, there is a morphological overlap, especially in high-grade ECs, which even complicates the distinction between the two main clinically significant histological categories; endometrioid or non-endometrioid. Furthermore, it has been clearly seen that patient management models based solely on histological subtypes are inadequate, both for the very rare and little-known EC subtypes (2-4), and

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for the more common and well-known EC subtypes, which may show prognostic inter-patient heterogeneity.

The original dualistic model does not fully reflect the clinical diversity of ECs, and recent molecular developments have highlighted the importance of incorporating molecular features into risk grouping algorithms, including for patients with EC, as has been done for some other cancer types. The Cancer Genome Atlas (TCGA) project categorised ECs into four distinct genomic subtypes through integration of mutational analysis, copy number variation, and mRNA expression results in 2013 (5).

In this review, the role of the TCGA molecular classification in the management of patients with EC are discussed, including how it is applied in clinical practice and the difficulties that may be encountered.

The Cancer Genome Atlas genomic classification of endometrial carcinoma

TCGA performed a genome-wide analysis of 373 ECs, including EEC (n=307), serous endometrial carcinomas (SEC) (n=13), and mixed endometrioid and serous (n=13) carcinomas. Based on the data obtained from the genomic, transcriptomic, and proteomic analysis of these cases, four distinct molecular subgroups of ECs with different clinical, pathological and molecular features, have been identified (5). Then, molecular classification models based on TCGA molecular subcategories of ECs were developed for further adaptation for clinical practice (6,7). The prognostic differences between these molecular subgroups, and the benefit for guidance for adjuvant therapy have been clearly demonstrated (8).

These molecular subgroups are the ultramutated subtype with mutation in the exonuclease domain of DNA polymerase epsilon (POLE) (7% of cases); the hypermutated-microsatellite instable (MSI) subtype characterized by deficiency of ≥1 mismatch repair proteins (MMRd) (28%); the copy number-high subtype/serous-like characterized by TP53 mutations (26%) and the copy number-low subtype [no specific molecular profile (NSMP)] (39%), which doesn't fit any of these molecular subclasses mentioned above. The molecular classification of ECs has provided a better clinicopathological approach to patients with EC in many regards, including tumor histological typing, prognostic and therapeutic guidance, and elucidation of hereditary carcinomas.

1. POLE-ultramutated endometrial carcinomas: During DNA replication, the synthesis of the DNA strand and the rereading of the synthesized DNA strand plays a crucial role in correcting errors. DNA polymerase is one of the key molecules in DNA replication. The *POLE*mut subgroup of ECs is characterized by pathogenic mutation in the exonuclease domain of the *POLE* gene, which is a DNA polymerase, leading to high tumour mutation load (exceeding 100 mutations per megabase) (9,10). This category includes 7-12% of ECs. Patients tend to be younger and have a normal body mass index. Although *POLE*mut ECs often have high-risk pathological features, such as extensive lymphovascular invasion (LVI) and high tumor grade, they have a very favourable clinical course, regardless of the tumor histotype and histological grade. Most, but not all, tumors in this group are of the endometrioid histotype. In the TCGA tumor cohort, 6.4% of low-grade EEC and 17.4% of high-grade EEC, but none of the mixed histology or serous carcinomas were *POLE*mut EC (5). High tumour grade, scattered tumour giant cells and prominent lymphocytic infiltrate are characteristic histological features. They may show intratumoral morphological heterogeneity and ambigious morphology with coexistence of both endometrioid and serous-like histological features (11).

Detection of *POLE* mutation in EC appears to lead reduction in therapy, as these carcinomas have a very favourable prognosis. These patients may also be candidates for anti-*PD1*/*PDL1* immune checkpoint inhibitory (ICI) therapies, when they are at advanced stage or have recurrent disease.

2. MSI/MMRd-hypermutated endometrial carcinomas: DNA mismatch repair (MMR) system repairs errors that occur during DNA replication, and has two heterodimers consisting of *MutS protein homologue 2 (MSH2)*/*MutS protein homologue 6 (MSH6)* and *MutL homologue 1 (MLH1)*/*PMS1 homologue 2 (PMS2)* (12). The MMR is responsible for the maintenance of genomic stability by correcting DNA replication errors (base mismatches or insertion-deletion errors) (13). MMR deficiency is characterized by deficiency of ≥1 MMRd. Microsatellites are short repeated DNA sequences found throughout the genome and DNA polymerases are more prone to make mistakes in these regions. Dysfunction of any *MMR* gene is first manifested by variations in the length of these microsatellite repeats, termed MSI. MSI is an indirect indicator of MMR dysfunction.

Initially, MSI/MMRd ECs were defined as hereditary cases, associated with Lynch syndrome (LS), a hereditary cancer syndrome characterized by autosomal dominant heterozygous germline mutations in one of the four major *MMR* genes and which has a 60% lifetime risk of developing EC (14). LS-related cancers develop following somatic loss of function of the other intact allele of the affected *MMR* gene. Only 10% of MMR deficiencies in ECs are inherited, and associated with LS. In the remaining sporadic cases, epigenetic deletion of the MLH1 promoter region by hypermethylation is the main mechanism preceding the majority of sporadic MMR deficiencies, and some are associated with somatic mutations in the *MMR* genes. MMR deficiency contributes to high tumor mutation load (>10 mutations/megabase), therefore ECs in this molecular subgroup are highly immunogenic tumors, though not as immunogenic as *POLE*mut ECs.

The MSI/MMRd EC subgroup accounts for 25-30% of all ECs, and consists predominantly of high grade EECs (15). In the TCGA tumor cohort, 28.6% of low-grade EEC and 54.3% of high-grade ECC were MSI/MMRd EC (5). Mucinous differentiation, "microcystic, elongated and fragmented" pattern of myometrial invasion and LVI are common histological features. Dense peritumoral and intratumoral lymphocytic infiltration usually accompany the tumor cells (16). This tumor type tends to involve the lower uterine segment and occur in a wide age range; those associated with LS occur at a younger age than sporadic cases.

MSI/MMRd ECs constitute the group with intermediate prognosis, among the four molecular subgroups, in which *POLE*mut ECs have the best prognosis and those with TP53 mutation have the worst. CCECs with MMR deficiency have been reported to have a more favourable prognosis than MMR proficient CCECs. Although they are in the non-endometrioid EC group which is expected to have a poor prognosis, they behave more like MMRd EECs and thus patients with MMRd CCEC are recommended to be managed in the same manner as MMRd EECs (17). Hormonal therapy, given in the context of a fertility-preserving approach, is not suitable for the MMRd group of ECs. As MMRd ECs have high propensity for LVI, sentinel or other lymph node procedures may be required (18,19). They respond well to radiotherapy (RT), but patients do not benefit from platinum-based chemotherapy (CT). They may be good candidates for ICI therapy. *PD-1/PD-L1* inhibitor therapy has been approved by The Food and Drug Administration for patients with recurrent or advanced MMRd EC and who do not have any other treatment option (20).

3. *P53* abnormal EC (*P53*abn EC)/copy number-high/serous-like: *P53*abn EC is characterized by the mutation of the tumor supressor gene-*TP53* and constitutes the most aggressive group among the four molecular subgroups, with a high number of somatic copy-number alterations. Most of the tumors in this group are high-grade tumors and have serous morphology. In the TCGA study, 97.7% of SECs, 75% of ECs with mixed histology, 19.6% of high-grade EECs, and 5.0% of low-grade EECs were included in this group (5). A relationship between SEC and hereditary breast and ovarian cancer syndrome, which is associated with germline mutations in the *BRCA1* or *BRCA2* genes, has been reported (21). For women with SEC, who have a family history of hereditary breast and ovarian cancer syndrome-related malignancy, this relationship should be considered and patients should be referred for germline *BRCA1/2* screening.

*P53*abn ECs tend to be seen at an older age in comparison to other molecular subgroups and are more likely to present at an advanced stage. They can spread to the adnexa and peritoneum without deep invasion of the uterine wall.

*P53*abn ECs have the worst prognosis within the four molecular subgroups. However, no significant prognostic difference was observed between *p53* wild type and *p53*abn CCECs, and thus similar management of these patients is recommended (17).

P53 abn ECs respond well to the combination of platinum-based CT and RT. Targeted therapies based on *Human epidermal growth factor receptor 2 (HER2)* (trastuzumab) or homologous recombination defects (HRD) poly-ADP ribose polymerase [(PARP) inhibitors] may provide treatment options (11).

4. No specific molecular profile endometrial carcinomas/copy number-low: Tumors in this molecular group do not harbor specific molecular features of other EC molecular groups, any pathogenic *POLE* mutation, MMR defect, or *p53* abnormality. Hence, after the exclusion of these molecular features, the tumor should be included in this molecular category, which is also the most common one, accounting for almost half of all ECs. Somatic copy number alterations and mutation load are low in this group (22).

The majority of NSMP ECs are typically low grade (grade 1 or 2), early stage EECs, that develop on the basis of endometrial atypical hyperplasia/endometrioid intraepithelial neoplasia and may respond to hormone therapy.

NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers to identify tumors with a high propensity for disease recurrence and thus requiring aggressive treatment, complicates the management of this patient group. A potential biomarker is β-catenin (CTNNB1) mutation status. Studies have shown that low-grade EECs harboring mutations in exon 3 of the *CTNNB1* gene have more aggressive outcome, with higher recurrence rates (23). Further studies are needed to elucidate predictive biomarkers that can identify patients with NSMP EC who require adjuvant therapy. NSMP ECs may be suitable candidates for hormonal therapy and they respond well to adjuvant therapy, which is given when there are poor prognostic features.

Distribution of EC histological types based on molecular subgroups is illustrated in Figure 1 (24). Most of the low-grade (FIGO grades 1 and 2) EECs correspond to the NSMP and MMRd molecular subgroups. In contrast, high-grade EECs show a heterogeneous molecular profile with a similar distribution rate across all genomic categories. All SECs are encompassed by the *p53*abn group.

Application of molecular classification in clinical practice

Molecular classification models based on TCGA molecular subcategories of ECs were developed to be adapted to clinical practice (6,7). Talhouk et al. (6) proposed a clinically applicable method for the molecular classification of ECs, the proactive molecular risk classifier for endometrial cancer (ProMISE). In the context of their study, consisting of 319 ECs,

four distinct prognostic molecular subgroups with significantly different survival profiles were identified using methods of MMR immunohistochemistry (IHC) to identify the MMRd group, *p53* IHC to identify *p53*abn group, and sequencing for POLE exonuclease domain mutations to identify *POLE*mut group (6). Thus, the molecular subtype of ECs can be successfully determined with high interobserver reproducibility (25), and a high level of compatibility is achieved between the molecular subclasses defined in endometrial biopsy and hysterectomy specimens (26). Studies of ECs with dual or multiple molecular classes (consistent with more than one molecular subgroup) have suggested a sequential algorithmic approach to identify the exact molecular subtype that will provide prognostic and therapeutic guidance in these tumors. This approach starts with *POLE* mutation analysis as the first step, MMRd/MSI analysis follows this and p53 mutation analysis is the last step (Figure 2) (11). Since *POLE* mutation analysis, the first step, is an expensive and not widely available test, it is usually not possible in routine practice to apply this algorithmic approach in this sequence. Hence, the *POLE* mutation test can be used in a limited fashion, only for patients in whom adjuvant therapy is planned. This may remain the situation until a cheaper and easier method, such as an IHC assay, is developed to investigate pathogenic *POLE* mutations.

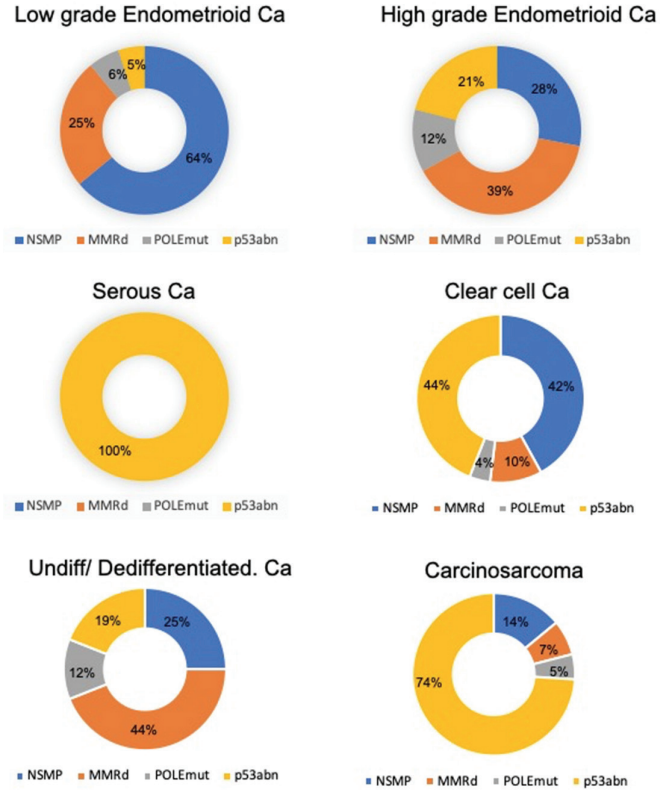

Figure 1. Distribution of endometrial carcinoma histological types by molecular subgroups
Ca: Carcinoma, MMRdef: Mismatch repair deficiency, POLEmut: Polymerase ε mutant, NSMP: No specific molecular profile

While the majority of EC are compatible with one of these four molecular categories, 3-6% show features of more than one molecular group and are termed "multiple classifier ECs" (27). Multiple classifier ECs harbor molecular features of different combinations of two molecular groups (*POLE*mut + MMRd/MMRd + *P53*abn/*POLE*mut + *P53*abn) or a combination of three groups (*POLE*mut + MMRd + *P53*abn). Concomitant TP53 mutation in *POLE*mut or MMRd ECs has been shown to be a passenger mutation that does not affect biological behavior of the tumor. It has been elucidated that these tumors do not have such a poor prognosis as single-classifier *p53*abn ECs and thus intensive treatment is not required. Since MMRd ECs with pathogenic *POLE* mutations are also found to have

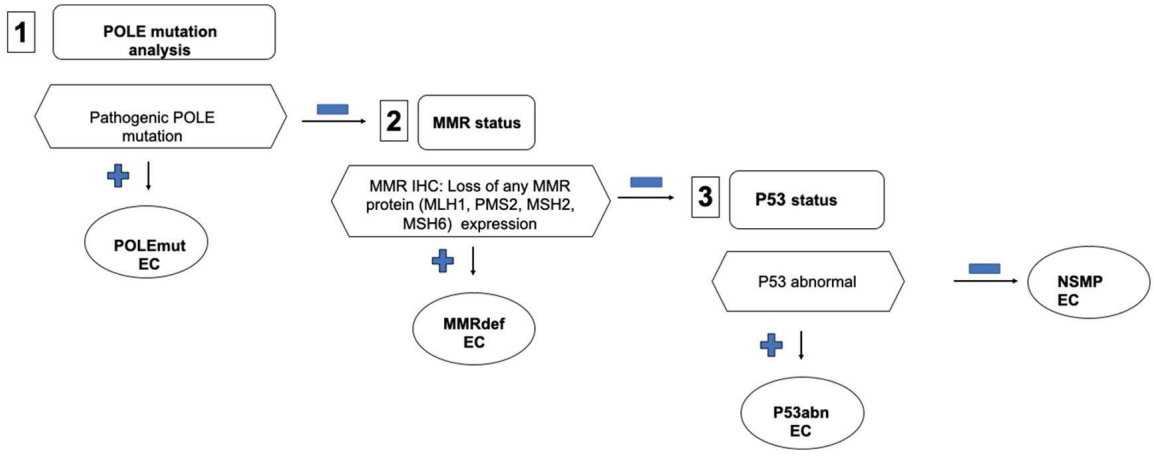

Figure 2. The proactive molecular risk classifier for endometrial cancer (ProMisE) technique to define the molecular class of endometrial carcinoma
EC: Endometrial carcinoma, POLE: Polymerase ε, POLEmut: Polymerase ε mutant, MMR: Mismatch repair, MMRdef: Mismatch repair deficiency, IHC: Immunochemistry

a good prognosis, like single classifier-*POLE*mut ECs, it is recommended to classify these tumors as single classifier *POLE*mut ECs (27). Therefore, *POLE* mutation analysis is the first step in the recommended algorithm to define the molecular subgroup of ECs.

a. *POLE* mutation testing: *POLE*mut ECs are diagnosed by detection of one of the 11 different pathogenic somatic missense mutations in the exonuclease domain of the *POLE* gene (Table 1) (28), P286R and V411L being the most common hot spot mutations. The method currently in use is DNA extraction from the tumor and sequencing of exons 9, 13, and 14 (or exon 9 through 14) by next-generation sequencing (NGS) or Sanger sequencing. Currently, there is no immunohistochemical assay to detect pathogenic *POLE* mutations, for the diagnosis of *POLE*mut ECs.

Table 1. Pathogenic mutations in the *POLE* gene, leading to the diagnosis of *POLE*mut EC

Protein change	Nucleotide substitution
P286R	c.857C>G
V411L	c.1231G>T/C
S297F	c.890C>T
S459F	c.1376C>T
A456P	c.1366G>C
F367S	c.1100T>C
L424I	c.1270C>A
M295R	c.884T>G
P436R	c.1307C>G
M444K	c.1331T>A
D368Y	c.1102G>T

POLE: DNA polymerase epsilon, *POLE*mut EC: *POLE* mutant endometrial carcinoma

b. MMR/MSI testing: It is recommended to peform MMR/MSI testing for all ECs, due to its diagnostic, prognostic and therapeutic contributions in the management of patients with EC (Figure 3). One of the purposes of MMR/MSI testing is the detection of the MMRd EC molecular subgroup, which has its own characteristics, in terms of treatment response and alternative treatment approaches. Since ICI inhibitor therapy has been approved for all advanced MMRd or MSI-high solid tumors, detection of MMR deficiency or high MSI in an EC raises the option of targeted therapy with ICI. Screening for LS is another indication. EC is often the first carcinoma type detected in patients with LS. Therefore, screening by MMR/MSI testing in ECs enables earlier detection of this syndrome, and provides follow-up of these patients, in terms of the development risk for more fatal carcinomas in the future (frequently colorectal carcinoma), and thus reduces the cancer-related mortality (29,30).

MMR defect is mainly detected using two methods. The first is IHC in which four major MMR protein expressions (*MLH-1, MSH-2, MSH-6, PMS-2*) are evaluated in tumor cells. The second method is a polymerase chain reaction (PCR) based technique, in which MSI analysis is performed. These two methods have approximately 95% compatibility, and IHC is the leading method with several advantages, such as being cheaper and more widely accessible, and with the capability to identify which *MMR* gene is likely defective.

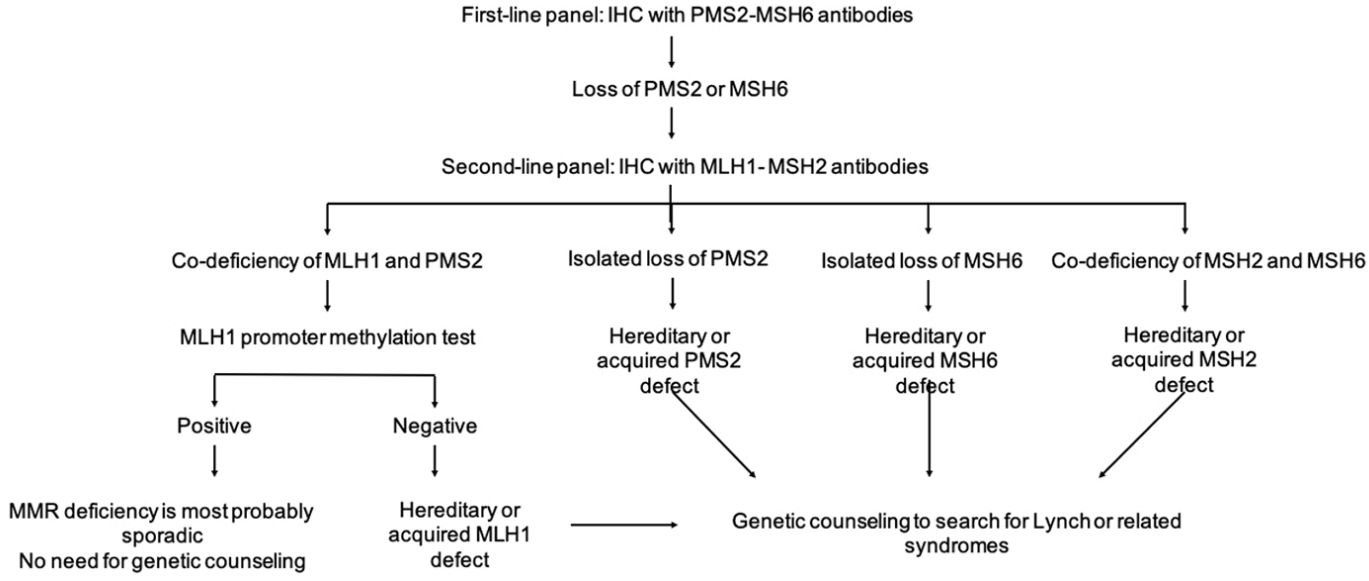

Figure 3. Steps of the MMR immunohistochemistry test and interpretation of possible results
IHC: Immunochemistry, MSH2: MutS protein homologue 2, MSH6: MutS protein homologue 6, MLH1: MutL homologue 1, PMS2: PMS1 homologue 2, MMR: Mismatch repair

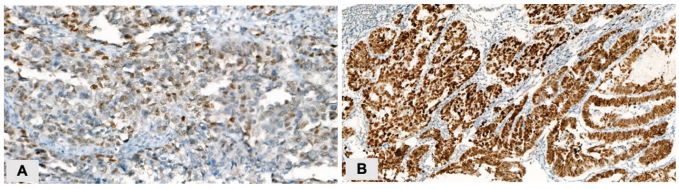

Figure 4. *p53* immunohistochemistry (A) "Wild type" *p53* staining patern. (B) *p53* overexpression (strong nuclear expression in more than 80% of tumor cell nuclei)

MMR proteins exist as heterodimers in which *MSH2* pairs with *MSH6* and *MLH1* pairs with *PMS2*. *MLH1* and *MSH2* can maintain their stability by forming heterodimers with other proteins in the cell, even in the absence of their own counterpart. However, *PMS2* and *MSH6* can maintain their stability only in the presence of *MLH1* and *MSH2* in the cell. Therefore, in order to reduce the cost of MMR IHC, it is recommended to perform a first-line panel consisting of only two antibodies first (*PMS2* and *MSH6*), and, if a defect is detected in any of these, to perform

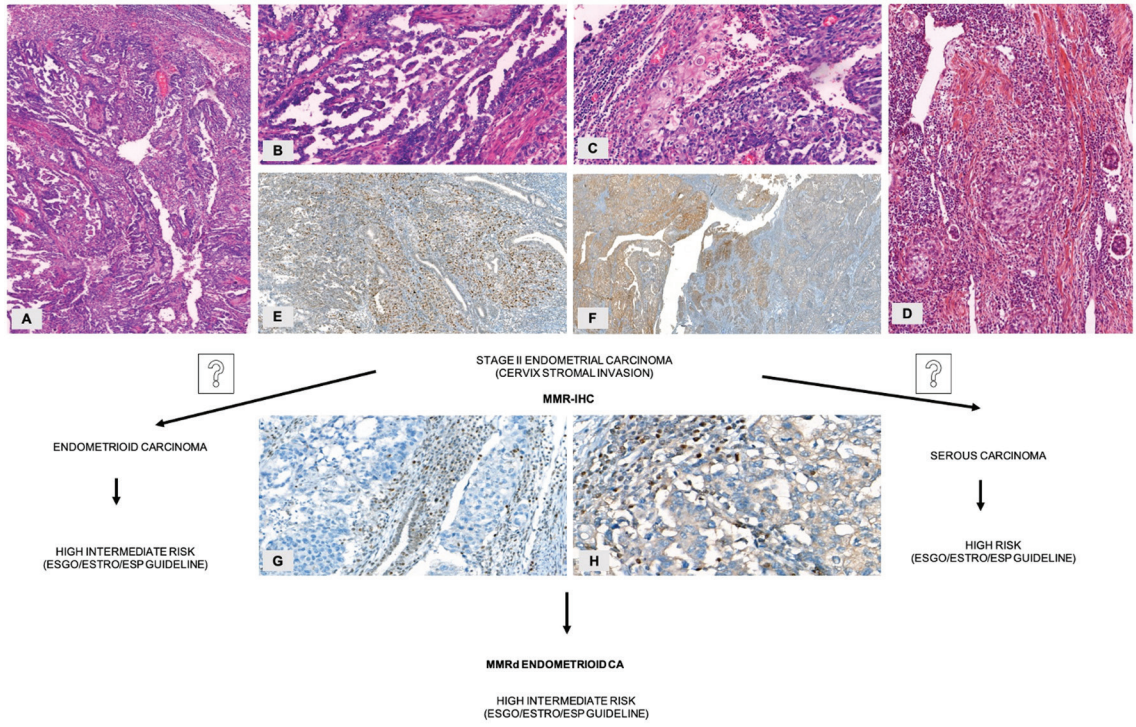

Figure 5. A 59 year old female patient. An exophytic mass with a long diameter of 9.5 cm in the uterine cavity. Endometrial carcinoma infiltrating the outer half of the myometrium and the cervical stroma is detected. (A, B) Serous carcinoma-like morphology. Tubulopapillary structures lined with cuboidal, polygonal tumor cells. However, the high degree of cytological atypia expected in serous carcinoma is not observed. (C) Squamous differentiation, which is not an expected histological feature for serous carcinoma, can be clearly seen in the tumor. (D) The tumor cells are accompanied by dense infiltration of immune cells and extensive lymphovascular invasion can be easily seen. (E, F) Immunohistochemical examination revealed "wild type" expression with p53 antibody, and patchy, scattered staining with p16 antibody. Immunohistochemical features were not supportive of serous carcinoma. Histological features are not consistent with endometrioid carcinoma, clear cell carcinoma, or any other type of endometrial carcinoma. With these findings, the tumor was classified as "stage 2 endometrial carcinoma" due to the invasion of cervix stroma, and a further histological typing could not be made, which is a required parameter for the ESGO/ESTRO/ESP guideline used for patient risk stratification in the management of ECs. According to this guideline, the patient will be evaluated in the "high-intermediate risk group" in case the tumor type is endometrioid carcinoma, and in the "high-risk group" in case of the tumor type is serous carcinoma. (G, H) MMR IHC is performed in the tumor and co-deficiency of MLH1 and PMS2 is detected, as shown in figures G and H, respectively. Since MMR deficiency is not an expected finding in serous carcinoma, the morphological suspicion of serous carcinoma is definitively ruled out and the tumor is reported as "MMRd endometrioid carcinoma". Thus, the tumor is included in the "high-intermediate risk group" according to the molecular classification integrated version of the ESGO/ESTRO/ESP guideline. In this case, the significant contribution of molecular classification to the clinical management of patients with endometrial carcinoma is clear, both to determine the histological type and the risk group of the patient.

ESGO/ESTRO/ESP: European Society of Gynaecological Oncology/European Society for Radiotherapy and Oncology/European Society of Pathology, EC: Endometrial carcinomas, MMRd: Deficiency of ≥1 mismatch repair proteins, IHC: Immunohistochemistry, MLH1: MutL homologue 1, PMS2: PMS1 homologue 2

the second step in which the other two antibodies (*MLH1* and *MSH2*) are added. Two-step IHC has been reported to have similar accuracy to a single-step four-antibody test (31).

Accordingly, isolated loss of *MSH6* or isolated loss of *PMS2* indicates hereditary or acquired *MSH6* or *PMS2* defects, respectively. On the other hand, the co-deficiency of *MLH1* and *PMS2* indicates hereditary or acquired *MLH1* defect; co-deficiency of *MSH2* and *MSH6* indicates hereditary or acquired *MSH2* defect. When reporting the MMR IHC result in the pathology report, the terminologies "normal" or "abnormal/defective/deficient" should be used. The use of ambiguous terminologies such as "positive, negative, present, absent, preserved, lost" should be avoided.

MSI PCR testing is a highly accurate and sensitive test, but in terms of its higher cost and poorer availability, it cannot be the first choice in routine practice, as the MMR IHC test is a cheaper and easily accessible test, and has a similar accuracy to the MSI test. As there is a high concordance of these two tests, co-administration of these two tests in all cases is unnecessary. It is recommended that the MSI PCR test is performed when an unexpected or unclear result is obtained with MMR IHC.

c. *P53* testing: Immunohistochemically different *p53* results are related to different types of *TP53* mutations (missense, frameshift, truncating mutations) (32). *TP53* missense mutations result in degradation resistant mutant proteins which accumulate in the tumor cell nucleus and reveal *p53* overexpression immunohistochemically (strong nuclear expression in more than 80% of tumor cell nuclei) (Figure 4). In contrast, non-sense or frameshift mutations result in premature termination codons that terminate translation and appear as complete loss of *p53* expression (null pattern) in tumor cells. The much rarer cytoplasmic *p53* expression pattern is usually caused by TP53 mutations that impair the nuclear localization of the protein. In the absence of TP53 mutation, a "normal, wild type" staining pattern is observed immunohistochemically. The "wild-type" staining pattern is characterized by varying rate (from a few positive tumor cells to the positivity of most tumor cell nuclei) and varying intensity of *p53* staining in tumor cells (Figure 4). In the "wild-type" staining pattern, the extent of *p53* staining varies from a few positive tumor cells to the positivity of most tumor cell nuclei and unlike the mutation immunophenotype of *TP53* gene, the intensity of *p53* staining differs intercellularly. The level of "wild type" expression depends on the differentiation status and proliferative activity of tumor cells. Highly proliferating tumors may show high levels of wild-type *p53* expression, and this profile may be difficult to distinguish from IHC *p53* overexpression seen in TP53 missense mutations. The sensitivity of *p53* IHC in the detection of TP53 mutation is quite high. The concordance of NGS and IHC for the detection of TP53 mutation is 88% (33). A small percentage of ECs harbouring TP53 mutation (truncating mutation) show "wild type" *p53* expression pattern immunohistochemically (32).

The significance of molecular classification in the clinical management of endometrial carcinomas and aspects that require improvement

1. It has been elucidated that accurate and reproducible histotyping, and even grading, of ECs, is not always possible by an approach based solely on histological features, and this issue is more problematic in high-grade ECs. The inclusion of molecular features in the risk stratification scheme appears to make a significant contribution to the clinical approach to patients with ECs, specifically the decision about whether any adjuvant treatment is needed or determination of the appropriate treatment approach (Figure 5). However, prospective validated clinical data are needed to provide therapeutic guidance from molecular classification in routine clinical practice.

2. Molecular subgroups also guide the therapy of patients with ECs. Since MMRd ECs tend to have high LVI, a conservative approach with hormonal therapy is not a good option. In addition, RT should be preferred to CT as a choice of adjuvant therapy in MMRd ECs, as these tumors do not respond well to CT. These patients may also be candidates for anti-PD-1/PD-L1 ICI therapies, when they are at advanced stage or have recurrent disease.

As *POLE*mut ECs often show aggressive histological features, such as higher histological grade, deeper invasion, or LVI, most of the studies regarding *POLE*mut ECs report results from patients who have already received adjuvant therapy. Despite the aggressive features of these tumours, patients with *POLE*mut EC show almost no recurrence or death. It is not clear whether this good clinical course is a result of a good response to treatment or regardless of the treatment (34). However, a recent meta-analysis revealed that most of the *POLE*mut ECs did not exhibit any recurrence or death, and neither type of adjuvant therapy (RT or CT) was associated with clinical outcome in these patients (35). For now, it has been suggested to reduce the treatment of *POLE*mut ECs and thus protect the patient from the toxicity of an unnecessary treatment. However, this approach again needs to be supported by evidence from prospective studies. Anti-PD-1/PD-L1 ICI therapy may be a good treatment option in recurrent or advanced *POLE*mut ECs, as in MMRd ECs.

The *p53*abn group benefits from platinum-based CT and RT. Among the four molecular subgroups, *p53*abn ECs get most benefit from the addition of CT to RT in adjuvant therapy, even at an early stage (36). *HER2/neu* amplification is closely related to the *p53*abn EC group, regardless of the histology.

Therefore, tumors with *TP53* abnormality and *HER-2/neu* amplification may benefit from the addition of trastuzumab to therapy, even in non-serous histology. Moreover, the success of PARP inhibitors in ovarian carcinomas has prompted the consideration of their use in the treatment of *p53*abn ECs. Studies are ongoing to identify the appropriate patient group with HRD for this treatment.

3. Current findings indicate that the presence of a pathogenic *POLE* mutation in an EC is the most important prognostic determinant among these molecular features, and in the presence of MMRd or *POLE*mut, p53 mutation is a passenger mutation that does not appear to affect prognosis. In addition, MMRd ECs also harboring pathogenic *POLE* mutations (multiple classifiers) were found to have a good prognosis, similar to single classifier *POLE*mut tumors. Therefore, the accepted best current knowledge is that the presence of any of the reported 11 POLE pathogenic variants in an EC can be considered the driver genomic feature for molecular classification and take precedent over other added molecular features as the prognostic determinant. Thus, there is a great need for more widely available and cheaper methods, such as IHC, to detect *POLE* mutations. Currently, the only method available for *POLE* mutation analysis is sequencing by NGS or Sanger, and this remains relatively expensive and difficult. Therefore, POLE sequencing is not suitable for all patients with ECs and its use may be limited to patients who are scheduled to receive adjuvant therapy. On the other hand, the presence of any *POLE* mutation other than the 11 reported pathogenic mutations has no prognostic effect and these tumors cannot be considered in the *POLE*mut EC category.

4. NSMP ECs are the largest and most heterogeneous molecular group. The lack of biomarkers to identify those with a high propensity for disease recurrence and thus requiring aggressive treatment, complicates the management of this patient group. it has been shown that NSMP ECs containing β-catenin (CTNNB1) mutations show a more aggressive course. Low-grade EECs harbouring mutations in exon 3 of *CTNNB1* gene have more aggressive outcome, with higher recurrence rate (23). Therefore, further studies are needed to identify prognostic subcategories in the molecular group of NSMP ECs. The presence of the CTNNB1 mutation provides a therapeutic option as well as these patients have been shown to benefit most from Bevacizumab treatment (37).

Conclusion

Molecular classification provided a major improvement in the management of patients with EC across diagnostic, prognostic and therapeutic aspects. Molecular classification has also been integrated into the patient risk stratification guidelines for ECs. MMR and *p53* analysis by IHC should be routinely performed in all ECs. Presence of any pathogenic *POLE* mutation in an EC plays a driver role in the determination of the molecular subgroup and constitutes the first step in the algorithmic approach, in which the MMRd/MSI and *p53* tests are subsequent steps to be performed. However, as *POLE* mutation analysis is expensive and not widely available, this test may be reserved for patients with EC who will be given adjuvant therapy, until a cheaper and easier method, such as an IHC assay, is developed. The significance of molecular classification of ECs should be validated prospectively and improved with further studies.

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References

1. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15: 10-7.
2. Sevim S, Enneli D, Serbes ED, Ersoz CC, Yuksel S. Primary adenosquamous carcinoma of the endometrium with glassy cell features. A diagnostic pitfall as a very rare tumour type in the endometrium. Pathologica 2022; 114: 332-8.
3. Deolet E, Van Dorpe J, Van de Vijver K. Mesonephric-Like Adenocarcinoma of the Endometrium: Diagnostic Advances to Spot This Wolf in Sheep's Clothing. A Review of the Literature. J Clin Med 2021; 10: 698.
4. Zhang C, Zhang H, Yang L, Wang Y, Li X, Guo J, et al. Primary squamous cell carcinoma of the endometrium in a woman of perimenopausal age: A case report. Medicine (Baltimore) 2018; 97: e13418.
5. Cancer Genome Atlas Research Network; Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013; 497: 67-73.
6. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. Cancer 2017; 123: 802-13.
7. Stelloo E, Nout RA, Osse EM, Jürgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. Clin Cancer Res 2016; 22: 4215-24.
8. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol 2018; 29: 1180-8.
9. Church DN, Briggs SE, Palles C, Domingo E, Kearsey SJ, Grimes JM, et al. DNA polymerase ε and δ exonuclease domain mutations in endometrial cancer. Hum Mol Genet 2013; 22: 2820-8.
10. Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer 2016; 16: 71-81.
11. Casey L, Singh N. POLE, MMR, and MSI Testing in Endometrial Cancer: Proceedings of the ISGyP Companion Society Session at the USCAP 2020 Annual Meeting. Int J Gynecol Pathol 2021; 40: 5-16.
12. Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite Instability as a Biomarker for PD-1 Blockade. Clin Cancer Res 2016; 22: 813-20.

13. Pećina-Šlaus N, Kafka A, Salamon I, Bukovac A. Mismatch Repair Pathway, Genome Stability and Cancer. Front Mol Biosci 2020; 7: 122.
14. Meyer LA, Broaddus RR, Lu KH. Endometrial cancer and Lynch syndrome: clinical and pathologic considerations. Cancer Control 2009; 16: 14-22.
15. Huvila J, Pors J, Thompson EF, Gilks CB. Endometrial carcinoma: molecular subtypes, precursors and the role of pathology in early diagnosis. J Pathol 2021; 253: 355-65.
16. Ersöz CC, Koltuk EK, Aras N, Kankaya D. Correlation of Microsatellit Instability with Morphological Findings in Endometrial Carcinomas. Ankara Üniversitesi Tıp Fakültesi Mecmuası 2022; 75: 322-7.
17. Travaglino A, Raffone A, Santoro A, Raimondo D, Angelico G, Valente M, et al. Clear cell endometrial carcinomas with mismatch repair deficiency have a favorable prognosis: A systematic review and meta-analysis. Gynecol Oncol 2021; 162: 804-8.
18. Crivellaro C, Baratto L, Dolci C, Ponti ED, Magni S, Elisei F, et al. Sentinel node biopsy in endometrial cancer: an update. Clin Transl İmaging 2018; 6: 91-100.
19. How JA, O'Farrell P, Amajoud Z, Lau S, Salvador S, How E, et al. Sentinel lymph node mapping in endometrial cancer: a systematic review and meta-analysis. Minerva Ginecol 2018; 70: 194-214.
20. Arora S, Narayan P, Ison G, Berman T, Suzman DL, Wedam S, et al. U.S. FDA Drug Approvals for Gynecological Malignancies: A Decade in Review. Clin Cancer Res 2022; 28: 1058-71.
21. de Jonge MM, Mooyaart AL, Vreeswijk MP, de Kroon CD, van Wezel T, van Asperen CJ, et al. Linking uterine serous carcinoma to BRCA1/2-associated cancer syndrome: A meta-analysis and case report. Eur J Cancer 2017; 72: 215-25.
22. Jamieson A, Bosse T, McAlpine JN. The emerging role of molecular pathology in directing the systemic treatment of endometrial cancer. Ther Adv Med Oncol 2021; 13: 17588359211035959.
23. Kurnit KC, Kim GN, Fellman BM, Urbauer DL, Mills GB, Zhang W, et al. CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence. Mod Pathol 2017; 30: 1032-41.
24. Santoro A, Angelico G, Travaglino A, Inzani F, Arciuolo D, Valente M, et al. New Pathological and Clinical Insights in Endometrial Cancer in View of the Updated ESGO/ESTRO/ESP Guidelines. Cancers (Basel) 2021; 13: 2623.
25. Plotkin A, Kuzeljevic B, De Villa V, Thompson EF, Gilks CB, Clarke BA, et al. Interlaboratory Concordance of ProMisE Molecular Classification of Endometrial Carcinoma Based on Endometrial Biopsy Specimens. Int J Gynecol Pathol 2020; 39: 537-45.
26. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A et al. Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: Earlier prognostic information to guide treatment. Gynecol Oncol 2016; 143: 46-53.
27. León-Castillo A, Gilvazquez E, Nout R, Smit VT, McAlpine JN, McConechy M, et al. Clinicopathological and molecular characterisation of 'multiple-classifier' endometrial carcinomas. J Pathol 2020; 250: 312-22.
28. León-Castillo A, Britton H, McConechy MK, McAlpine JN, Nout R, Kommoss S, et al. Interpretation of somatic POLE mutations in endometrial carcinoma. J Pathol 2020; 250: 323-35.
29. Crosbie EJ, Ryan NAJ, Arends MJ, Bosse T, Burn J, Cornes JM, et al. The Manchester International Consensus Group recommendations for the management of gynecological cancers in Lynch syndrome. Genet Med 2019; 21: 2390-400.
30. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective Lynch syndrome database. Gut 2017; 66: 1657-64.
31. Raffone A, Travaglino A, Cerbone M, Gencarelli A, Mollo A, Insabato L, et al. Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer. Pathol Oncol Res 2020; 26: 1417-27.
32. Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB, McCluggage WG. Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility. Int J Gynecol Pathol 2019; 38 Suppl 1: S123-31.
33. Thiel KW, Devor EJ, Filiaci VL, Mutch D, Moxley K, Alvarez Secord A, et al. TP53 Sequencing and p53 Immunohistochemistry Predict Outcomes When Bevacizumab Is Added to Frontline Chemotherapy in Endometrial Cancer: An NRG Oncology/Gynecologic Oncology Group Study. J Clin Oncol 2022; 40: 3289-300.
34. Corr B, Cosgrove C, Spinosa D, Guntupalli S. Endometrial cancer: molecular classification and future treatments. BMJ Med 2022; 1: e000152.
35. McAlpine JN, Chiu DS, Nout RA, Church DN, Schmidt P, Lam S, et al. Evaluation of treatment effects in patients with endometrial cancer and POLE mutations: An individual patient data meta-analysis. Cancer 2021; 127: 2409-22.
36. León-Castillo A, de Boer SM, Powell ME, Mileshkin LR, Mackay HJ, Leary A, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. J Clin Oncol 2020; 38: 3388-97.
37. Aghajanian C, Filiaci V, Dizon DS, Carlson JW, Powell MA, Secord AA, et al. A phase II study of frontline paclitaxel/carboplatin/bevacizumab, paclitaxel/carboplatin/temsirolimus, or ixabepilone/carboplatin/bevacizumab in advanced/recurrent endometrial cancer. Gynecol Oncol 2018; 150: 274-81.

Gestational trophoblastic neoplasia with concurrent metastasis to the mother and child: a systematic literature review

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Abstract

Gestational trophoblastic neoplasia (GTN) arising in the placenta and presenting as a metastatic disease concurrently in the mother and the baby is extremely rare. GTN poses a diagnostic dilemma to the treating clinicians. In the current review, an electronic search of Scopus, PubMed, Embase and other databases was conducted for case reports and case series of GTN co-existing or metastatic to both the mother and the baby, published to date. Globally, a total of twenty-two cases of GTN with metastasis to both the mother and baby was found. The previous history of histopathology confirmed molar pregnancy was present in 4/22 cases. The median time to diagnose GTN in the mother was six weeks post-partum. In the majority of cases, diagnosis of maternal disease was made after the infant presented with clinical manifestation. Overall survival was reported in 17/22 mothers up to varying latest follow-up and in 6/22 infants. A knowledge of the varied clinical presentation, eliciting a history of previous pregnancy loss/term pregnancy and serum beta human chorionic gonadotrophin (β-hCG) estimations were helpful for early diagnosis. The concurrent presence of GTN in the mother and baby is a rare entity and poses a diagnostic dilemma. Diagnosis in the mother often follows diagnosis in the baby after an infant presents with clinical manifestations. GTN is a highly chemo-sensitive tumour, but the main prognostic factors determining survival are the time to diagnosis following previous pregnancy and serum β-hCG levels.

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Keywords: Choriocarcinoma, concurrent to mother and foetus, GTN, infantile choriocarcinoma

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Introduction

Gestational choriocarcinoma is a malignant trophoblastic tumour arising from any gestational event. It is generally observed in reproductive-age females. Choriocarcinoma (CC) is often a clinical masquerade, and almost every case has a distinct clinical presentation in different individuals, making diagnosis difficult. Gestational trophoblastic neoplasia (GTN) may present after any gestational event, be it normal delivery, miscarriage or, rarely, a partial mole coexistent with normal fetus (1). Regression of the primary tumour after metastasis is also known. However, one-third of patients may manifest complications of metastatic disease. CC is a highly chemo-sensitive tumour, responding well to chemotherapy with a response rate of up to 80%, even in the presence of brain metastasis (2). The predominant determinant of survival is the time to diagnosis from the presentation (3).

Primary infantile or neonatal CC is an extremely rare condition, and most of the cases presenting in infancy are metastatic CC from the placenta to the fetus (4). Infantile CC usually presents between 0 and six months of age with the clinical picture of anaemia and hepatomegaly (5), which was characteristically described in 1968 by Witzleben and Bruninga (6). Commonly, the manifestations are widespread and rapidly progressing metastatic disease involving the lungs, brain, and subcutaneous tissue. Sometimes, precocious puberty may be present, related to the elevated serum beta human chorionic gonadotrophin

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(β-hCG). The unusual tendency of infantile malignancies to seed cutaneous tissue can generate vivid clinical presentations (5). The prognosis of infantile CC is usually unfavourable, with most cases being fatal within three to four weeks of diagnosis (7).

GTN arising in the placenta and presenting as a metastatic disease concurrently in the mother and the baby is extremely rare. It is observed that approximately 5% of all cases of intra-placental CC have metastasis in the fetus (7). Most mothers of babies with infantile CC are asymptomatic during pregnancy, the diagnosis being confirmed retrospectively with manifestations in the infant (7). Due to the condition's rarity, the literature available is only in the form of case reports. We report a systematic review of the literature search that yielded 22 case reports of concurrent CC in mothers and infants. The primary aim was to identify clinical signs and symptoms that can facilitate the early clinical suspicion of CC, and prompt timely investigation and appropriate therapy, thereby improving survival in both the mother and baby.

Material and Methods

Being a systematic review of case reports, ethics committee approval was not sought as systematic reviews are exempt from ethics review. The systematic review was planned and reported according to the Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines.

An electronic search of Scopus, PubMed, Embase and other databases was conducted for case reports and case series of GTN co-existing or metastatic to both the mother and the baby, published to date. The electronic search strategy was done using keywords such as "gestational trophoblastic neoplasia" OR "choriocarcinoma" OR "infantile choriocarcinoma" OR "placental site trophoblastic tumour" OR "PSTT" and "mother and baby" OR "mother and fetus" and "case reports" OR "case series" OR "concurrent choriocarcinoma" OR "infantile metastatic choriocarcinoma". We analyzed the title and abstracts of all case reports identified by the initial search. The reference lists of relevant reports were also explored. Two reviewers double-checked the data independently and simultaneously to avoid duplication. The third reviewer resolved any conflicts.

Published case reports and case series of GTN co-existing or metastatic to both the mother and the baby, published from inception till September 2022, were included in this systematic review. GTN cases localized to the placenta or confined only to the mother or the fetus were excluded. Patients of all published case reports/case series of infantile CC only were studied in detail for the information regarding the mother so that no cases of the concurrent disease in mother and infant were missed. Review articles, original articles, clinical trials, conference abstracts, editorials, cases with incomplete information, and papers in languages other than English or commentary were excluded.

We extracted information, such as geographical distribution or country of occurrence of the case, year of publication, and the type of GTN. Details of the mother, including her obstetric history, with particular attention to the previous history of molar pregnancy or CC, were recorded. A note was made of what brought the patient to clinical attention, their clinical features, serum β-hCG levels at the time of diagnosis, sites of metastasis and their follow-up, survival and treatment received. Information regarding the baby included the sex, age of presentation, presenting complaints, how and when the diagnosis was confirmed, serum β-hCG levels at the time of diagnosis, sites of metastasis and the outcome. A note was also made of the primary misdiagnosis, if available.

Descriptive statistics were used to calculate simple frequency, percentage, and proportion out of the total case reports. The survival analysis was performed by Kaplan Meier analysis, using the Statistical Package for Social Sciences for Windows, version 16 (IBM Inc., Armonk, NY, USA). Death was taken as the event for overall survival (OS) analysis.

Results

A total of twenty-two cases of GTN with metastasis to both the mother and baby had been reported globally (Figure 1). Of these, 21 cases were of CC, and one was a placental site

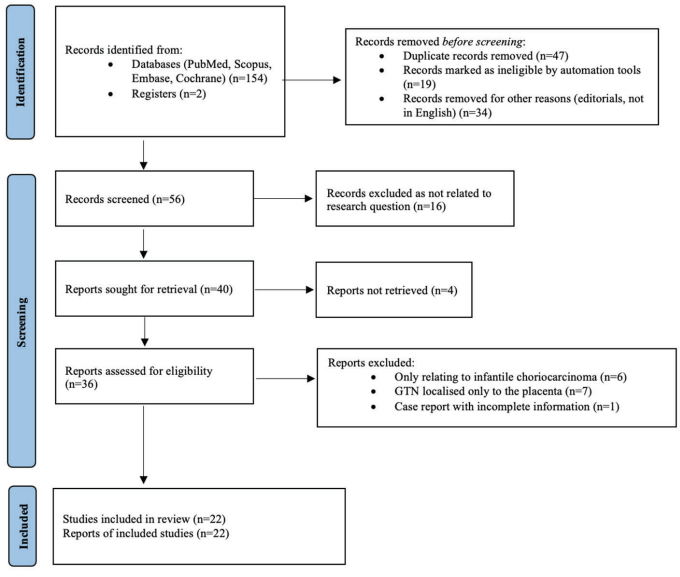

Figure 1. PRISMA flowchart of the screening process used for the present systematic review
PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analyses

trophoblastic tumour (PSTT). Out of these 22 children reported, the male:female ratio was 1.2:1. The median birthweight was recorded in 14 children whose data was available as 2965 grams [interquartile range (IQR): 2615.5, 3325]. The case details are shown in Table 1, 2, respectively.

Previous molar pregnancy

The previous history of histopathologically confirmed molar pregnancy was present in 4/22 cases. Furthermore, in 3/22 cases, a history of prior molar pregnancy cannot be excluded as the author has reported that the patient had a history of abortion before the index pregnancy. Nevertheless, no details regarding the histopathology report of the products of conception were reported. There was no history of previous molar pregnancy given in 9/22 cases. In 6/22 cases, the details regarding the last pregnancy were not mentioned.

Time of diagnosis in mother and baby

The median time to diagnose GTN in the mother was six weeks post-partum. Two out of these twenty-two cases were diagnosed at the time of delivery, both due to the abnormal gross appearance of the placenta, for which a pathological examination was requested. In one case, the diagnosis was suspected in the antenatal period at around 36 weeks of gestation due to multiple lung metastases. In one case, the diagnosis was made at thirteen months after delivery on β-hCG follow-up; the patient, in this case, was on follow-up because of a diagnosis of infantile CC in the baby. The median age of presentation in infants was 1.75 (IQR: 0.1, 6.75) weeks. The median age of diagnosis in the child was 5.00 (IQR: 3.55, 8) weeks. In 10/22 children, the diagnosis was suspected at birth. For 1/22 children, the diagnosis was suspected because the mother was diagnosed with GTN. In the remaining 21 children, the primary diagnosis was made in the infant, and retrospectively mother was diagnosed as having the disease. The median time to diagnosis from symptom onset was 1.7 (IQR: 0, 3.65) weeks in the infants.

What brought the patient to clinical attention?

In 8/22 cases, the diagnosis was suspected after the infant presented with symptoms. In 4/22, dilatation and curettage due to abnormal uterine bleeding led to pathologic confirmation in the post-partum period. In 6/22, the diagnosis was suspected either after imaging or β-hCG estimation was done for a tumour of unknown origin, which showed widespread metastasis. In five cases, the diagnosis was suspected in the peripartum period, predominantly due to abnormal gross appearance of the placenta or post-partum haemorrhage after delivery (2/22) and significant feto-maternal haemorrhage (1/22). In one case, the patient was on β-hCG follow-up due to a previous history of molar pregnancy.

In infants, the primary complaints at the time of presentation were pallor, vomiting, skin nodules, and poor feeding observed in 11/22 (50%), 3/22 (13.7%), 2/22 (9%), and 2/22 (9%), respectively. Intrauterine foetal demise was noted in 2/22 (9%). Hematemesis, melena, abdominal distension, generalized swelling and cold were each observed in one child. Hepatomegaly was observed in 13/22 (59%). Other clinical findings were failure to thrive, facial mass, jejunal mass and cutaneous tumours, each in one child. Complications noted were severe anaemia (haemoglobin <8 g/L) in 11/22 (50%), respiratory failure, tumor bleeding and cardiac failure in 3/22 (13.7%) each, and respiratory distress, intracranial haemorrhage, and multi-organ dysfunction in 2/22 (9%) each. Complications, such as pneumothorax, hypovolemic shock, hemoperitoneum, severe acidosis, thrombocytopenia, tumor rupture, pulmonary haemorrhage and hypoproteinaemia were each recorded in one child. The liver was involved in 17/22 (77.35%). Metastases to the lung were recorded in 16 (72.7%), brain and gastrointestinal tract in 4 (18.2%), bone and skin/subcutaneous site in 3 (13.7%), eyes in 2 (9%) and kidney in one child.

The diagnosis of CC was made post-mortem in 9/22 (40.9%) infants by autopsy or post-mortem histopathology of liver mass. The antemortem diagnosis was made in 13 children; seven by biopsy of the mass, four by β-hCG estimation and two by a combination of biopsy and β-hCG. The possibility of CC was strongly considered in 4 children (18%). Before arriving at the final diagnosis, the differential diagnosis was available for 20 children and included hepatic angiosarcoma, haemangioma, hepatoblastoma, haemolytic anaemia, feto-maternal haemorrhage, haemangioblastoma, haemangioendothelioma, hepatocellular carcinoma, and sepsis in 3, 3, 2, 2, 2, and 1 child, respectively.

Treatment received

The details of treatment were available for 20 mothers. Chemotherapy alone was provided in 13, chemotherapy and surgery in four, chemotherapy and irradiation in one, and surgery alone in two. In three patients, single-agent methotrexate (MTX) was administered, while the rest received combination chemotherapy. In four patients, MTX, actinomycin and etoposide combination chemotherapy was provided. Other drugs used were cisplatin, cyclophosphamide, and vincristine.

Definitive treatment was administered in 14 children (63.7%), which included chemotherapy alone in seven, a combination of chemotherapy and surgery in five and surgery only in two children, respectively. The most common treatment regimen

Table 1. Gestational trophoblastic neoplasia co-existing in mother and baby: Maternal characteristics

S. no	Author, year of publication	Previous h/o molar pregnancy	When the diagnosis was made? (weeks post-partum)	How was diagnosis made?	Symptoms in the mother at diagnosis	Maternal serum β-hCG at diagnosis	F/u of mother	Treatment received
1	Mercer et al. (8), 1958	Not mentioned	8 weeks	Dilatation and curettage due to AUB	Bleeding p/v off and on since 6 wk. pp	NA	Death at 8 months pp	TAH + BSO followed by immediate cobalt teletherapy and full course of pelvic irradiation
2	Brooks and Nolting (2), 2014	Not mentioned	6 weeks	After confirmation of diagnosis in the baby	Not mentioned	Not mentioned	Not specified	CT (details not available)
3	Hanson et al. (9), 2011	Not mentioned	6 weeks	After confirmation of diagnosis in the baby	Not mentioned	>60,000 mIU/mL	Survived	Total abdominal hysterectomy f/b chemotherapy (Single agent MTX X 8 doses)
4	Sashi et al. (10), 1996	Yes	7 weeks	After death of baby, multiple lung and liver tumours and raised high urine hCG	Not mentioned	Urine hCG - 435.84x 104 IU/L)	Survived till14 months from diagnosis	CT (details not available)
5	Andreitchouk et al. (11), 1996	Yes	4 months	Clinical features. After death of baby, its autopsy report	General fatigue, back pain, and intermittent genital bleeding, severe weight loss and abdominal distention, Hepatomegaly, Severe anaemia	Urine hCG, 5,300,000 mIU/mL; Serum hCG, 4,358,400 mIU/mL Serum hCG-P, 20,000 ng/mL	Remission	Chemotherapy (EMACO X 14 courses in 7 months (Etoposide, 100 nig/m^2/day on days 1 and 2; Methotrexate, 100 mg/m^2/day on day 1; Dactinomycin, 0.5 mg/m^2/day on days 1 and 2 Cyclophosphamide, 600 mg/m^2/day on day 9 Vincristine 1 mg/m^2/day on day 9 and combined with folic acid 30 mg/day on days 2, 3, 4
6	Avril et al. (12), 1986	No	At delivery	Pathologic examination of placenta due to a 5 cm abnormal growth on the uterine surface.	NK	16,000 IU/L	Alive at 14 months No further details available	Chemotherapy with methotrexate, actinomycin D, vincristine, and cisplatinum.

Table 1. Continued

7	Bolze et al. (13), 2013	No	13 months	On β-hCG follow-up after diagnosis in baby	Intermittent headache, partial loss of vision, elevated β-hCG	107 IU/L	Normal at 3 years f/u. No evidence of relapse on MRI, PET and hCG levels at 3 years follow-up	Resection of brain metastasis + chemotherapy with etoposide, actinomycin D and cisplatinum (3 courses) + stereotactic brain irradiation (39 grays in 13 fractions)
8	Flam et al. (14), 1989	No	10 weeks	D and C d/t persistent bleeding	PPH at 4 weeks pp	24,200 IU/L	Alive at 7 years	Chemotherapy (methotrexate and folinic acid) 3 cycles. hCG not detectable after second cycle
9	Rzanny-Owczarzak et al. (15), 2021	H/o abortion 4 months before present pregnancy, HPE NA	5 weeks	After diagnosis in baby, D and C done in mother	Vaginal bleeding	37,600 IU/mL	Alive and pregnant at 1 year	Chemotherapy for 6 months (dactinomycin, etoposide, methotrexate)
10	Liu and Guo (16), 2006	H/o three abortions before current pregnancy	5 weeks	D and C i/v/o persistent vaginal bleeding	At the 24th, 29th and 37th day postpartum, the patient had unexplained vaginal bleeding	hCG-33648 mIU/mL at day 51, and the β-hCG-14899.9 mIU/mL	Normal at 1 year f/u	Uterine arteriography and transcatheter arterial chemotherapy (VCR + FUDR + KSM + VP16)
11	Tsukamoto et al. (17), 1986	H/o 2 abortions, No h/o molar pregnancy	3 weeks	β-hCG done after confirmation of fetal diagnosis on autopsy.	Intermittent bloody discharge p/v	1270 rig/mL at 4 weeks, 1590 rig/mL at 5 weeks, Urinary hCG 3000 IU/L at 3 weeks, 5000 IU/L at 4 weeks	Asymptomatic at 9 months f/u	Combination chemotherapy with MAC 7 courses (methotrexate 15 mg im, actinomycin-D 0.5 mg iv, and cyclophosphamide 100 mg iv q.d. x 5 days), X 7 weeks 50 mg of methotrexate was injected into each internal iliac artery.
12	Buckell and Owen (18), 1954	No	9 weeks	Had Abnormal uterine bleeding post-delivery, D and E done for retained products of conception, HPE suggestive	Continuous vaginal bleeding for 9 weeks following birth		Alive at 13 months pp	TAH, BSO and pelvic RT

Table 1. Continued

13	Kruseman et al. (19), 1977	No	4 weeks	FMH Autopsy of fetus s/o CC Secondary PPH at 4 weeks d/b D and C	PPH at 4 weeks pp	345,000 IU/24-hour urine	Alive at 6 months	9 courses of CT (methotrexate and folinic acid)
14	Mosayebi and Movahedian (20), 2016	No	6 weeks (after death on autopsy)	Severe anaemia and fatigue in pregnancy	Not mentioned	Not mentioned	Died at 30 days pp	None
15	McNally et al. (21), 2002 Heath and Tiedemann (22), 2001	Yes	At delivery	H/o molar pregnancy, Abnormal appearance of placenta	Not significant	12,34,000	Alive and healthy at 3 years	Craniotomy + EMA/EP regime (etoposide, methotrexate and actinomycin D alternating weekly with cisplatin and etoposide) 6 cylces + 2 cycles of intrathecal methotrexate f/b 7 cyles of etoposide and paclitaxel f/b 3 cycles of paclitaxel
16	Daamen et al. (23), 1961	No	5 months	Primary and Secondary PPH at delivery f/b palpable tumour at 5 months pp.	A large round, taut and cystic tumour filled the entire true pelvis and was continuous with the neoplasm palpable from the outside, Arising from right ovary		Died 7 months pp	Laparotomy f/b chemotherapy with methotrexate
17	Aozasa et al. (24), 1981	Details not available		Chest X-ray s/o multiple metastasis and elevated β-hCG	A varix was noted on uterus at the time of delivery, Pyrexia, amenorrhea, Sudden unconsciousness and death	1280 IU/L	Died at 5 months pp	Not mentioned
18	Fraser et al. (25), 1992	Not mentioned	5 weeks	Elevation in β-hCG		8 weeks pp-day 0-2000, day 11-950, day 20-300, and day-37-1450	Alive and healthy at 2 years	4 courses of cisplatinum and VP 16

Table 1. Continued

19	Getrajdman et al. (26), 2012	No	1 month postpartum During pregnancy at 36 weeks was suspected	CT scan s/o metastasis in lung, liver and spleen a/w elevated β-hCG	Cramping at 12 weeks, Haemoptysis at 28 weeks, and rib pain at 36 weeks. Later again presented 1 month pp with debilitating shoulder pain, shortness of breath and abdominal distension	4.9 million mIU/mL	Alive and healthy at 2 years	Emergent selective hepatic embolization and splenic artery embolization f/b CT with EMA-EP alternatively for 6 weeks
20	Kishkurno et al. (27), 1997	Yes	13 weeks	Large liver and lung tumours with elevated hCG	Large hepatic tumour and small multiple metastases to the lungs	Urinary 4,213,500 IU/L	Alive and healthy	CT
21	Picton et al. (28), 1995	Not mentioned	8 weeks	Heavy vaginal bleeding, multiple lung metastasis with elevated hCG	Heavy vaginal bleeding with clots for 8 weeks	1112000 IU/L	Alive and healthy at 11 months	CT with methotrexate with folinic acid rescue followed by IV actinomycin-D and etoposide for 3 days for 6 cycles
22	Monclair et al. (29), 2002	h/o 2 abortions, HPE not mentioned	8 months	D and C following recurrent vaginal bleeding	Irregular vaginal bleeding	1270 IU/L	Alive and healthy at 26 months	TAH

GTN: Gestational trophoblastic neoplasia, CC: Choriocarcinoma, PSTT: Placental site trophoblastic tumour, CT: Chemotherapy, RT: Radiotherapy, hCG: Human chorionic gonadotropin, NK: Not known, NA: Not available, D and C: Dilatation and curettage, HPE: Histopathologic examination, FMH: Feto-maternal haemorrhage, PPH: Postpartum haemorrhage, TAH: Total abdominal hysterectomy, BSO: Bilateral salpingo-oophorectomy, pp: Postpartum, MAC: Methotrexate, actinomycin-D and cyclophosphamide

used was bleomycin, etoposide, and cisplatin in four children. MTX alone and dexamethasone doxorubicin combination were used in two children, respectively. A combination of MTX, etoposide and cisplatin, etoposide and MTX, etoposide alone and a combination of multiple drugs were administered in one child each. Complications secondary to chemotherapy were reported in one child, which were hearing loss and adrenal insufficiency.

Follow-up and survival

The duration of follow-up mentioned in each of the cases varied widely. Excluding the 4/22 mothers who died, the follow-up time mentioned in the cases was as follows. The duration of follow-up was around one year (11-14 months) in 5/22, around two years (24-26 months) in 3/22, three years in 2/22 and seven years in 1/22. In 2/22 cases, the follow-up was less than nine months. In 4/22 cases, although the authors mentioned that the patients survived, the duration of follow-up was not mentioned. 4/22 mothers expired within six to seven months of pregnancy, with a mortality rate of 18.2%. One patient expired 30 days after delivery, one each after five months, six months and seven months, respectively. 17/22 mothers survived up to the most recent follow-up reported (shortest reported follow-up reporting survival & longest reported follow-up), and in 1/22 cases, no information regarding survival was mentioned. The 12-month OS in mothers was 71.8±10.7% (Figure 2).

Out of 20 live-born children, six (30%) survived to latest reported follow-up, ranging from seven months to three years in children who survived. The OS was 22.2±9.8% (Figure 3). Out of seven children who received multi-agent chemotherapy, including a platinum agent, six children survived (85.7%).

Table 2. Gestational trophoblastic neoplasia co-existing in mother and baby: infant details (n=22)

S. no	Author and year of publication	Presenting complaints & clinical features at time of diagnosis	Time of diagnosis in weeks	Complications	Method of confirmation of diagnosis	Site of metastasis	Treatment received	Outcome & follow -up
1	Mercer et al. (8), 1958	Small red nodule in the upper anterior alveolar ridge	6	Profuse haemorrhage	Biopsy	Upper maxilla, nasal fossa and later head and neck	Surgery	Died
2	Brooks and Nolting (2), 2014	Right-sided facial mass, Recurrence of facial mass after resection	5	Tumour bleed	Biopsy	Lung	Surgery, embolization and chemotherapy	Survived
3	Hanson et al. (9), 2011	Fever, pallor, and fatigue, Recurrent severe anaemia, hepatomegaly	8	Severe anaemia, hypovolemic shock, respiratory failure	Biopsy and β-HCG	Liver	Chemotherapy and liver transplantation	Survived
4	Sashi et al. (10), 1996	Anaemia, abdominal distension, tachycardia, cyanosis, liver tumours	5	Multi-organ dysfunction syndrome	Biopsy	Liver, lung, brain	Nil	Died
5	Andreitchouk et al. (11), 1996	Severe anaemia Hepatomegaly	5	Congestive cardiac failure, respiratory distress	Autopsy of liver mass, β-HCG	Liver, lung, brain.	Chemotherapy	Died
6	Avril et al. (12), 1986	Cutaneous lesions Disseminated cutaneous tumours, hepatomegaly, lung rales	0.85	Hypoproteinaemia, pulmonary haemorrhage	Biopsy	Skin, lung, bone, pelvis	Chemotherapy	Died
7	Bolze et al. (13), 2013	Dyspnea and anaemia, Liver mass, mediastinal lymphadenopathy	21.8	Not mentioned	β-HCG	Liver, lung, mediastinal lymph nodes	Chemotherapy and hepatectomy	Died
8	Flam et al. (14), 1989	Anaemia Liver tumour	3	Rupture of liver tumour	Post-mortem histopathology of liver mass	Liver	None	Died
9	Rzanny-Owczarzak et al. (15), 2021	Hematemesis Liver tumour	4	Multi-organ dysfunction syndrome respiratory failure	Autopsy	Lung, liver, intestine, lymph nodes	Chemotherapy	Died
10	Liu and Guo (16), 2006	Unexplained melena, Jejunal mass	3.4	Nil	Biopsy of jejunal mass	lung, jejunum,	Chemotherapy	Survived
11	Tsukamoto et al. (17), 1986	Unexplained intrauterine fetal death Metastatic liver disease	0.1	Intrauterine fetal death	Autopsy	Liver, lungs, hilar lymph nodes, diaphragm, and subcutaneous tissue of the head.	None	Intrauterine fetal death
12	Buckell and Owen (18), 1954	Vomiting, abdominal distension, Anaemia, epigastric mass	7	Severe anaemia	Autopsy	Liver, ribs and nodes	Blood transfusion	Died

Table 2. Continued

13	Kruseman et al. (19), 1977	Still birth, polyhydramnios, tumour in left kidney 1.2x1.8 cm	0.1	Massive feto-maternal haemorrhage	Autopsy	Kidney	None	Intrauterine fetal death
14	Mosayebi and Movahedian (20), 2016	Recurrent vomiting and poor feeding, sick child, decreased neonatal reflexes, systolic murmur, bilateral megalocornea and leukocoria	8	Severe anaemia, encephalopathy, intracranial haemorrhage and herniation	β-HCG and mother's diagnosis	Brain, lung, liver and eye	Blood transfusion	Died
15	Heath and Tiedemann (22), 2001	Solitary liver nodule	0.1	None	β-HCG	Liver	Chemotherapy, surgery	Survived
16	Daamen et al. (23), 1961	Fevere, severe anaemia, firm tumour in the upper abdomen, leucocytosis, recurrent anaemia, jaundice	26.2	Severe anaemia	Biopsy of liver tumour	Liver, peritoneum, abdominal muscles and skin, lung, subcutaneous layers and adrenal	Laparotomy, Chemotherapy, multiple transfusions	Died
17	Aozasa et al. (24), 1981	Weakness of feeding and edema in the right inguinal and labial region, hepatomegaly, abdominal distension, thrombocytopenia	8	Congestive cardiac failure	Biopsy of liver tumour	Liver, lung	Right hepatic artery embolization and excision	Died
18	Fraser et al. (25), 1992	Anaemia and lethargy, Hepatomegaly	9 weeks	Nil	Biopsy of liver mass, β-HCG	Liver, lung	Chemotherapy, segmental resection of liver mass	Survived
19	Getrajdman et al. (26), 2012	Pallor Hepatomegaly and anaemia	4 weeks	Tumour bleed	Elevated serum β-HCG	Liver, lung, eyes	Chemotherapy	Survived
20	Kishkurno et al. (27), 1997	Anaemia, hepatomegaly	5.4	Recurrent severe anaemia, congestive cardiac failure	Autopsy, biopsy of liver mass	Liver, brain, lung	Chemotherapy	Died
21	Picton et al. (28), 1995	Feeding difficulty, poor weight gain, anaemia, vomiting Failure to thrive, hepatosplenomegaly	4.5	Intracranial haemorrhage, respiratory failure, severe anaemia, hemoperitoneum, severe acidosis	Autopsy	Liver, lung	None	Died
22	Monclair et al. (29), 2002	General malaise, common cold, Hepatomegaly, dyspnoea, tachycardia, pneumothorax,	19.4	Respiratory failure secondary to bilateral pneumothorax	Autopsy	Liver, lung, mesentery	None	Died

HCG: Human chorionic gonadotropin, NK: Not known, NA: Not available

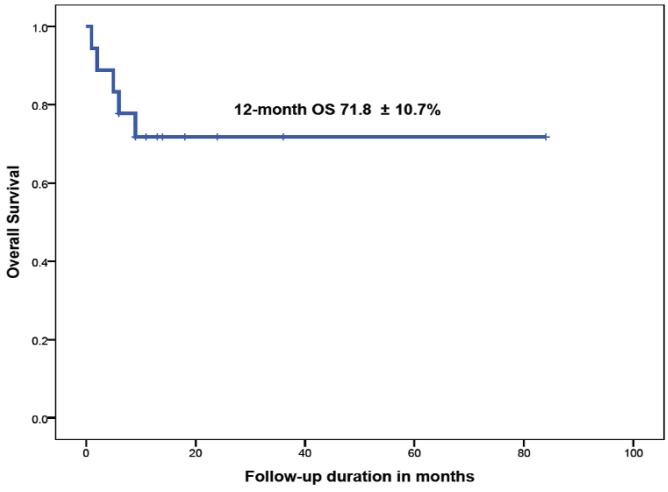

Figure 2. Overall survival of mothers with choriocarcinoma, coexisting or metastatic to the child
OS: Overall survival

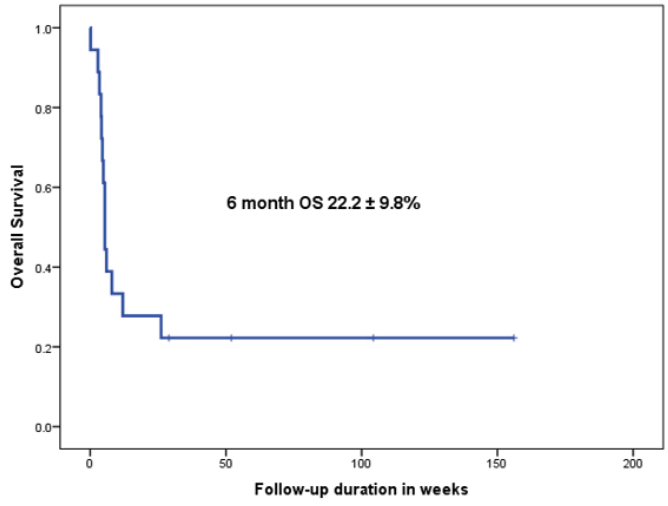

Figure 3. Overall survival of children with choriocarcinoma coexisting in the mother
OS: Overall survival

Discussion

We present a comprehensive systematic review of all published case reports and short case series of GTN co-existing or metastatic to both the mother and the baby published up to September 2022. Worldwide, only 22 such cases were published in English, indicating the rarity of this entity. Therefore, this systematic review will help understand the epidemiology, clinical presentation, and OS and prognosis of mother and baby. It would also help to identify a possible clinical clue that may help treating physician to identify the condition early, thereby improving prognosis and survival.

The transmission of maternal cancer to the fetus/neonate is an extremely rare phenomenon, with the reported incidence being one infant per 500,000 mothers with cancer (30). Approximately 1 in 1000 live births involves a mother with cancer (31). Eighteen cases have been reported in the literature (32), and the malignancies include those of the skin, lung, cervix, and hematologic system, predominantly leukaemia. Most of these reported cases are non-GTN. Either of the following hypothesis can explain the transmission of cancer from mother to the fetus/neonate. One mechanism is by transplacental hematogenous dissemination and the other by direct transmission (via contact or aspiration) at the time of delivery (33).

Though extremely rare, GTN arising from the trophoblastic tissue and metastatic to both the mother and baby can be explained by the fact that malignant tissue arising from the trophoblast can enter the systemic circulation and then through the placenta to the fetus. Trophoblastic tissue embolizing into the systemic circulation was first reported by Schmorlin in 1893 in a post-mortem study of eclamptic patients (34). There have also been isolated case reports of trophoblastic embolism during curettage or caesarean section (35,36). Trophoblastic pulmonary embolism is also common, predominantly in patients with difficult parturition, following manual removal of placenta, in cases of placenta praevia, preeclampsia and eclampsia and in cases of uterine tears (34).

GTN is considered one of those malignancies with a good prognosis because of its high chemo-sensitivity. Survival rates have been reported to vary from 85% to 95%. However, in the present review, the OS was lower as the 12-month OS in mothers was 71.8±10.7%. The six-month OS in cases where the infantile CC co-exists with the maternal CC or is a result of metastasis was 22.2±9.8%. The survival of infants was similar to that reported in previous studies, at approximately 20% (5). Still, there seems to be an earlier onset of the disease, with the median age of presentation being 1.75 weeks. In comparison Blohm et al. (5) reported age at presentation to be four weeks. Although not much studied, the disease co-existing in the mother and baby or fetal/neonatal metastasis could be an important factor determining poorer prognosis. Hence, our review found that it seems to be a more rapidly progressing disease when it co-exists in mother and baby.

We found that the median age to diagnosis in the mother was six weeks post-partum. A previous history of molar pregnancy was present in 4/22 patients. In 9/22 mothers, the last pregnancy details were either unavailable or not confirmed (h/o abortion present, but molar pregnancy not ruled out on histopathology). GTN is staged and scored as per the WHO Scoring System, which divides it into two groups: (i) low-risk group (score ≤6) intimating single-agent chemotherapy; and (ii) high-risk group (score >6) mandating multi-agent chemotherapy (37). Risk is defined as developing drug resistance (methotrexate or MTX) as determined by the WHO Prognostic Scoring System. All patients

with non-metastatic disease and patients with risk scores <7 are considered low-risk. Patients with a score >6 are deemed to have high-risk disease. It is notable that two of the parameters in the risk scoring include the antecedent pregnancy, whether it was a mole, abortion or a normal term pregnancy and the time interval from the end of index pregnancy to the diagnosis of GTN. It, therefore, becomes imperative to determine if the present GTN developed following which pregnancy, or which is the antecedent pregnancy. The tumour DNA would be identical to the neonate if it arose following the current pregnancy and will be different if the index pregnancy was a previous abortion, mole or previous full-term pregnancy. There is always a possibility that undetected gestational trophoblastic disease from an earlier pregnancy may have progressed to CC during the index pregnancy. Genetic analysis should therefore be performed to confirm the origin of the tumour, as this would confer a significant prognostic value. If the tumour is gestational in origin, it would contain both maternal and paternal genes, and its genetic composition would be the same in the mother and fetus or the neonate. This also rules out the possibility that the tumour arose de novo in the infant and is not a metastasis from the mother. The time between the index pregnancy and development of CC has been reported to range widely from 4 weeks to as long as 25 years (38). Therefore, in any adult female, the origin of GTN being a previous pregnancy always arises. At times, it may be challenging to localize the antecedent pregnancy causing GTN as the malignant placental or uterine tissue may be extruded entirely or devitalized, leaving no trace of its presence (39). Therefore, the so-called primary tumour may, in all possibility, be a metastasis. Establishing the causative pregnancy, which may also be any other than the current pregnancy, has a clear prognostic value, more so in cases of PSTT or epithelioid trophoblastic tumour, where a time interval of less or greater than four years affects future survival (40,41).

Blohm et al. (5) published one of the most extensive and informative literature reviews regarding infantile CC in 2004 (21). A few salient observations in the review included: (a) equal male-to-female ratio; (b) major clinical features of anaemia, hepatomegaly, failure to thrive, respiratory distress or pulmonary haemorrhage; (c) 33% presented in the neonatal period and 67% up to 5 months of age; (d) liver, lung, brain and skin involvement in 77%, 67%, 27% and 10%, respectively; (e) diagnosis was established at autopsy in 32% of live-born infants; (f) elevated β-hCG in all tested children that contributed to the confirmation of diagnosis; (g) anti-neoplastic therapy was administered in 15/28 live-born infants and combination chemotherapy including a platinum agent along with surgery appeared to be the most effective strategy; and (h) 5/30 children (17%) survived. In our review, the observations of equal male-to-female ratio, clinical features, sites of involvement, and proportion of live-born infants diagnosed in the autopsy were similar; however, in cases in which the infantile CC was co-existent with the maternal CC or was a result of metastasis from it, there seems to be an earlier onset of disease with the median age of presentation being 1.75 weeks. In comparison, it was four weeks reported by Blohm et al. (5). Hence, it seems to be a more rapidly progressing disease when it co-exists in mother and baby. It should be noted that 13 children reported in this review had a differential diagnosis of malignancy. The common differentials were hepatic angiosarcoma, haemangioma and hepatoblastoma. These observations highlight the lack of awareness among general paediatricians and those working in paediatric oncology regarding infantile CC, probably due to its rarity. However, severe anaemia refractory to multiple transfusions, multi-systemic involvement and multi-organ dysfunction, especially pulmonary haemorrhage in the presence of hepatic tumour, are key signs for suspecting CC in an infant, even in the absence of disease activity in the mother. In the current study, liver metastasis was the common presentation in the fetus.

The predominant localization of metastatic CC in the fetal liver can be explained easily by understanding the prenatal blood supply to the fetus. The liver gets around 70 to 80% of all the blood in the umbilical vein, and only 20-30% is shunted through the ductus venosus (42).

In CC, β-hCG is universally elevated and is a simple, easy, affordable and accessible investigation. In the reports included in this review, β-hCG was performed in only 6/20 children and was elevated in all. This observation further highlights that CC is not one of the initial differentials considered in an infant with hepatic tumours. Measurement of β-hCG can prevent a risky, life-threatening procedure like a biopsy, as CC is very friable and well-known for intrinsic tumour rupture. In seven children included in this review, doing a β-hCG measurement could have speeded and facilitated diagnosis.

Another notable finding of this review was that although all 22 reported CC in both mother and infant, in only one child, CC was suspected because of previous GTN in the mother. In most cases, the disease was suspected in the mother only after the diagnosis was made in the baby. However, on closer analysis, in 11/20 (55%) mothers in whom the time of diagnosis was available, the disease was confirmed within two weeks of diagnosis in the baby. Hence, it is plausible that the majority of disease manifestations are concurrent in mother and baby. For early diagnosis in both mother and baby, it is essential for effective communication and collaboration between healthcare providers and physicians managing mother and infant.

In adults, the management of CC is risk-stratified and tailored. Patients with low-risk disease are treated with single-agent (MTX or actinomycin D) therapy. In contrast, patients with

high-risk disease are treated with multi-agent chemotherapy and surgery or radiation as per requirement. Survival in adults is remarkable, reaching up to 100% in low-risk and 90-95% in high-risk disease (43). In our review, the superior survival of mothers, when compared to children, underlines the remarkable chemo-sensitivity of the tumour. However, CC following-term pregnancy is usually considered high-risk because of metastasis and high β-hCG levels. In a study from the Netherlands on post-term CC, the survival was 86% (44). All patients were either medium or high-risk in their cohort as per their clinical staging system. As per the country protocol, treatment was multi-agent chemotherapy consisting of EMA/CO (etoposide, MTX, actinomycin D, cyclophosphamide and vincristine). They identified a long symptom to treatment interval of 16 weeks and high MTX resistance (75%). Hence, they suggested that multi-agent chemotherapy is justified in post-term CC. Similarly, authors from China have also reported high complete remission (CR) rates of 87.8% with combination chemotherapy (45).

In children, as in other germ cell tumours, the management of CC consists of multi-agent neo-adjuvant chemotherapy, re-assessment after 2-4 cycles, surgery of persistent disease and adjuvant chemotherapy. The principle of this complex therapy is to control the metastatic nature of the disease and prevent relapse. Upfront chemotherapy helps control the disease, especially when a child has multi-systemic involvement and is not fit for surgery. The excellent survival of children treated in this fashion in this review re-iterates the principles of therapy to be undertaken. The guidelines are well-established and readily available (46).

Significant predictors of survival identified are the time to diagnosis and serum β-hCG levels (37,47). A study by Ma et al. (45) of 123 patients with post-partum CC observed an interval between pregnancy and diagnosis of fewer than four months, and a β-hCG titre less than 1000 IU/L was associated with good outcome. In our review, although the mean duration from delivery to diagnosis was short at six weeks, high β-hCG levels were observed in most (15 patients), which may have contributed to the lower survival.

The following babies should be kept on β-hCG follow-up:

1. Babies of mothers with a history of a hydatiform mole in a previous pregnancy;
2. Babies of mothers with CC in the current or past pregnancy;
3. History of feto-maternal haemorrhage;
4. Birth of baby with severe anaemia;
5. Recurrent anaemia associated with hepatomegaly (25);
6. Haemangioma or a vascular lesion, presenting in the early months of life;
7. Any liver tumour in the baby presenting in the early months of life;
8. Any abnormality in the gross appearance of the placenta.

Equally important is screening mothers of babies with CC, mainly manifesting in the first six months of life (47). Although there are no specific and clear-cut recommendations at present (38), assuming that a majority of cases occur within six months of delivery, measurement of β-hCG every two weeks for the first six months, and monthly after that up to two years in the neonate has been recommended (5). However, it should be noted that recurrence of the disease, even long after CR (48), and secondary malignancies following chemotherapy for GTN (49), have also been reported in the literature.

Study limitations

The present systematic review provides the most recent and comprehensive overview of epidemiology, clinical manifestations, OS and prognosis in GTN co-existing in or metastatic in the mother and baby. Extensive electronic database searches were attempted to identify all globally published cases in English. However, the data were heterogeneous, and the analysis was descriptive, which are limitation of this systematic review. Case reports and series published in other languages were not included, which might be another limiting factor.

Implications for future research: This review highlights the need for more extensive research into the aetiology, progression, genetic aspects, management and long-term prognosis of cases with concurrent GTN in mother and infant.

Conclusion

The concurrent presence of GTN in the mother and baby is a rare entity and poses a diagnostic dilemma. Maternal diagnosis often follows diagnosis in the baby after an infant presents with clinical manifestations. A knowledge of the varied clinical presentation, eliciting a history of previous pregnancy loss/term pregnancy and serum β-hCG estimations are helpful for early diagnosis. GTN is a highly chemo-sensitive tumour, but the main prognostic factors determining survival are the time to diagnosis following previous pregnancy and serum β-hCG levels.

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References

1. Mangla M, Kaur H, Khoiwal K. Partial mole with coexistent live fetus: A systematic review of case reports. J Turk Ger Gynecol Assoc 2022; 23: 83-94.
2. Brooks T, Nolting L. Cutaneous manifestation of metastatic infantile choriocarcinoma. Case Rep Pediatr 2014; 2014: 104652.
3. De Lucia DR, Castaldo A, D'Agostino V, Ascione R, Pesce I, Coppola L, et al. Metastatic choriocarcinoma with hemorrhagic complications and spontaneous ovarian hyperstimulation syndrome: A case report. Radiol Case Rep 2021; 16: 3868-74.
4. Sebire NJ, Lindsay I, Fisher RA, Seckl MJ. Intraplacental choriocarcinoma: experience from a tertiary referral center and relationship with infantile choriocarcinoma. Fetal Pediatr Pathol 2005; 24: 21-9.
5. Blohm ME, Calaminus G, Gnekow AK, Heidemann PH, Bolkenius M, Weinel P, et al. Disseminated choriocarcinoma in infancy is curable by chemotherapy and delayed tumour resection. Eur J Cancer 2001; 37: 72-8.
6. Witzleben CL, Bruninga G. Infantile choriocarcinoma: a characteristic syndrome. J Pediatr 1968; 73: 374-8.
7. Jiao L, Ghorani E, Sebire NJ, Seckl MJ. Intraplacental choriocarcinoma: Systematic review and management guidance. Gynecol Oncol 2016; 141: 624-31.
8. Mercer RD, Lammert AC, Anderson R, Hazard JB. Choriocarcinoma in mother and infant. J Am Med Assoc 1958; 166: 482-3.
9. Hanson D, Walter AW, Dunn S, Rittenhouse DW, Griffin G. Infantile choriocarcinoma in a neonate with massive liver involvement cured with chemotherapy and liver transplant. J Pediatr Hematol Oncol 2011; 33: e258-60.
10. Sashi R, Sato K, Hirano H, Tomura N, Watarai J, Ishida A, et al. Infantile choriocarcinoma: a case report with MRI, angiography and bone scintigraphy. Pediatr Radiol 1996; 26: 869-70.
11. Andreitchouk AE, Takahashi O, Kodama H, Morita M, Sankawa T, Saito K, et al. Choriocarcinoma in infant and mother: a case report. J Obstet Gynaecol Res 1996; 22: 585-8.
12. Avril MF, Mathieu A, Kalifa C, Caillou C. Infantile choriocarcinoma with cutaneous tumors. An additional case and review of the literature. J Am Acad Dermatol 1986; 14: 918-27.
13. Bolze PA, Weber B, Fisher RA, Seckl MJ, Golfier F. First confirmation by genotyping of transplacental choriocarcinoma transmission. Am J Obstet Gynecol 2013; 209: e4-6.
14. Flam F, Lundström V, Silfverswärd C. Choriocarcinoma in mother and child. Case report. Br J Obstet Gynaecol 1989; 96: 241-4.
15. Rzanny-Owczarzak M, Sawicka-Metkowska J, Jończyk-Potoczna K, Gowin E, Sosnowska-Sienkiewicz P, Mańkowski P, et al. Simultaneous Occurrence of Choriocarcinoma in an Infant and Mother. Int J Environ Res Public Health 2021; 18: 1934.
16. Liu J, Guo L. Intraplacental choriocarcinoma in a term placenta with both maternal and infantile metastases: a case report and review of the literature. Gynecol Oncol 2006; 103: 1147-51.
17. Tsukamoto N, Matsumura M, Matsukuma K, Kamura T, Baba K. Choriocarcinoma in mother and fetus. Gynecol Oncol 1986; 24: 113-9.
18. Buckell EW, Owen TK. Chorionepithelioma in mother and infant. J Obstet Gynaecol Br Emp 1954; 61: 329-30.
19. Kruseman AC, van Lent M, Blom AH, Lauw GP. Choriocarcinoma in mother and child, identified by immunoenzyme histochemistry. Am J Clin Pathol 1977; 67: 279-83.
20. Mosayebi Z, Movahedian AH. Infantile and Maternal Choriocarcinoma : A Case Report and Review of Literature 2016; 7: 7-10.
21. McNally OM, Tran M, Fortune D, Quinn MA. Successful treatment of mother and baby with metastatic choriocarcinoma. Int J Gynecol Cancer 2002; 12: 394-8.
22. Heath JA, Tiedemann K. Successful management of neonatal choriocarcinoma. Med Pediatr Oncol 2001; 36: 497-9.
23. Daamen CB, Bloem GW, Westerbeek AJ. Chorionepithelioma in mother and child. J Obstet Gynaecol Br Emp 1961; 68: 144-9.
24. Aozasa K, Ito H, Kohro T, Ha K, Nakamura M, Okada A. Choriocarcinoma in infant and mother. Acta Pathol Jpn 1981; 31: 317-22.
25. Fraser GC, Blair GK, Hemming A, Murphy JJ, Rogers P. The treatment of simultaneous choriocarcinoma in mother and baby. J Pediatr Surg 1992; 27: 1318-9.
26. Getrajdman J, Kolev V, Brody E, Chuang L. Case of maternal and infantile choriocarcinoma following normal pregnancy. Gynecol Oncol Case Rep 2012; 2: 102-4.
27. Kishkurno S, Ishida A, Takahashi Y, Arai H, Nakajima W, Abe T, et al. A case of neonatal choriocarcinoma. Am J Perinatol 1997; 14: 79-82.
28. Picton SV, Bose-Haider B, Lendon M, Hancock BW, Campbell RH. Simultaneous choriocarcinoma in mother and newborn infant. Med Pediatr Oncol 1995; 25: 475-8.
29. Monclair T, Abeler VM, Kaern J, Walaas L, Zeller B, Hilstrøm C. Placental site trophoblastic tumor (PSTT) in mother and child: first report of PSTT in infancy. Med Pediatr Oncol 2002; 38: 187-91; discussion 92.
30. Greaves M, Hughes W. Cancer cell transmission via the placenta. Evol Med Public Health 2018; 2018: 106-15.
31. Smith LH, Danielsen B, Allen ME, Cress R. Cancer associated with obstetric delivery: results of linkage with the California cancer registry. Am J Obstet Gynecol 2003; 189: 1128-35.
32. Arakawa A, Ichikawa H, Kubo T, Motoi N, Kumamoto T, Nakajima M, et al. Vaginal Transmission of Cancer from Mothers with Cervical Cancer to Infants. N Engl J Med 2021; 384: 42-50.
33. Herskovic E, Ryan M, Weinstein J, Wadhwani NR. Maternal to fetal transmission of cervical carcinoma. Pediatr Radiol 2014; 44: 1035-8.
34. Lapaire O, Holzgreve W, Oosterwijk JC, Brinkhaus R, Bianchi DW. Georg Schmorl on trophoblasts in the maternal circulation. Placenta 2007; 28: 1-5.
35. Tews G, Yaman C, Ebner T. Fatal trophoblastic embolism during cesarean section. Int J Gynaecol Obstet 2002; 76: 179-80.
36. Kamoi S, Ohaki Y, Mori O, Satomi M, Takahashi H, Kawamura T, et al. Placental villotrophoblastic pulmonary emboli after elective abortion: immunohistochemical diagnosis and comparison with ten control cases. Int J Gynecol Pathol 2003; 22: 303-9.
37. Gehrig PA, Van Le L. Gestational trophoblastic disease: An update. Curr Probl Obstet Gynecol Fertil 2002; 25: 150-68.
38. Mangla M, Singla D, Kaur H, Sharma S. Unusual clinical presentations of choriocarcinoma: A systematic review of case reports. Taiwan J Obstet Gynecol 2017; 56: 1-8.
39. Fisher RA, Savage PM, MacDermott C, Hook J, Sebire NJ, Lindsay I, et al. The impact of molecular genetic diagnosis on the management

of women with hCG-producing malignancies. Gynecol Oncol 2007; 107: 413-9.

40. Froeling FEM, Ramaswami R, Papanastasopoulos P, Kaur B, Sebire NJ, Short D, et al. Intensified therapies improve survival and identification of novel prognostic factors for placental-site and epithelioid trophoblastic tumours. Br J Cancer 2019; 120: 587-94.
41. Schmid P, Nagai Y, Agarwal R, Hancock B, Savage PM, Sebire NJ, et al. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: a retrospective observational study. Lancet 2009; 374: 48-55.
42. Kiserud T, Rasmussen S, Skulstad S. Blood flow and the degree of shunting through the ductus venosus in the human fetus. Am J Obstet Gynecol 2000; 182: 147-53.
43. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. Lancet 2010; 376: 717-29.
44. Lok CA, Ansink AC, Grootfaam D, van der Velden J, Verheijen RH, ten Kate-Booij MJ. Treatment and prognosis of post term choriocarcinoma in The Netherlands. Gynecol Oncol 2006; 103: 698-702.
45. Ma Y, Xiang Y, Wan XR, Chen Y, Feng FZ, Lei CZ, et al. The prognostic analysis of 123 postpartum choriocarcinoma cases. Int J Gynecol Cancer 2008; 18: 1097-101.
46. Göbel U, Calaminus G, Schneider DT, Schmidt P, Haas RJ; MAKEI and MAHO Study Groups of the German Society of Pediatric Oncology and Hematology, and the SIOP CNS GCT Study Group. Management of germ cell tumors in children: approaches to cure. Onkologie 2002; 25: 14-22.
47. FIGO Committee on Gynecologic Oncology. Current FIGO staging for cancer of the vagina, fallopian tube, ovary, and gestational trophoblastic neoplasia. Int J Gynaecol Obstet 2009; 105: 3-4.
48. Jones WB, Lewis JL Jr. Late recurrence of gestational trophoblastic disease. Gynecol Oncol 1985; 20: 83-91.
49. Das JK, Lalmuanawmi C, Devi YS, Singh LJ. Acute myeloid leukemia following chemotherapy for gestational trophoblatic neoplasia. J Obstet Gynaecol India 2014; 64: 55-6.

What is your diagnosis?

A 55 year-old, post-menopausal woman presented to the gynecology outpatient department with a complaint of gradual, painless distension of the abdomen over the preceding three months. She also reported that for the last six months, her husband had complained of severe pain in the penile region after intercourse. The husband had undergone a complete genito-urinary examination, which was completely normal. There was no history of post-menopausal or post-coital bleeding. There was no history of shortness of breath, loss of appetite or weight, or bowel and bladder disturbances. The patient had become menopausal 15 years earlier, and she was not on any hormone replacement therapy. Her obstetric history revealed that she had two pregnancies, both of which were conceived spontaneously without any history of infertility treatment. Her last childbirth was 25 years earlier. She was hypertensive, treated with amlodipine 5 mg once daily for the previous two years. There was no history of any other chronic medical illness in the past.

The patient was conscious and coherent on examination, moderately built with stable vitals, and general and systemic examinations were unremarkable. Per-abdominal examination revealed a cystic, abdominopelvic mass of 22-24 weeks gravid uterus, occupying the hypogastrium, right and left iliac fossa, with restricted mobility. It had a smooth surface and regular borders. Fluid thrill was present, but there was no shifting dullness. Per speculum examination, the cervix was flushed with the vagina and taken up, and vaginal walls appeared atrophic and pale. On bimanual examination, the uterus was normal in size, and the same cystic mass of approximately 20x20x10 cm was palpated; it was not fixed to the uterus or pelvic side wall. These findings were reconfirmed on rectal examination. Rectal mucosa, recto-vaginal septum, and parametrium were healthy on P/V/R examination. Blood test results for tumor markers were CA-125 16 U/mL and CA-19-9 4.6 U/mL. Lactate dehydrogenase, carcinoembryonic antigen, alpha-fetoprotein and human chorionic gonadotropin were within normal limits.

A radiologist with more than 15 years of experience in gynecologic ultrasound (USG) performed a transabdominal USG scan. An irregular, lobulated, unilocular cystic lesion measuring 184x104 mm was found, arising from the left adnexa and extending into the lower abdomen. The lesion had thin walls with echogenic mobile contents. An incomplete, thin septation was present showing minimal vascularity (CS-2). There were no solid components. The inner margins appeared irregular, with focal wall thickening. There was no evidence of ascites or para-aortic lymphadenopathy. Neither ovary could be visualized. The uterus was unremarkable (Figure 1). All these features favored a lesion of intermediate risk (Ovarian-Adnexal Reporting Data System USG score 4). The patient was referred for cross-sectional imaging for better characterization and detection of additional findings. A contrast-enhanced computed tomography of the abdomen was performed. It revealed a large (220x180x120 mm) lobulated, cystic lesion with a thin septation, arising from the left adnexa and extending into the abdomen. The wall of the lesion showed focal areas of thickening with enhancement. Enhancement of the septation was present. Two enhancing papillary projections (13x10 mm) were noted in the posterior wall of the lesion. There was no ascites or para-aortic lymphadenopathy. The right ovary and uterus were normal but the left ovary was not separately identifiable. Features favored a left ovarian cystic mass lesion of intermediate concern (Figure 2). A pap smear was negative for malignant cells. USG of breast and upper and lower gastrointestinal endoscopies were unremarkable.

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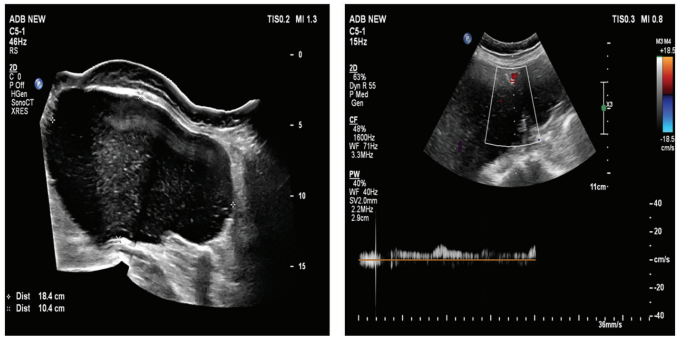

Figure 1. Greyscale ultrasound image of the pelvis showing an irregular, lobulated, unilocular cystic lesion measuring 184x104 mm, arising from the left adnexa extending into the lower abdomen. Note the presence of thin walls with echogenic mobile contents. An incomplete thin septation showing minimal vascularity (CS-2) was also present

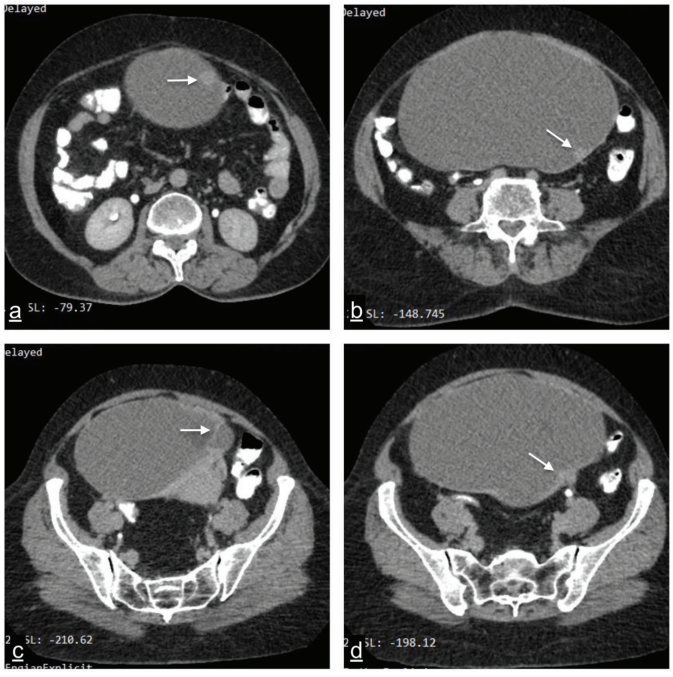

Figure 2. CECT images show a large (220x180x120 mm) lobulated cystic lesion with a thin septation in the left adnexa extending into the abdomen. The wall of the lesion showed focal areas of thickening with enhancement. Enhancement of the septation was present. Two enhancing papillary projections (13x10 mm) were noted in the posterior wall of the lesion

CECT: Contrast-enhanced computed tomography

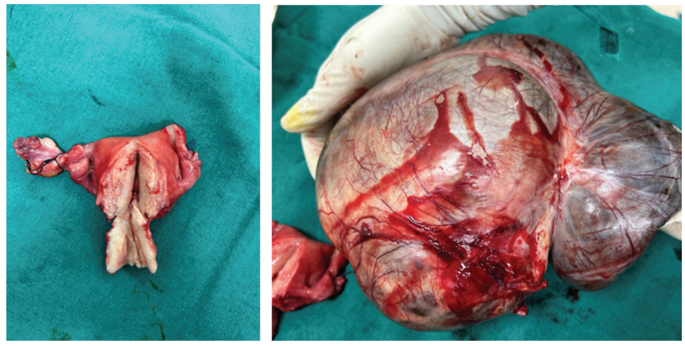

Figure 3. Intraoperative images showing normal uterus and right adnexa and 20x18x10 cm, uninoculated left ovarian cyst with fallopian tube stretched over it

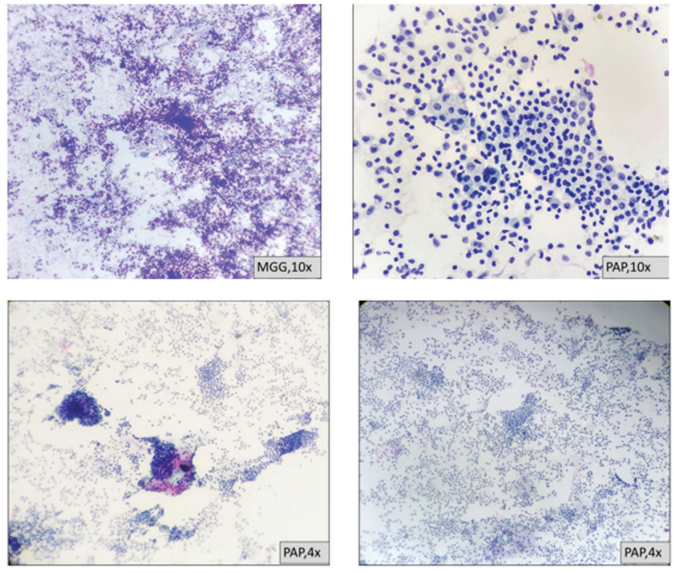

Figure 4. Ascitic fluid cytology negative for malignant cells

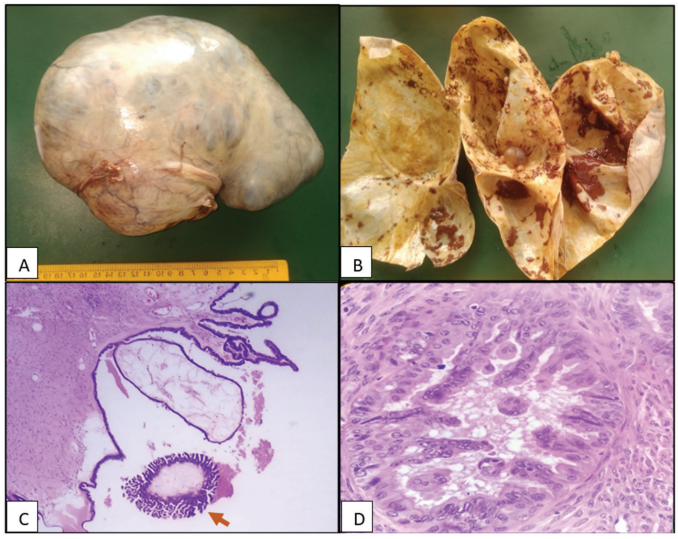

Figure 5. Large cystic lesion of the ovary with a smooth external surface (A) with unilocular cyst with a few papillary excrescences (B). Microscopically elongated "snakes" of epithelial cells without fibrovascular cores arising from papillae, resembling a Medusa-head (C). Tufted micropapillae in the large bulbous papillary structure (D). C and D are magnified at x100 and x400, respectively

Answer

A staging laparotomy was performed through a midline vertical incision. There was minimal straw-colored peritoneal fluid present which was sent for cytopathological examination. A left ovarian, unilocular, cystic mass, measuring 20x18x11 cm and weighing 2.25 kg, with an intact capsule and tube stretching over it was seen occupying the pelvic cavity (Figure 3). The uterus was average size and the right fallopian tube and ovary were healthy. There were no suspicious areas on the omentum, liver, or under the surface of the diaphragm. Total abdominal hysterectomy was performed with bilateral salpingo-oophorectomy (BSO) with pelvic and para-aortic lymphadenectomy and omental and peritoneal biopsy.

A pathologist with more than ten years experience in gynecologic pathology performed the histopathologic examination. The ascitic fluid was negative for malignant cells (Figure 4). Gross analysis of the ovary showed a thin-walled, cystic lesion with a smooth outer surface. The cut section showed an uninoculated cyst filled with viscous hemorrhagic fluid with incomplete septations and multiple papillary excrescences studded on the inner aspect of the wall (Figure 5). No solid component was noted. Histology showed that a single-layered, tubal columnar epithelium lined the cyst. The papillary growths were entirely exophytic, with no invasion at the base of the fronds. Micropapillae arose from the large bulbous papillary structures, lacking a fibrovascular core and were entirely comprised of large eosinophilic cells with distinct cell borders. The height of these micropapillary structures was more than five times the width. There was no evidence of stromal invasion; nuclear atypia and mitosis were inconspicuous. There were no macroscopic or microscopic implants over the fallopian tubes or on the contralateral ovary. Omentum, lymph nodes, and peritoneum were free of tumor. Based on the characteristic micropapillary pattern, the diagnosis of micropapillary serous carcinoma was made, which is a subset of borderline serous tumors, pT1aN0M0. The patient refused any further molecular or genetic testing due to economic constraints. The patient has now been on regular follow-up with pelvic examination, transvaginal USG, and monitoring of CA-125. There is no evidence of recurrence at the time of writing.

Discussion

With an annual prevalence of 1.8-4.8/100,000, borderline ovarian tumors (BOTs) account for approximately 15% of all epithelial ovarian cancers (1,2). BOTs were first described in 1929 by Taylor (3) as a "semi-malignant ovarian tumor". In 1971, the International Federation of Obstetrics and Gynecology identified BOTs as a "low-grade malignant tumor" completely different from ovarian cancer. In the World Health Organization classification of female genital tumors in 2014, the word "low-grade malignant tumor" was replaced by "borderline tumor" or "atypical proliferative tumor" (4).

BOTs are enigmatic neoplasms, and apprehension in the patient and the treating doctor is understandable. Although USG, in combination with color and power Doppler, is one of the best imaging modalities in differentiating benign from malignant ovarian masses with a specificity reaching up to 90% in expert hands (5), its ability to accurately diagnose an adnexal mass as borderline is limited. The sonographic appearance of BOTs range from unilocular cysts to masses with solid and fluid components, and papilla formation is typical (6,7). A retrospective study analyzing 383 ovarian tumors, including 27 borderline ones, found that BOTs have more similarity on USG to malignant lesions (absence of anechoic pattern, presence of diffuse internal echoes, and intra-cystic papillae) than to benign tumors (absence of septa, absence of solid or heterogeneous pattern) (8). However, papillary projections are known to be the most typical USG features of non-invasive (borderline and low-grade) malignant serous tumors. In contrast, solid components but no papillations favor invasive disease (9,10). Studies have shown that USG holds promise for differentiating varieties of BOTs, serous borderline ovarian tumors (SBOTs) from mucinous borderline ovarian tumors (MBOTs). Fruscella et al. (11) found that SBOTs and endocervical-type MBOTs had very similar sonographic features and usually presented as unilocular-solid lesions with a higher color score than intestinal-type MBOTs. Intestinal-type MBOTs were usually multilocular (with >10 locules) when compared with endocervical-type

MBOTs (11). The value of computed tomography and magnetic resonance imaging features in differentiating BOTs from malignant tumors is also relatively limited. Still, characteristics, such as papillary growth pattern with internal branching, higher signal intensity on T2-weighted images, and higher apparent diffusion coefficient values may be considered characteristic features of solid components in BOTs (12-14).

The predominant treatment is surgery. For patients who do not desire future fertility, complete resection with surgical staging, including total hysterectomy and BSO, peritoneal washing, omentectomy, and resection of grossly visible metastases, is the surgery of choice. As these tumors tend to occur in a younger age group, fertility-sparing surgery, rather than complete surgical staging, may be considered for patients desiring to maintain future fertility, as this has shown favorable outcomes in recurrence and disease-free survival (15). However, the National Comprehensive Cancer Network advises considering completion surgery after childbearing for patients with a remaining ovary (16). Routine lymphadenectomy and chemotherapy do not have a significant role in managing BOTs. The studies regarding adjuvant chemotherapy have contradictory results, with some showing benefits while others showing none.

Micropapillary features in BOTs are associated with an increased likelihood of invasive peritoneal implants, lymph node metastasis, and recurrence. However, using this as an independent factor to predict survival in BOTs remains controversial (12,13). Our patient did not have any risk factors for the development of BOT, as there was no history of intake of either ovulation-inducing drugs or hormone replacement therapy. The prognosis of BOTs, even at the advanced stage, is usually good, with a 5-year survival of more than 75%, even at stage 4 (17). The risk of malignant transformation is still unclear, and progression to invasive cancer may represent true transformation or even de novo development of ovarian or peritoneal cancer.

BOT presenting as asymptomatic ovarian cyst is not uncommon. The present case was, however, unique as the chief complaint was dyspareunia in the sexual partner. Since the genito-urinary examination of the husband was completely normal, the most probable cause was the ovarian cyst, bulging into the vagina, causing difficulty in penetration during intercourse. The present case is probably the first reported case of BOT presenting as dyspareunia for the sexual partner. Publishing such rare clinical features of a relatively common condition is essential to make clinicians aware that patients can sometimes present with these unusual presenting signs.

Borderline tumors pose a diagnostic challenge as they lack characteristic radiologic criteria compared with benign or malignant tumors. In this case, the patient was a post-menopausal female and thus the decision to undertake a complete staging laparotomy with BSO was straightforward. The dilemma of deciding the best treatment and surgery arises if the same tumor occurs in a young, reproductive age woman. A preoperative suspicion that the tumor may be borderline could be beneficial for fertility preservation. Healthcare providers should also be aware that specific histopathologic subtypes, such as micro-papillary variants, as in the presented case, are associated with an increased risk of recurrence and therefore determine poorer prognosis. Such patients should be monitored closely with regular comprehensive follow-up.

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References

1. Skírnisdóttir I, Garmo H, Wilander E, Holmberg L. Borderline ovarian tumors in Sweden 1960-2005: trends in incidence and age at diagnosis compared to ovarian cancer. Int J Cancer 2008; 123: 1897-901.
2. Akeson M, Zetterqvist BM, Dahllöf K, Jakobsen AM, Brännström M, Horvath G. Population-based cohort follow-up study of all patients operated for borderline ovarian tumor in western Sweden during an 11-year period. Int J Gynecol Cancer 2008; 18: 453-9.
3. Taylor HC. Malignant and semi-malignant tumours of the ovary. Surg Gynecol Obstet 1929; 48: 204-30.
4. Hauptmann S, Friedrich K, Redline R, Avril S. Ovarian borderline tumors in the 2014 WHO classification: evolving concepts and diagnostic criteria. Virchows Arch 2017; 470: 125-42.
5. Daraï E, Fauvet R, Uzan C, Gouy S, Duvillard P, Morice P. Fertility and borderline ovarian tumor: a systematic review of conservative management, risk of recurrence and alternative options. Hum Reprod Update 2013; 19: 151-66.
6. Valentin L. Use of morphology to characterize and manage common adnexal masses. Best Pract Res Clin Obstet Gynaecol 2004; 18: 71-89.
7. Valentin L, Ameye L, Testa A, Lécuru F, Bernard JP, Paladini D, et al. Ultrasound characteristics of different types of adnexal malignancies. Gynecol Oncol 2006; 102: 41-8.
8. Pascual MA, Tresserra F, Grases PJ, Labastida R, Dexeus S. Borderline cystic tumors of the ovary: gray-scale and color Doppler sonographic findings. J Clin Ultrasound 2002; 30: 76-82.
9. Moro F, Baima Poma C, Zannoni GF, Vidal Urbinati A, Pasciuto T, Ludovisi M, et al. Imaging in gynecological disease (12): clinical and ultrasound features of invasive and non-invasive malignant serous ovarian tumors. Ultrasound Obstet Gynecol 2017; 50: 788-99.

10. Ludovisi M, Foo X, Mainenti S, Testa AC, Arora R, Jurkovic D. Ultrasound diagnosis of serous surface papillary borderline ovarian tumor: A case series with a review of the literature. J Clin Ultrasound 2015; 43: 573-7.
11. Fruscella E, Testa AC, Ferrandina G, De Smet F, Van Holsbeke C, Scambia G, et al. Ultrasound features of different histopathological subtypes of borderline ovarian tumors. Ultrasound Obstet Gynecol 2005; 26: 644-50.
12. Yang S, Tang H, Xiao F, Zhu J, Hua T, Tang G. Differentiation of borderline tumors from type I ovarian epithelial cancers on CT and MR imaging. Abdom Radiol (NY) 2020; 45: 3230-8.
13. deSouza NM, O'Neill R, McIndoe GA, Dina R, Soutter WP. Borderline tumors of the ovary: CT and MRI features and tumor markers in differentiation from stage I disease. AJR Am J Roentgenol 2005; 184: 999-1003.
14. Kawaguchi M, Kato H, Hatano Y, Tomita H, Hara A, Suzui N, et al. MR imaging findings of low-grade serous carcinoma of the ovary: comparison with serous borderline tumor. Jpn J Radiol 2020; 38: 782-9.
15. Daraï E, Fauvet R, Uzan C, Gouy S, Duvillard P, Morice P. Fertility and borderline ovarian tumor: a systematic review of conservative management, risk of recurrence and alternative options. Hum Reprod Update 2013; 19: 151-66.
16. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. Ovarian Cancer, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2021; 19: 191-226.
17. Trimble CL, Kosary C, Trimble EL. Long-term survival and patterns of care in women with ovarian tumors of low malignant potential. Gynecol Oncol 2002; 86: 34-7.

No birth sex ratio difference between Mexican and non-Mexican births in Mexico

To the Editor,

The sex ratio at birth is expected to be approximately 0.515, calculated as male births divided by total births (M/T), thus a slight excess of males. Acute stress in the general population may cause M/T depressions, as evidenced by the M/T dip observed after the Great Recession of 2007 in the United States (1). Significant racial differences have been noted and attributed to innate and minor physiological differences, but chronic stress has been proposed as a possible cause (2). A recent paper showed that M/T was depressed in Mexico when compared to a global reference dataset (3,4). This study was carried out in order to ascertain whether Mexican M/T was similar to M/T in non-Mexican births in the same study cohort (4).

Ethical approval was not required as data was comprised of free and anonymous datasets from the Instituto Nacional de Estadística y Geografía, Mexico's National Institute of Statistics and Geography. For the same reason, informed consent was not obtained. Live births by sex, year and nationality [Mexican or non-Mexican ("Extranjero")] were available for the period January 2010-December 2020.

The equations of Fleiss (binomial) were used to calculate 95% confidence intervals (CI) for proportions. A bespoke Excel sheet was used to perform chi square tests. A p-value <0.05 was taken to represent a statistically significant result. Mexican and non-Mexican births by sex, and M/T with 95% CIs are shown in Table 1. There was no significant M/T difference between the two groups. While racial disparities in M/T could theoretically be caused by innate physiological differences, it is also possible that the differences seen may be due to chronic stress (2). Support for this comes from a comparison of racial M/T in the United States which showed that M/T was higher in Whites than in American Indian/Alaska Native, and Black/African American births (5). This was probably due to the fact that in the United States, race remains the primary determinant of socioeconomic status and stress. This accords with the Trivers-Willard hypothesis of male foetal loss in this type of stress (2).

Table 1. Mexican and non-Mexican births by sex, and M/T with 95% confidence intervals

	Mexican	Non-Mexican	Total
M	13,327,784	52,711	13,380,495
F	13,006,783	51,609	13,058,392
Total	26,334,567	104,320	26,438,887
UCI	0.5063	0.5083	0.5063
M/T	0.5061	0.5053	0.5061
LCI	0.5059	0.5022	0.5059

M: Male, F: Female, T: Total, UCI: Upper incidence interval, LCI: Lower incidence interval

This study indirectly supports the hypothesis that innate physiological differences do not appear to affect M/T, as there were no significant differences between the low Mexican M/T and non-Mexican M/T. However, the study was limited by small numbers and a lack of breakdown of what races or ethnicities constituted the non-Mexican births.

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References

1. Grech V. The Great Recession of 2007 in the United States and the male: female ratio at birth. J Turk Ger Gynecol Assoc 2015; 16: 70-3.
2. Grech V. A socio-economic hypothesis for lower birth sex ratios at racial, national and global levels. Early Hum Dev 2018; 116: 81-3.
3. Grech V, Mamo J. What is the sex ratio at birth? Early Hum Dev 2020; 140: 104858.
4. Grech V. Regional differences in the sex ratio at birth in Mexico. J Turk Ger Gynecol Assoc 2023; 24: 1-4.
5. Grech V. Evidence of socio-economic stress and female foeticide in racial disparities in the gender ratio at birth in the United States (1995-2014). Early Hum Dev 2017; 106-107: 63-5.

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Vaginal malignant melanoma: surgical challenge and need for combination treatment

To the Editor,

We read the article entitled "Laparoscopic radical hysterectomy and total vaginectomy for vaginal malignant melanoma with cervical metastasis" by Vardar et al. (1) with a great deal of interest. The authors present a step-by-step surgical approach for such a challenging case. Mucosal melanomas are a rare type of melanomas in comparison to cutaneous melanomas (2). For this reason, optimal management of locally advanced vaginal melanoma is poorly understood, including exenteration procedures, adjuvant radiotherapy, chemotherapy and immunotherapy with agents such as ipilimumab or nivolumab (3).

A recent systematic review analyzed 15 patients with vaginal melanoma and reported that, in contrast to cutaneous melanoma, there were fewer BRAF mutations and more PI3K/AKT/mTOR pathway alterations, and so early stage and surgical extirpation affected the prognosis (4). However, a combination treatment including application of immune checkpoint inhibitors, radiotherapy and/or anti-angiogenic therapy, may have a synergistic effect in the treatment of patients with advanced vaginal melanoma (5). Moreover, a recent large cohort study from MD Anderson Cancer Center found that mitotic rate >10/mm^2, nodal involvement and non-vulvar anatomic subsite were related to poor outcomes, independently of the combination of treatment (6). More specifically, the study reports 46% local control, 53% nodal control, 36% distant metastasis-free survival, 49% melanoma-specific survival and 48% overall survival (6).

Once again, we would like to congratulate the authors for their excellent anatomic approach/surgery and for raising awareness of such a rare entity.

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References

1. Vardar MA, Khatib G, Güzel AB, Küçükgöz Güleç Ü, Mısırlıoğlu M. Laparoscopic radical hysterectomy and total vaginectomy for vaginal malignant melanoma with cervical metastasis. J Turk Ger Gynecol Assoc 2023; 24: 84-5.
2. Musheyev Y, Fazylov M, Abramowitz C, Ikhuoriah TA, Rogu P, Levada M. Melanoma in the Vulva of a 71-Year-Old Patient: A Case Report. Cureus 2022; 14: e32698.
3. Tarhini AA, Hanayneh WB, Powers JJ, Segura CMM, Conejo-Garcia JR, Lam CA, et al. Case Report: Durable complete pathologic response and organ preservation following ipilimumab and nivolumab for locally advanced primary vaginal mucosal melanoma. Front Oncol 2022; 12: 1044587.
4. Khayyat A, Esmaeil Pour MA, Mousavi S, Khalili-Toosi AR, Amin A. Primary Malignant Melanoma of the Genitourinary System: A Systemic Review and Report of Eight Cases. Cureus 2022; 14: e30444.
5. Yin P, Ma X, Zhang Y, Song Y, Wang Y, Lu Z. Primary vaginal malignant melanoma successfully treated with combination therapy: A case report. Oncol Lett 2022; 24: 430.
6. Mitra D, Farr M, Nagarajan P, Ho J, Bishop AJ, Jhingran A, et al. Gynecologic tract melanoma in the contemporary therapeutic era: High rates of local and distant disease progression. Gynecol Oncol 2022; 167: 483-9.

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Author's Response

Dear Editor,

The best method to increase our knowledge about rare diseases is sharing data and information. Therefore, we would like to thank Iavazzo et al. for their valuable contribution, and literature-based details about this rare disease.

Yours sincerely,

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:
https://www.emedevents.com/obstetrics-and-gynecology)

September 10-13, 2023	International Federation of Fertility Societies (IFFS) World Congress, Athens, Greece
October 01-04, 2023	European Society for Gynaecological Endoscopy (ESGE) 32nd Annual Congress, Brussels, Belgium
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:
https://www.kongreuzmani.com/2023)

September 15-17, 2023	1. Akdeniz Jinekoloji ve Obstetrik Kongresi, Adana, 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