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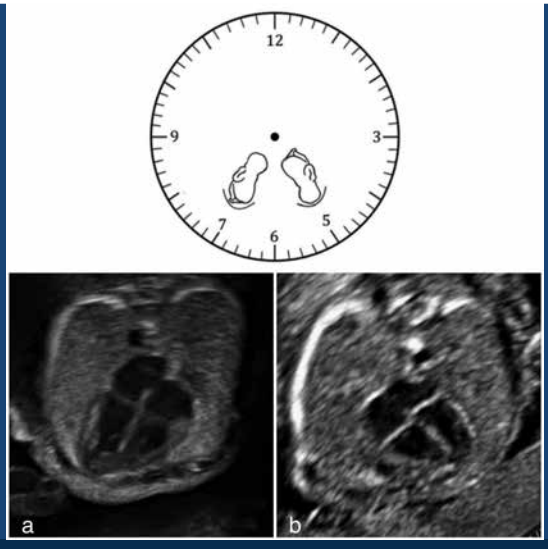

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Cover Picture: Sezgin Dursun and Fatih Aktoz. Clock method to determine fetal heart axis

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Contents

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Editorial

Dear Colleagues,

I am delighted to introduce the third issue of the "Journal of the Turkish-German Gynecological Association *(J Turk Ger Gynecol Assoc)*" in the publishing year of 2020.

As you know, vaginal microbiota are complex ecosystems of more than 200 bacterial species affected by many factors. The lactobacilli dominating the healthy vagina support a defense system. Here you will read a paper investigating the association of microbiota and human papilloma virus.

You will also read an interesting paper investigating the effectiveness of pegylated liposomal doxorubicin (PLD), beta-carotene, and PLD + beta-carotene combination therapy in two different human choriocarcinoma cell lines. PLD seems to be a promising chemotherapeutic drug for treatment of choriocarcinoma. Also you will get the occasion to read a new technique for determining the axis of the fetal heart; "Clock position method" which may be a good option for clinicians at every level of experience.

You will also watch a demonstrative video describing the essential steps to perform a transvaginal adnexal abscess drainage with a step-by-step explanation.

Dear authors and reviewers,

Journals are beeing evaluated for at least 75 years. After invention of the journal "impact factor (IF)", editors and publishers use it for academic evaluation. It is used to measure the importance or rank of a journal by calculating the times it's articles are cited. According to the 2020 Clarivate Analytics reports, the estimated impact factor of J Turk Ger Gynecol Assoc is 1.04 in 2019, which reflects 12% increase compared to last year (IF=0.92).

We ensure the quality and wide dissemination of obstetrics and gynecological knowledge - through robust review, and the publication of high quality researchs and reviews. Our journal has become an important platform by the support of researchers and reviewers. We all thank you in advance,

Best regards,

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

Dr. Dirk Wildemeersch

MD, PhD

1944 - 2018

Dr. Dirk Wildemeersch MD, Phd (1944-2018) gynecologist, scientist and the inventor of the frameless IUDs: Gynefix, GYN-CS, ReLARC, Fibroplant and Femilis CU & Femilis LNG (https://www.wildemeersch.com/products/).

Born in Belgium, he completed his medical training at the University of Ghent and graduated at the end of his internship in South Africa at Verwoerd University hospital in Pretoria and Tygerberg university hospital in Cape Town after which he started his gynecology practice. In 1985, he commenced his research activities aimed to manage, develop and study innovative delivery technologies. His research goal was to find improved methods for prevention and treatment of gynaecological conditions, improvements to birth control methods and higher levels of safety, user acceptability and compliance. It was his mission to provide women with gynaecological solutions of the highest possible level of comfort, safety and quality of life, for which he continued researching new developments and technologies until he passed away in November 2018.

In 2015, Dr. Wildemeersch started a collaboration with several doctors and professors in Turkey. He gave presentations and training sessions to Turkish doctors and Universities in İstanbul, Ankara, Antalya and İzmir, amongst others. He also attended several congresses throughout Turkey.

Starting from 2016 until the present day, there are several clinical trials for his IUDs (Gazi University Ankara, Zeynep Kamil University Hospital, ...). Some of these studies have already gathered quite a bit of international interest with their publications:
Comparison of expulsions following intracesarean placement of an innovative frameless copper-releasing IUD (Gyn-CS) versus the TCu380A: A randomized trial., Clinical experience with a novel anchored frameless copper-releasing contraceptive device (Gyn-CS) for intracesarean insertion to prevent displacement and expulsion - A 3-month study.

Expulsion rate of intrauterine device: mediate vs. immediate puerperium period

Juliano Terra Hochmuller[1], Karina Souza Lopes[1], Cristina Aparecida Falbo Guazzelli[2], Mariana Kefalas Oliveira Gomes[3], Edward Araujo Júnior[2,4], Alberto Borges Peixoto[1,3]

[1]Mário Palmério University Hospital, University of Uberaba (UNIUBE), Uberaba-MG, Brazil

[2]Department of Obstetrics, Paulista School of Medicine - Federal University of São Paulo (EPM-UNIFESP), São Paulo-SP, Brazil

[3]Department of Obstetrics and Gynecology, Federal University of Triângulo Mineiro (UFTM), Uberaba-MG, Brazil

[4]Medical course, Municipal University of São Caetano do Sul (USCS), São Paulo-SP, Brazil

Abstract

Objective: To evaluate the rate of expulsion of intrauterine device (IUD) inserted during the immediate and mediate puerperium. To evaluate whether the type of delivery is a predictor of expulsion of the IUD when inserted in the puerperium period.

Material and Methods: This was a prospective observational study. Patients whose IUD (TCU-380 copper) was placed during the puerperal period were divided in two groups according to the time of placement: immediate and mediate puerperium. The decision regarding the time of IUD insertion was made in a non-randomized manner. Analysis was performed using chi-square, Mann-Whitney U, and Spearman's correlation tests and logistic regression analysis.

Results: The total rate of IUD expulsions was 28.8% (49/170). There was no significant association between the occurrence of expulsion and the timing of IUD insertion (immediate vs mediate puerperium; 26.6% vs 34.78%, p=0.296). Among patients experiencing expulsion, 79.6% (39/49) underwent insertion after vaginal delivery and 20.4% (10/49) during cesarean section (CS). The type of delivery was a significant predictor for IUD expulsion (p<0.0001). Vaginal delivery was fourfold more likely to be associated with IUD expulsion inserted in the puerperal period than CS (odds ratio: 4.23, 95% confidence interval: 1.94-9.25). There was no significant correlation between the period between IUD insertion and the diagnosis of expulsion in regard to number of pregnancies (r=-0.160, p=0.271) or gestational age at delivery (r=-0.058, p=0.939).

Conclusion: Vaginal delivery was the most prevalent type of delivery in patients who underwent IUD insertion during the immediate and mediate puerperium. The risk of IUD expulsion after vaginal delivery was greater than CS. (J Turk Ger Gynecol Assoc 2020; 21: 143-9)

Keywords: Intrauterine device, contraception, puerperium, delivery, expulsion, postpartum

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Introduction

The postpartum period is marked by a transition for the woman and her family, during which the new mother experiences physical adjustments as she returns to her prepregnancy state and psychosocial changes as a result of the presence of a new family member (1). Currently, the World Health Organization recommends and emphasizes early medical follow-up during this period, with the goal of preventing and reducing neonatal and maternal morbidity in this stage of the female reproductive cycle (2,3). One concern during the postpartum period is the possible occurrence of a new pregnancy in a short period of time, which may not only cause maternal-fetal complications but may also have serious psychological, social, and economic repercussions.

Pregnancy and the postpartum period are appropriate times to discuss contraception, because there is an increased motivation for its use. This moment favors the patient-physician relationship and the evaluation of individual contraceptive needs. Physician guidance directly influences the woman's decision about the

Address for Correspondence: *Edward Araujo Júnior*
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use and type of contraception. Currently, some of the most commonly recommended options include long-term methods (e.g., long-acting reversible contraceptives), such as a copper or hormonal intrauterine device (IUD) and implant.

The copper IUD is one of the most widely used reversible contraceptive methods in the world, and it has an extremely low failure rate, with failure occurring in less than 1 in 100 women in the first year of use. It is indicated because of its ease of use, high efficiency, and association with safety and few side effects. One study showed that its use immediately after delivery was safe for both the mother and the newborn, with advantages including convenience and ease of insertion (3).

The literature indicates that the need for effective contraception in women immediately after delivery has been underestimated because, unfortunately, unplanned pregnancies can occur during this period. Recent research has shown that almost 50% of mothers return to having sex within six weeks of delivery, and many of these women do not use contraception (4).

Thus, there is a need for greater dissemination and understanding of the use of the IUD in the immediate postpartum period, as well as an assessment of its advantages and disadvantages. These questions encouraged us to analyze the use of this method in our practice.

Material and Methods

This was a prospective observational study conducted in the Gynecology and Obstetrics Sector, from June 2018 to September 2019. The study was approved by the local research ethics committee (CAAE: 20502719.5. 0000.5145). Patients whose IUD were placed during puerperal period were divided in two groups according to the time of placement: immediate puerperium and mediate puerperium. A signed consent form was obtained from all patients.

The inclusion criteria included pregnant women ≥18 years old who expressed a desire to insert the IUD in the puerperal period. The exclusion criteria were the following: 1) IUD insertion between >72 hours and less than four weeks after delivery; 2) chorioamnionitis; 3) rupture of membranes >18 hours; 4) HIV; 5) puerperal sepsis; 6) puerperal endometritis; 7) abnormal uterine bleeding with uninvestigated etiology; 8) Mullerian anomaly; 9) postpartum hemorrhage; and 10) extensive laceration of the vagina.

The decision regarding the timing of IUD insertion (immediate puerperium period: between 10 and 15 minutes after placental delivery and 48 hours after delivery; mediate puerperium period: between 48 hours and six weeks after delivery) was made in a non-randomized manner. We used the TCU-380 copper IUD, which was inserted by medical residents in gynecology and obstetrics (first, second, and third years). We defined partial expulsion as the protrusion of the IUD from the external cervical os or visualization on transvaginal ultrasound of the distal end of the IUD below the internal os of the cervix (5).

Insertion technique after vaginal delivery in anesthetized patients without the need for additional instruments

After uterine massage and prior to perineal repair, new sterile gloves were placed and oxytocin was administered [10 IU intravenously (iv)]. No specific antibiotic prophylaxis was used for the procedure. Subsequently, the IUD was removed from the insert. The IUD was placed between the index and middle fingers, and the opposite hand was inserted to stabilize the uterus externally. In the period between 10 and 15 minutes after placental delivery, the IUD was introduced until contact was made with the uterine fundus. To confirm that the IUD came into contact with the uterine fundus, the examiner used manual tactile perception through the placement of one hand on the uterine fundus. As the inner hand was being removed, a rotation of about 45 degrees clockwise or counterclockwise was performed, an act that was used to prevent the exteriorization of the IUD. Then, the threads were cut at the height of the external orifice of the cervix. The threads were visualized and trimmed on the follow-up visit four weeks after insertion. All cases in which the IUD wire was not visible inside the vaginal canal were referred for ultrasound examination to assess the position of the IUD. This technique was used in all patients who underwent vaginal delivery with peridural anesthesia.

Insertion technique after vaginal delivery in non-anesthetized patients using Foerster or De Lee Forceps

This technique was used posteriorly and consisted of putting on sterile gloves, uterine massage, perineal repair, and administration of antibiotic prophylaxis and oxytocin (10 IU iv) as routine. Immediately after these steps, the IUD was removed from the insert. The IUD was then captured using De Lee's Forceps, taking care not to activate the rack and to avoid damaging the copper, so that the sphere of the stem and the wires were parallel to the forceps. The upper tip of the IUD was placed flush with the tip end of the forceps. The wires were positioned away from the axis of the forceps, thus preventing them from becoming tangled or attached to the instrument when it was removed from the uterus. Then, using a hand or Doyan valve, the anterior lip of the cervix was exposed and visualized. A soft grip of the anterior lip of the cervix was performed using another De Lee's Forceps. The cervix was pulled slightly, the IUD was inserted under direct visualization. Soon after, the hand that pulled the cervix was repositioned on the abdomen to stabilize the uterine fundus. Then, the IUD was precipitated to the uterine fundus, and the funicular

position was confirmed with both the abdominal hand and the insertion hand. As the inner hand was being removed, a rotation of about 45 degrees clockwise or counterclockwise was performed, which was used to prevent the exteriorization of the IUD. The threads were cut at the height of the external orifice of the cervix and then visualized and trimmed during the follow-up visit four weeks after insertion. All cases in which the IUD wire was not visible inside the vaginal canal were referred for ultrasound examination to assess the position of the IUD.

Insertion technique for cesarean delivery

After placental delivery, the IUD was inserted at the top of the uterine fundus either manually or using De Lee's Forceps. Before hysterorrhaphy, the threads were incorporated in the lower segment of the uterus to allow them to hang naturally through the cervix during the puerperium period. Before hysterorrhaphy was performed, the IUD was confirmed as being retained in the fundus. The threads were visualized and trimmed during the follow-up visit four weeks after insertion. All cases in which the IUD wire was not visible inside the vaginal canal were referred for ultrasound examination to assess the position of the IUD.

The following variables were evaluated: age, number of pregnancies, number of deliveries, number of abortions, gestational age at delivery, type of delivery, time between IUD insertion and ultrasound examination, rate of IUD expulsion, time to diagnosis of IUD expulsion, rate of false path of IUD.

Statistical analysis

The data were analyzed using SPSS, version 20.0 (IBM Inc., Chicago, IL, USA) and Prisma GraphPad version 7.0 (GraphPad Software, San Diego, CA, USA). The quantitative variables were initially subjected to the Kolmogorov-Smirnov normality test and were presented as median and the interquartile range; 25th and 75th percentiles. Categorical variables were described with absolute and percentage frequencies and represented in tables and graphs. To assess the difference between categorical variables and their proportions, a chi-square test was used. The Mann-Whitney U test was used to analyze continuous variables. To perform the correlation between continuous variables, Spearman's correlation test was used. Logistic regression was performed to determine the best predictors for IUD misplacement. The level of significance (p) for all tests was <0.05.

Results

Between June 2018 and September 2019, 1,939 deliveries occurred in our service. During this period, 322 copper-T IUDs were inserted. A total of 152 cases were excluded because of lack of clinical follow-up after insertion. Thus, 170 cases were included in the final statistical analysis. The included cases were divided into two groups according to the IUD insertion period: insertion during the immediate puerperium period (n=124) and insertion during the mediate puerperium period (n=46; Figure 1).

There was no significant difference between IUD insertion timing groups (immediate vs mediate puerperium) and age (p=0.174), number of pregnancies (p=0.855), parity (p=0.896), number of abortions (p=0.570), gestational age at delivery (p=0.570), time between IUD insertion and ultrasound examination (p=0.179), and time between IUD insertion and the diagnosis of IUD expulsion (p=0.751; Table 1).

A significant association was observed between the IUD insertion and type of delivery (p=0.044). The rate of vaginal deliveries was higher in those undergoing immediate puerperium IUD insertion (52.42 vs 47.58%) and mediate puerperium IUD insertion (69.57 vs 30.43%) compared with the rate of cesarean sections (Figure 2).

In the whole cohort the overall rate of expulsion of IUDs was 28.8% (49/170). There was no significant association found between the occurrence of expulsion of the IUD and the timing of insertion (immediate puerperium vs mediate puerperium; 26.6% vs 34.78%, p=0.296), either clinically or with transvaginal ultrasound (Table 1). There was no significant association of IUD false path with either the immediate or mediate puerperium groups (1.6% vs 0%, respectively) (Table 1). There were no cases of endometritis in either group.

A significant association was observed between the type of delivery and IUD position (p=0.0002). Among patients diagnosed with IUD expulsion, 79.6% (39/49) underwent insertion after vaginal delivery, whereas 20.4% (10/49) had

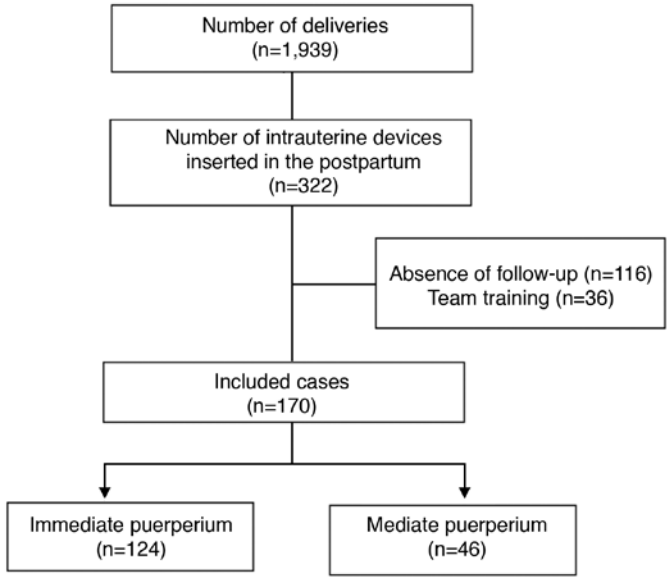

Figure 1. Study recruitment flowchart showing included and excluded cases

Table 1. Clinical, obstetric, and intrauterine device characteristics of the studied population. Data are shown as median (interquartile range) or count and frequency (n, %)

	Immediate puerperium (n=124)	Mediate puerperium (n=46)	p
Age (years)	26 (22-32)	24 (21-30)	0.174*
Number of pregnancies	2 (1-3)	1 (1-3.25)	0.855*
Number of deliveries	2 (1-3)	2 (1-3)	0.896*
Number of abortions	0 (0-1)	0 (0-0)	0.570*
Gestational age at delivery (weeks)	40 (39-40.4)	40 (38.6-40.6)	0.831*
Type of delivery	-	-	0.044**
Vaginal	65 (52.42%)	32 (69.57%)	-
Cesarean section	59 (47.58%)	14 (30.43%)	-
Time between IUD insertion and ultrasound examination (days)	51 (37-70)	45 (32-61.5)	0.179*
IUD expulsion	33 (26.61%)	16 (34.78%)	0.296**
Time to diagnosis of IUD expulsion (days)	49 (30-80)	41 (16-71)	0.751*
Uterine perforation	2 (1.61%)	0 (0%)	>0.999

n: Absolute number of cases included and each study group, IUD: Intrauterine device, IQR: Interquartile range, *Mann-Whitney U test-median (25^{th}-75^{th}), p<0.05, **chi-square-frequency (percentage), p<0.05

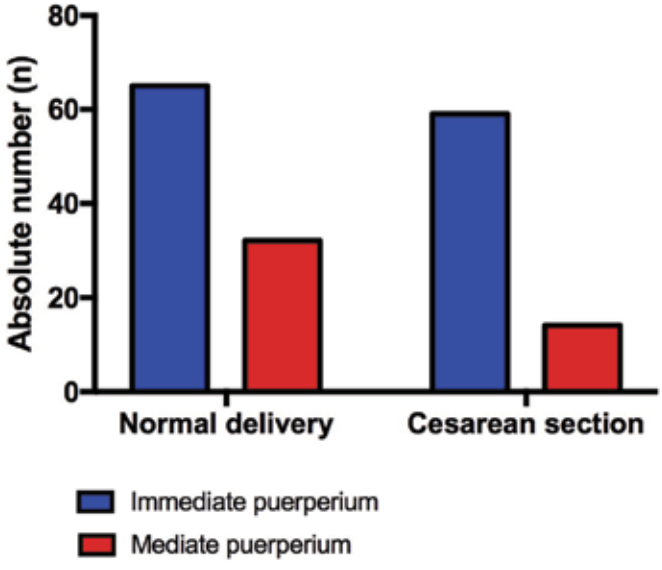

Figure 2. Bar graph showing the association between intrauterine device insertion and type of delivery. Chi-square, p<0.05

the IUD inserted during cesarean section. However, both for patients who underwent IUD insertion after vaginal delivery (59.80% vs 40.20%) and for those who underwent insertion during cesarean section (86.30% vs 13.70%), the rate of a correctly positioned IUD was higher than the expulsion rate (Table 2).

All IUDs were inserted by medical residents in gynecology and obstetrics, in the first (n=4), second (n=4), and third (n=4) years of residency, and none had previous experience inserting an IUD in the puerperal period. Physicians received only theoretical training prior to the start of the study. The first case of IUD insertion after vaginal delivery in the immediate puerperium period, the first case inserted after vaginal delivery in the mediate puerperium period, and the first case inserted after cesarean section by each physician were excluded. No significant association was found between the level of training of the medical residents, who had the same level of IUD placement training, and the rate of IUD expulsion (p=0.626; Table 3).

There was no significant correlation between the time between IUD insertion and the diagnosis of IUD expulsion and number of pregnancies (r=-0.160, p=0.271) or gestational age at delivery (r=-0.058, p=0.939; Figure 3).

A binary logistic regression was performed to determine whether the type of delivery in patients who had an IUD inserted in the puerperal period was a predictor of IUD expulsion. The model containing the type of delivery was significant for the prediction of IUD expulsion [$\chi^2(1)$: 15.14, p<0.0001, R^2 Nagelkerke: 0.12). Patients who delivered vaginally were 4.23

Table 2. Association between type of delivery and intrauterine device positioning

	Vaginal delivery (n=97)	Cesarean section (n=73)	p*
			0.0002
IUD expulsion	39 (40.20%)	10 (13.70%)	-
IUD positioned	58 (59.80%)	63 (86.30%)	-
*Chi-square-frequency (percentage) p<0.05, IUD: Intrauterine device			

Table 3. Association between the level of training of medical residents in gynecology and obstetrics and the rate of intrauterine device expulsion in the puerperal period

	MR1	MR2	MR3	p*
				0.626
IUD expulsion	30 (29.12%)	11 (25%)	3 (18.75%)	
IUD positioned	72 (70.58%)	33 (75%)	13 (81.25%)	
MR1: Medical resident of first year, MR2: Medical resident of second year, MR3: Medical resident of third year, *chi-square, p<0.05, IUD: Intrauterine device				

times more likely than patients who had cesarean sections to expel the IUD when it was inserted in the puerperal period [odds ratio (OR): 4.23, 95% confidence interval (CI): 1.94-9.25; Table 4).

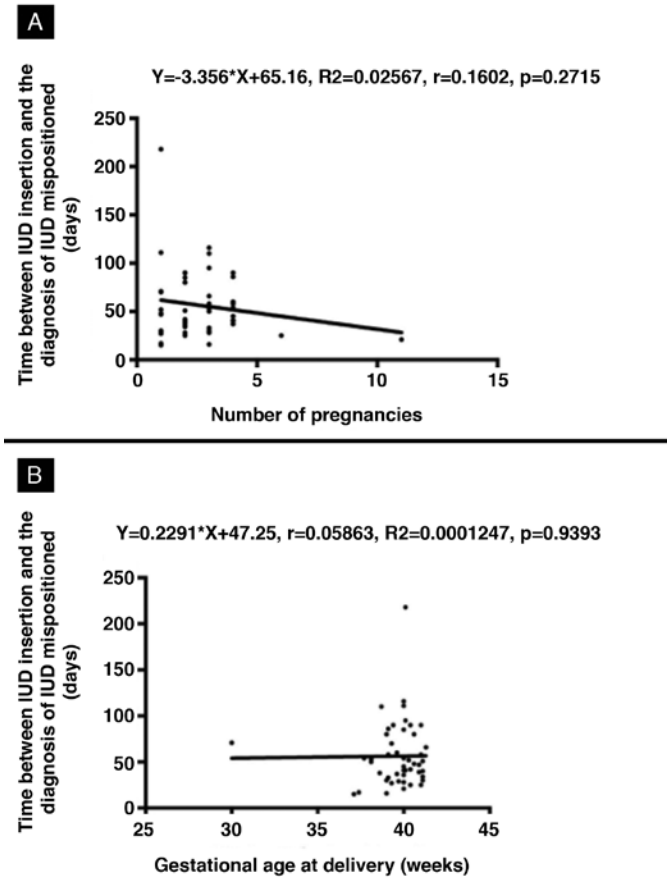

Figure 3. Scatter plot showing the correlation between the number of days between intrauterine device (IUD) insertion and the diagnosis of IUD expulsion in terms of number of pregnancies (A) and gestational age at delivery (B). Linear regression and Spearman's correlation test, p<0.05

Table 4. Odds ratio of intrauterine device expulsion in patients who underwent insertion in the puerperal period after vaginal delivery compared with cesarean delivery

	OR	CI 95%	R^2 Nagelkerke	p*
IUD insertion in the puerperal period	4.23	1.94-9.25	0.12	<0.0001
IUD insertion in the immediate puerperium	8.17	2.89-23.11	0.22	<0.0001
IUD insertion in the mediate puerperium	0.94	0.25-3.50	0.000	NS

OR: Odds ratio, CI: Confidence interval, IUD: Intrauterine device, NS: Not significant, *Binary logistic regression, p<0.05

A binary logistic regression analysis was also performed to determine whether the type of delivery in patients who had an IUD inserted in the immediate puerperium period was a predictor of expulsion of the IUD. The model containing the type of delivery was significant for the prediction of expulsion of the IUD when it was inserted in the immediate puerperium period [$\chi^2(1)$: 20.58, p<0.0001, R^2 Nagelkerke: 0.22). Patients who delivered vaginally were 8.17 times more likely than patients who had cesarean sections to expel the IUD when it was inserted in the immediate puerperium period (OR: 8.17, 95% CI: 2.89-23.11). In contrast, the model containing the type of delivery was not significant for the prediction of expulsion when the IUD was inserted in the mediate puerperium period [$\chi^2(1)$: 0.008, p<0.930, R^2 Nagelkerke: 0.000; Table 4).

Discussion

This study evaluated if the type of delivery was a predictor of expulsion and rate of expulsion of IUD inserted during the immediate and mediate puerperium. The results have shown that the insertion of IUD during puerperal period is a safe technique, even when it is performed by medical professionals with no previous experience in puerperal IUD insertion. The copper-T IUD is classified as a long-acting, reversible contraceptive and is recommended by the American College of Obstetrics and Gynecology (ACOG) as one of the best contraceptive options in the immediate postpartum period (6). ACOG's guideline on this method aims to improve the spacing of pregnancy, thus contributing to the improvement of maternal and child health care, especially in developing countries.

There was a low acceptance of IUD insertion in the mediate or immediate postpartum period in the population studied, with only 16.6% of women agreeing to its use. Another important finding was the loss of 47.2% of women who chose to insert an IUD after delivery and did not return for follow-up. Similar values have been reported previously with between 10% and 40% of puerperal women not returning for follow-up and that 40% to 70% of those who planned to use an IUD were unable to have it placed (7,8). These results show the benefit of offering this contraceptive method during the immediate postpartum period, as puerperal women have a lower likelihood of undergoing IUD insertion because of the difficulty of the procedure and lack of follow-up.

Our study found that IUD insertion in the immediate postpartum period was safe, which is in keepig with the findings of other studies (9,10). The advantages of this procedure include adequate retention of the IUD inside the uterus and the fact that is a safe and efficient, long-term contraceptive method. In our cohort, the rate of uterine perforation was low (1.6%). Conversely, previous studies have shown high rates of IUD translocation when the insertions were performed in the

puerperal period (11,12). Moreover, there were no cases of endometritis diagnosed during the period of the present study.

One of the objectives was to evaluate the positioning of the IUD after its insertion, via ultrasound or physical examination, performed in the first 60 days. We found no difference in relation to age, number of pregnancies, or age among women of both groups. Interestingly, our findings showed that pregnant women who underwent vaginal delivery had a higher incidence of IUD acceptance than those who delivered via cesarean section.

One concern about IUD insertion after delivery is the higher rate of expulsion when compared with IUD insertion during other time periods. The rate of IUD expulsion was 28.8%, as determined by ultrasound or physical examination in the first 60 days. However, there was no difference in the frequency of IUD expulsion between insertions that took place during the mediate and immediate postpartum periods.

The definition of IUD expulsion is not always clear, and diagnostic criteria are rarely reported. Expulsions, as reported in the literature, may have been determined by a variety of methods, including clinical, physical, or ultrasound examinations. In a systematic review published in 2018, the authors reported that, depending on the time of IUD placement, there was a change in the incidence of expulsion. In the immediate, early, and interval of IUD insertion, the expulsion rate was 10.0%, 29.7%, and 1.9%, respectively, including all follow-up periods (13). The expulsion rate was 14.9% for vaginal deliveries, 3.6% higher than for cesarean deliveries, at all follow-up intervals (13). Similarly, in our study, patients who underwent IUD insertion after vaginal delivery were more likely to a have poorly positioned IUD than those who underwent cesarean section (OR: 8.17, 95% CI: 2.89-23.11).

Braaten et al. (14) reported that in approximately 10% of their users, the IUD was mispositioned. Currently, good positioning is considered when the end of the vertical IUD nail is above the internal os (15). In cases suspicious for poorly positioned IUD on ultrasound examination, symptoms such as pain and increased or irregular bleeding should trigger further investigation. There was no change in IUD expulsion rate when the IUD was inserted 4-6 weeks after delivery.

With regard to the type of delivery as a factor for IUD expulsion, in the present study 79.6% (39/49) of women underwent insertion after vaginal delivery, whereas 20.4% (10/49) had the IUD inserted during cesarean section, and in both types of delivery, the rate of adequate positioning was higher than the rate of poor positioning. A randomized comparative study, in which both immediate and late postpartum IUD insertions were performed, demonstrated that the failures were not influenced by the timing of insertion, cervical dilation, or distance between the apex of the IUD and the fundus of the uterine cavity (16).

In the present study women who underwent vaginal deliveries were 4.23 times more likely than women who underwent cesarean sections to expel the IUD when it was inserted in the puerperal period. Aoun et al. (17) reported the following risk factors for expulsion: history of previous expulsion of another copper-T IUD associated with a probability of a new expulsion of 30%, increased menstrual flow, and severe dysmenorrhea. Lopez et al. (18) in a systematic review compared immediate (within 10 minutes of placental delivery) versus early postpartum placement of the IUD and found no difference in expulsion rates. A trial from Uganda showed that expulsion was more likely in the immediate group, although the estimate was imprecise. In a meta-analysis, expulsion by six months post-delivery was more likely for the immediate group, but the confidence interval was wide (OR: 4.89, 95% CI: 1.47-16.32; participants: 210 in four separate studies). The authors concluded that the benefit of effective contraception immediately after delivery may outweigh the disadvantage of increased risk for expulsion.

O'Hanley and Huber (19) proposed that previous training was not necessary for IUD placement, which is in agreement with our study wherein the IUDs were placed by professionals with no previous experience in inserting puerperal IUD. Similarly, Cwiak and Cordes (20) reported that both experienced clinicians and interns can successfully insert IUDs within a medical residency program for gynecology and obstetrics. There was no significant association between the level of medical resident training in gynecology and obstetrics and the rate of expulsion of the puerperal IUD in the present study ($p=0.626$). Although earlier studies have shown that previous experience was not necessary for IUD insertion, in our opinion the overall high expulsion rates demonstrated in our study may have been in part due to the lack of prior training of the professionals. We did not evaluate the necessary experience in IUD insertion in the training period required to obtain lower expulsion rates. However, the low level of experience of the professionals in our study reflects the reality for many institutions sited in regions of low socio-economic status, where IUD insertion in the puerperal period can contribute to better family planning.

Conclusion

In summary, vaginal delivery was the most prevalent type of delivery in patients who underwent IUD insertion during the immediate and mediate puerperium. The risk of IUD expulsion after vaginal delivery was greater than after cesarean section.

Ethics Committee Approval: *Local research ethics committee (CAAE: 20502719.5. 0000.5145).*

Informed Consent: *A signed consent form was obtained from all patients.*

Peer-review: *Externally peer-reviewed.*

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

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Significant improvement of laparoscopic knotting time in medical students through manual training with potential cost savings in laparoscopy - an observational study

Sebastian Findeklee[1,2], Georg-Peter Breitbach[1], Julia Caroline Radosa[1], Emanuela Morinello[3], Elmar Spüntrup[4], Erich-Franz Solomayer[1], Carolin Spüntrup[1,5]

[1]Clinic for Gynaecology, Obstetrics and Reproductive Medicine, Saarland University Hospital, Homburg, Germany
[2]MVZ Fertility Center Hamburg, Hamburg, Germany
[3]Clinic for Anaesthesiology, Intensive Care and Pain Management, Saarland University Hospital, Homburg, Germany
[4]Clinic for Radiology, Saarbrücken Hospital, Saarbrücken, Germany
[5]Pelvic School Saarbrücken, Saarbrücken, Germany

Abstract

Objective: Laparoscopy is a standard procedure in operative gynaecology, but laparoscopic simulator training for novices/junior surgeons is not currently well-established. The aims of this study were to demonstrate that a laparoscopic knot course for trainees can significantly shorten the knotting time and to perform a counter-value calculation for the clinic's costs.

Material and Methods: An observational study was performed with exercises on a laparoscopic box trainer as part of the practical clerkship in gynaecology and obstetrics between 07.10.2019-31.01.2020. At the beginning and at the end of the exercises, the participants made a laparoscopic knot and the difference in knotting time, Δt in seconds (s) was measured.

Results: Eighty-eight medical students needed an average of 247.1 s for the first laparoscopic knot at the beginning of the course and an average of 45.43 s for the second at the end of the course. Mean shortening of the knotting time was 201.67 s or 81.6% (p=0.02). Calculating costs of an average of €40-50 for an operation minute would mean a cost saving of at least €120-150 for a partial node.

Conclusion: Trainees can significantly improve their operative skills in a short time with the aid of surgical simulation training. Such training can be beneficial for clinics by reducing the operating time if the basics, such as sewing and instrument guidance, are learned on a simulator. We therefore suggest that operative simulation training should be mandatory in medical education. (J Turk Ger Gynecol Assoc 2020; 21: 150-5)

Keywords: Laparoscopy, laparoscopic knotting, surgery simulator, equivalent calculation

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Introduction

Minimally invasive endoscopic surgery is now considered a standard procedure in many surgical fields, especially in gynaecology and urology, because it is associated with shorter convalescence and an improved cosmetic result. At the same time, it is characterized by a low peri- and post-operative complication rate (1). However, this depends less on the technical equipment in the operating room than on the training status of the surgeon (2).

According to Gallagher and Satava (3), laparoscopic surgeons can be divided into novices, juniors and experts with regard to their training status. Novices have performed less than 10 operations, juniors 10-100 and experts performed more

Address for Correspondence: *Sebastian Findeklee*
e.mail: Sebastian.Findeklee@uks.eu ORCID: orcid.org/0000-0002-2921-0664

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than 100 operations (3). Laparoscopic surgery is sometimes characterized by relatively flat learning curves. Somewhat complex surgical steps, such as the laparoscopic knot should therefore not primarily be learned and practiced on humans. On the other hand, it seems to be essential to familiarize young doctors and possibly also medical students with the laparoscopic approach and to allow them to practise laparoscopic steps in order to be able to generate enough experienced young surgeons for the future. In order to reach this goal, various surgical simulators have been developed over the past few years. With the help of simulators, surgical skills can be practiced without endangering the patient (4). Unfortunately, training on surgical simulators has so far not been part of student teaching or medical training in operative subjects. Therefore, surgical training on simulators has so far been sporadic and not standardized (5).

The aim of this study was to measure the influence of manual training on the knotting time of medical students, as part of the practical clerkship in gynaecology and obstetrics. A classic box trainer working with a tablet camera was used and manual exercises were carried out in a defined sequence. Figure 1 shows the box trainer.

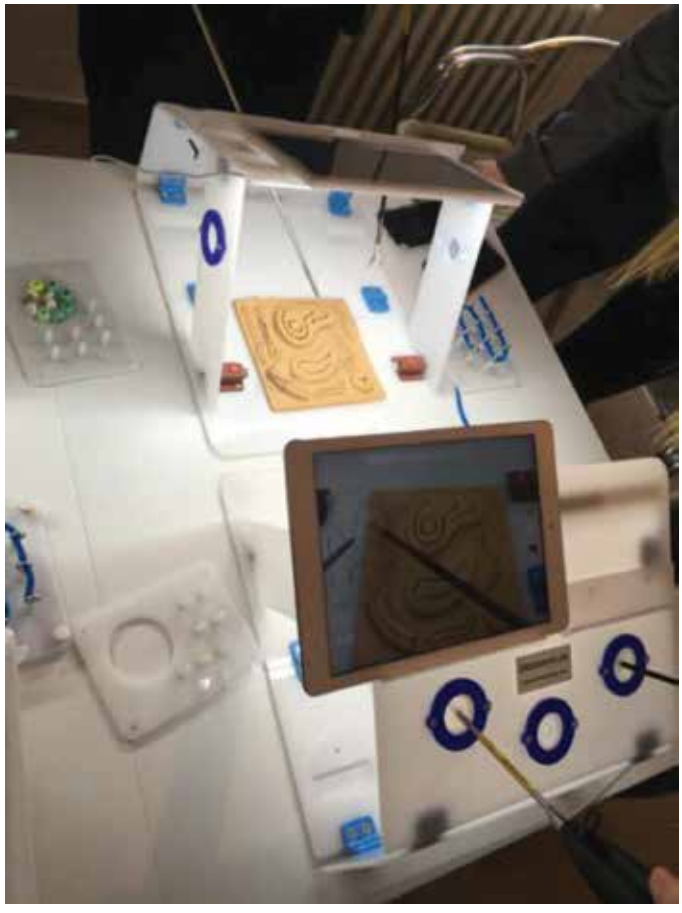

Figure 1. Structure of the laparoscopy simulator

Material and Methods

This was an observational study involving medical students as part of the practical clerkship in gynaecology and obstetrics in the winter semester 2019/2020. The study period was from 07.10.2019 to 31.01.2020.

The training protocol was as follows. There was one tutor (study doctor with experience in laparoscopic surgery) for a group of six to eight students. At the beginning of the course, the course participants were asked to perform a laparoscopic knot with a wrapping on a self-developed, laparoscopy simulator, including a box trainer and a tablet camera. This wrapping and the functionality of the instruments were explained to the students in advance. After the students had briefly familiarized themselves with the instruments, they started with their knot. The knotting time in seconds (s) was measured by the study doctor. For this first part of the training, including instruction by the doctor and knotting, a total of 30 minutes were allowed.

The following second training step lasted 30 minutes. Here, the students carried out hand-eye coordination exercises with one and two arms and skill training according to a defined protocol. This protocol included picking up tacks with a pair of pliers in the box trainer, threading beads onto a stick and running a ring over a splint without touching it using a laparoscopic needle holder.

Subsequently, clamping a needle in the correct way was demonstrated and the looping for the knot was repeated. The students were now asked to carry out different kinds of seams (interrupted suture, continuous rows of seams). The seams were secured with a double-strand knot and a counter-rotating single knot. This third training session also took 30 minutes. The respective seams and knots were made with braided and thin monofilament threads, so that the students could also develop a feeling for different thread sizes and thread types.

Finally, in the last 30 minutes of the knotting training, the students were asked to perform a laparoscopic knot on the simulator again. The study doctor measured the knot time in seconds for a second time. The difference between the mean knotting time of the first and last laparoscopic knot (Δt) was measured. At the end, every student gained feed-back from study doctor.

The training with the four modules, each 30 minutes long, lasted a total of two hours. The course of the laparoscopic knotting training is summarized in Figure 2.

Statistical analysis

The paired t-test was used to check whether there was a significant difference between the mean knotting time of the first endoscopic node (t1) and the mean knotting time of the

second laparoscopic node (t2) (significance level $p<0.05$). Statistical analysis was performed with the aid of SPSS version 24 (IBM, Armonk, New York, USA).

Informed consent and ethics

All study participants signed an informed consent form for further processing the obtained data anonymously before participating in the training course. The local institutional review board was contacted to ask for an ethics vote for the study, but this was not required for the study since it was a regular course belonging to the practical clerkship in gynaecology and obstetrics within the student curriculum.

Results

Between 07.10.2019 and 31.01.2020, a total of 88 medical students took part in the laparoscopic node course with the laparoscopy simulator. The mean time to complete the first laparoscopic knot at the beginning of the course was 247.1 s (minimum: 45 s, maximum: 1,290 s, range: 1,245 s, median: 790 s). An average of 45.43 s (minimum: 7 s, maximum: 280 s, range: 273 s, median: 150 s) was required for the second laparoscopic knot at the end of the course. Thus the knotting time was shortened by 201.67 s or 81.6% due to the learning success with the help of the course (Table 1). The difference between the mean first knotting time and the mean second knotting time (Δt) was statistically significant in the paired t-test ($p=0.02$). Calculating costs of an average of €40-50 for an operation minute would mean a cost saving of €120-150.

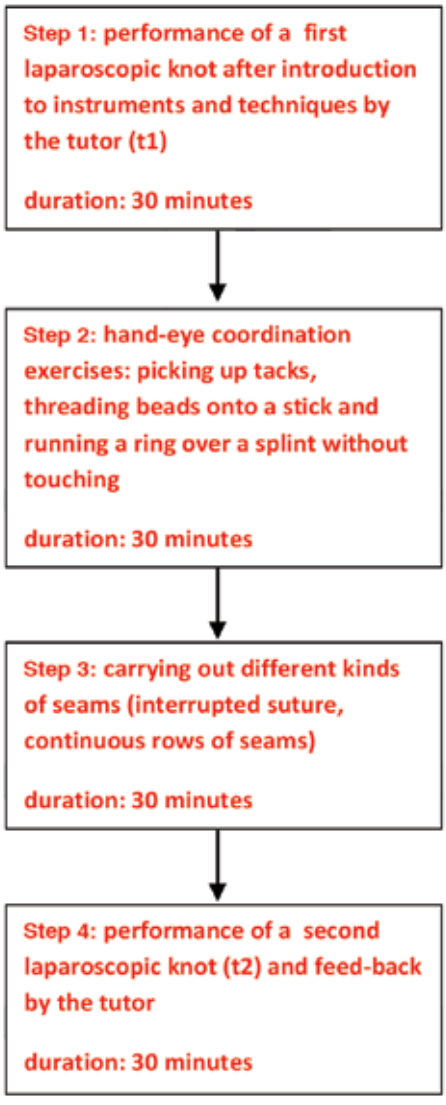

Figure 2. Course of the laparoscopic knotting training

Discussion

Our study shows that medical students can significantly reduce their knotting time by an average of 81.6% after attending a laparoscopic knotting course using a laparoscopy simulator, performing skill exercises and with adequate demonstration and explanation of the knotting technique. In this specific case, the clinic could save €120-150 for each laparoscopic partial node performed if one node had calculated costs of €40-50 per minute of surgery time, which seems realistic with regard to the published literature (6).

Whereas laparoscopic surgery in general possesses a low operative complication rate, it is self-evident that expertise increases with the surgeon's experience. From an ethical point of view, it should not be a requirement for scientific evidence of an economic benefit for the clinic before implementing laparoscopic simulation courses into specialist training for medical doctors, improved clinical performance and safety should be sufficient. In our opinion, it does not appear ethically responsible to carry out complex surgical interventions in humans with the risk of serious complications without having practiced the individual surgical steps beforehand. Even if there are no complications intra-operatively, such as organ injury, the longer duration of the operation with longer anaesthesia can pose a risk to the patient, especially in the case of pre-existing diseases. There is evidence that complication rates are directly related to surgical duration in gynaecological surgery (7). As laparoscopic simulator training enables a significant shortening

Table 1. Comparison of knot times before and after the course

Criteria	Knotting time (t1) (s)	Knotting time (t2) (s)
Median	247.1	45.43
Minimum	45	7
Maximum	1,290	280
Range	1,245	273
Total	21,745	3,998

of surgical partial steps like knotting it also has the potential of reducing operative complication rates.

Since longer anaesthesia also means an increase in costs for the clinic, these examples stress how closely linked the medical and economic consequences of longer operation times are. In the same context it has to be stressed that ethical and economic aspects are not mutually exclusive. Resources in every health care system are limited - hence a responsible use of health care resources, such as operating time, is important so that limited health care resources can be available to as many patients as possible.

Comparable to the pilot training that has been established for decades, there are currently numerous simulators for laparoscopic operations available with which comparable successes have been demonstrated with respect to learning curves (8-10). However, training on the simulator in the surgical curriculum - in contrast to pilot training - is not intended for surgeons. In contrast to pilots, the costs must also be borne by the trainee her- or himself. Hospital providers often argue that they cannot cover the costs of surgical skill training on the simulator, because a counter value calculation cannot be directly derived. In addition, up to now there is insufficient data on whether both surgery time, as the most cost-intensive factor, and the operative complication rate can be reduced by surgeons trained on the simulator, so that ultimately patients, hospital operators and young surgeons could benefit from the simulated training (3,11).

Our study provides new data on this issue. It was possible to measure a significant reduction in knotting time through structured simulator-based training. This could result in potential savings in the three-digit Euro range for each laparoscopic partial node. A countervalue calculation also has to consider the exact costs of simulation training. At the same time, it must be emphasized that the costs of structured laparoscopic knotting training on the operation simulator are very low. The technical equipment with box trainer and tablet camera amounts to less than €1,500 (box trainer €280, tablet camera approximately €200, laparoscopic instruments including needle holder, grasping forceps and scissors approximately €1,000). The costs of the suture material are about €250 per package including 36 pieces. Suture material and endoscopic instruments are also available in every clinic. It is difficult to calculate the costs for the tutor because the study doctor is employed at the clinic and can take the course during working time. There was no necessity for an additional salary. The training programme for all 88 participants at our institution incurred costs of approximately €2,250. Fortunately, the technical equipment is re-usable for future trainings. Calculating the operating costs at €50 per minute, this would mean that the simulator training would have paid for itself if cumulative knotting time of all 88 participants together could be reduced by at least 45 minutes. The effect of our training was a total reduction in knotting time of about 296 minutes. We therefore recommend our training concept, based on our experience, as it is clinically beneficial for the trainees and is also financially worthwhile. Our training concept also allows a variety of other exercises relevant to the operating room, such as of spatial imagination within the laparoscopic site or hand-eye coordination.

A decrease in the rate of operative complications after training with the simulator cannot be directly derived from our study. However, if we regard the handling of the instruments by the students before and after the course, it does not seem unconscionable to expect a potential for reduced surgical complications due to an increase in confidence utilizing laparoscopy instruments. For example, after the training, the seam pad, possessing a toughness comparable to that of intestinal tissue, showed significantly fewer tears of the monofilament threads. Perhaps, this could result in a lower rate of anastomotic leakage in the case of surgical interventions on the intestine.

There are several other studies demonstrating that laparoscopic techniques like knotting or sutures can also be learned by novices with the aid of simulation courses (12-15). In contrast to previous studies, we have introduced laparoscopic simulation training into the gynaecological practical clerkship which offered the opportunity to observe a significant number of participants and to make a comparison of the time saving of laparoscopic knotting time with a potential saving in operation costs. Our study results therefore provide new evidence for young surgeons as well as medical faculties and teaching hospitals to make laparoscopic simulation courses an obligatory part of medical training or to have the course costs reimbursed by the employer.

Although surgical simulators are not a new invention, they are still not very widespread, because of the reasons given above. Basically, animal models as well as self-made plastic simulators without material from living beings can be used for practicing surgical interventions (16). From an ethical point of view, simulators made of non-biological material should be preferred. In addition, they offer the opportunity to practice surgical steps repetitively without time or space restrictions.

Laparoscopy simulators have been successfully evaluated in the past. Among other things, it could be shown that repetitive training has a greater influence on the success of learning the endoscopic knot than talent factors such as manual work or the desire to work in a surgical subject in the future (17). In addition, Ghesquière et al. (18) and Madec et al. (19) showed that surgical simulators are a suitable methodology for teaching

surgeons appropriate laparoscopic technique. However, disadvantages of these studies are the relatively small number of participants and their monocentric character.

Study Limitations

Our observational study also has some limitations. With 88 students, the number of participants is limited and does comprise just one centre. The examined laparoscopic node represents only a partial step of a minimally invasive surgical intervention. It is not possible to infer the improvement in the time required for the entire operative procedure. Besides, there was no further analysis of the characteristics of the students. For example, it could be that a disproportionately large number of students have already worked in a surgical field and therefore had easier access to the laparoscopic node. However, the "talent factor" argument was already invalidated by our publication from 2019 (17). It should also be considered that the number of participants in our study is distinctly higher than the previous publications that have dealt with this topic. Additionally, we are aware that the long-term retention of technical skills acquired during simulation training is a problem. For improving retention of skills, further regular training sessions are recommended and necessary. This circumstance also has to be factored in the cost analysis. Fortunately, repetitive training at an existing simulator is possible without obstacles and does not provide additional costs. Just the costs for the suture of about €250 per package including 36 pieces have to be estimated. A pragmatic solution could be seen in the utilization of expired sutures.

Furthermore, it has to be taken into account that different health care systems in different countries may go along with different health care costs including the costs for operating time. For example, a literature review performed by Chen et al. (20) revealed differences by a factor of two in operating costs in the different regions of the world (operating room costs per minute differing from $13.90 in Europe, the Middle East and Africa to $24.83 in North and South America) (20). Perhaps, this could lower the economic efficacy of simulation training in other countries independent from the ethical point of view.

In our opinion, the special finding of our observational study is that it is not just demonstrating a significant shortening of the time for a laparoscopic knot performed by inexperienced surgeons, but also provides a countervalue calculation for the potential saving of operation costs. If one assumes that a total laparoscopic hysterectomy requires at least two laparoscopic knots, this operation alone could save the clinic €250-300 with the aid of surgical skill training. For urogynaecological interventions with a mesh insert, this saving could be increased to €700-1,000. This could create an argument for the assumption of costs for operative simulation training by hospital authorities, from which doctors and patients would ultimately benefit. The fact that students regularly do not perform laparoscopic knots in real life may limit the impact of our findings. Nevertheless, the students from today are the doctors of tomorrow and also students during practical clerkship assist in the operating room with the opportunity of benefiting from acquired practical skills. The lesson we learned from our laparoscopic training course is that it will be a mandatory component of the practical clerkship in the subject gynaecology and obstetrics at our faculty. Additionally, the simulator is currently used by young residents in our clinic. It is imperative to carry out practical exercises on the simulator under supervision before an operation can be performed in the operating room. Furthermore, the surgical and the urological clinic at our university are planning to implement a similar surgical skills training based on the positive experience we have gained with our course.

Finally, our study could help to further support the spread of operative simulation training, both in the context of training young surgeons and in the curriculum for teaching medical students at the universities.

Conclusion

Young surgeons can significantly improve their operative skills in a short time with the aid of surgical simulation training. Such training on the simulator can be beneficial for the clinics by reducing the operating time if the basics such as sewing and instrument guidance are learned on the simulator. We therefore suggest that operative simulation training should be widely implemented in medical education.

Ethics Committee Approval: *Institutional Review Board of Saarland consulted. The local institutional review board was contacted to ask for an ethics vote for the study, but this was not required for the study since it was a regular course belonging to the practical clerkship in gynaecology and obstetrics within the student curriculum.*

Informed Consent: *It was obtained from all study participants.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: C.S., G.P.B., S.F.; Concept: C.S., S.F., E.F.S.; Design: S.F., C.S., E.M., E.F.S., J.C.R.; Data Collection or Processing: J.C.R., C.S., E.S.; Analysis or Interpretation: S.F., J.C.R.; Literature Search: S.F., C.S., E.S., E.M.; Writing: S.F.*

Conflict of Interest: *All authors declare no conflict of interest.*

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En-bloc rectosigmoid and mesorectum resection as part of pelvic cytoreductive surgery in advanced ovarian cancer

Juan Luis Alcazar[1], Matias Jurado[1], Jose Angel Minguez[1], Enrique Chacon[1], Fernando Martinez-Regueira[2]

[1]Clinic of Obstetrics and Gynecology, Clinica Universidad de Navarra, Pamplona, Spain
[2]Clinic of Surgery, Clinica Universidad de Navarra, Pamplona, Spain

Abstract

Objective: "En-bloc" resection of pelvic tumor in ovarian cancer (OC) is still controversial. The aim was to analyze results in an OC series from a single center, all of whom underwent "en-bloc" resection as part of cytoreductive surgery.

Material and Methods: Clinical and surgical records from sixty patients with ovarian carcinoma who underwent "en-bloc" resection surgery were retrospectively analyzed.

Results: Patients' mean age was 56 years; 36 patients had primary disease and 24 had recurrent disease. Carcinomatosis was present in 46.7% of patients. Primary surgery was performed in 49 and interval debulking surgery in eleven. Complete cytoreduction was achieved in 55.0% and optimal in 38.3% of patients. Carcinomatosis significantly decreased the probability of complete cytoreduction [odds ratio (OR): 0.22; p=0.021]. Mesorectal infiltration occurred in 83% of patients. Risk of death was non-significantly higher (hazard ratio: 1.9) in women with mesorectal infiltration. Median overall survival was longer for patients without infiltration (46.1 vs 79.1 months; p=0.15). Eighty-five percent suffered from mild to moderate complications and colorectal anastomosis (CRA) leak occurred in two patients (3.6%) with CRA below 6 cm. Diaphragm resection had >5 times the risk for major complications (OR: 5.35; p=0.014). There was no three month mortality.

Conclusion: When contiguous gross extension of disease to pelvic peritoneum and sigmoid colon is found, in patients with advanced OC, microscopic involvement of the mesorectum and intestinal wall is present in most cases making "en-bloc" resection necessary if complete cytoreduction is to be achieved. The associated morbidity is acceptable. (J Turk Ger Gynecol Assoc 2020; 21: 156-62)

Keywords: Ovarian cancer, surgery, rectosigmoid

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Introduction

Optimal cytoreduction followed by adjuvant chemotherapy with carboplatin and paclitaxel is currently considered as the standard treatment for primary advanced ovarian cancer (AOC) (1,2). To achieve this goal, maximum surgical effort is needed. This may include several intra-peritoneal and extra-peritoneal procedures (3-5). The role and potential benefits of a similar surgical therapeutic approach for recurrent OC is still under debate although results from some studies are encouraging (6). Contiguous extension to pelvic peritoneum and sigmoid colon may need a radical "en-bloc" resection of pelvic tumor with or without colorectal anastomosis (CRA). This procedure has been progressively incorporated into surgical practice and it should be currently considered the standard approach for achieving optimal cytoreduction (7-12). Nevertheless, some studies have assessed the real benefit of this more radical procedure balanced against the potential risk of complications (13-22). It is well known that the spread pattern of ovarian cancer that infiltrates the intestinal wall resembles that of primary colon

Address for Correspondence: *Juan Luis Alcazar*
e.mail: jlalcazar@unav.es ORCID: orcid.org/0000-0002-9700-0853

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carcinoma (8,10). This finding would imply that resection of regional lymph nodes of the involved organs, as is undertaken for primary colorectal carcinoma, may be appropriate in optimal debulking surgery (DS) for patients with OC.

The aim of this study was to analyze the results from a series of patients with primary or recurrent OC who underwent "en-bloc" pelvic disease resection with extraperitoneal CRA as part of cytoreductive surgery, in order to investiagte there is oncological justification for this procedure. In addition, the morbidity associated with this surgical approach was documented.

Material and Methods

Sixty patients with OC, 36 women (60.0%) with a primary tumor and 24 women (40.0%) after recurrence, all of whom underwent "en-bloc" pelvic disease resection with extraperitoneal CRA were retrospectively studied. Clinical and surgical records were retrieved from the hospital database. Institutional Ethical Review Board approval from Clinica Universidad de Navarra (approval number: 2018.001) was obtained for this study. Due to the retrospective design, patients' informed consent was waived.

The inclusion criterion for this study was any patient with histologic diagnosis of epithelial OC who underwent an "en-bloc" pelvic disease resection with CRA below the Douglas peritoneal reflection.

All patients were followed-up until the time of death or until December 2017, if alive. There was no patient lost to follow-up.

Surgical procedure

All patients underwent radical oophorectomy according to Bristow et al. (23) technique. The extension of mesorectal resection is partial since the rectum is transected at the same level as the mesorectum, 2 to 3 cm below the palpable tumor and avoiding a cone effect. According to some publications, the oncological outcome of partial mesorectal excision is safe, with a lower risk of anastomotic leak (24).

All CRA were end-to-end and performed with a circular end-to-end anastomotic staple device. Both excised doughnuts were inspected for integrity and macroscopic normality and CRA integrity was checked by a bubble test, and tension and color of the bowel were evaluated. Proximal diversion was not performed. Surgical Complexity Score (SCS) was used for determining the surgical complexity (25).

Preoperatively, most patients received a full mechanical bowel preparation the day before surgery. Standard antibiotic prophylaxis during surgery was delivered. In patients with gross intra-operative fecal contamination, broad-spectrum antibiotic coverage was administered for 72 hours postoperatively.

Low molecular weight heparin at a dose of 3500 U/day was given during the first four weeks and antiembolism stockings and pneumatic sequential compression device were routinely placed during surgery.

Outcomes analyzed

Primary outcome was oncological outcome. Secondary outcome was associated morbidity.

Statistical analysis

Statistical analysis was performed with the SPSS for Windows, version 20 (IBM Inc., Chicago, IL., USA). Continuous data are presented as mean with standard deviation or median with interquartile range depending on data distribution. Categorical data are presented as the number of cases and percentages. Categorical data were compared using two-tailed Fisher's exact test. Kruskal-Wallis test was used where appropriate. Continuous data were compared using Mann-Whitney U test when data distribution was not normal and One-Way analysis of variance when distribution was normal. Odds ratio (OR) with 95% confidence intervals (CIs) for predicting morbidity were calculated for several prognostic factors by using a binary logistic regression analysis, choosing a forward stepping model procedure.

Overall survival (OS) was measured in months from the date of surgery to the time of death or last follow-up appointment. Survival Free of Disease (DFS) was measured from the date of surgery to the time of the first failure observed after surgery. Survival analysis was done with the Kaplan-Meier method, compared by the Log-rank and Breslow statistical method, estimated from the first day after surgery. Univariate Cox proportional hazard ratio (HR) analysis was performed to identify potential prognostic factors and multivariate Cox proportional HR was performed introducing only those statically significant variables found in the univariate analysis. A p value <0.05 was considered statistically significant for all tests.

Sample size calculation was not performed.

Results

Patients' demographics, and tumor features are summarized in Table 1. Overall, mean age was 56 years, and patient's performance was good in 60.0% of cases, Upfront DS was performed in 49 women (82%) and interval DS in eleven (18%). CRA at >6 cm from the anal verge was performed in 96% of patients.

Table 2 shows all surgical procedures performed for achieving maximal cytoreduction. All sixty patients underwent an "en-bloc" pelvic disease resection followed by a CRA below the Douglas reflection. No patient underwent protective fecal stream diversion.

Table 1. Patients' and tumor characteristics

	All (n=60)	Primary (n=36)	Recurrent (n=24)
Age mean (SD), years	56 (12.1)	57.6 (11.6)	53.8 (12.7)
BMI mean (SD)	26.0 (4.3)	25.8 (4.1)	25.6 (4.7)
ASA n (%)			
1-2	36 (60.0)	61	58
>3	24 (40.0)	38	42
FIGO n (%)			
≤ II a	8 (13.3)	0	8 (33.3)
II b-IV	52 (86.7)	36 (100)	16 (66.7)
Histology n (%)			
Serous	44 (77.3)	29 (80.6)	15 (62.5)
Endometrioid	6 (10.0)	4 (11.1)	2 (8.3)
Mucinous	3 (5.0)	2 (5.6)	1 (4.2)
Clear cell	3 (5.0)	0	3 (12.5)
Squamous	1 (1.7)	0	1 (4.2)
Mixed	2 (3.4)	0	2 (8.3)
Other	1 (1.7)	1 (2.8)	0
Grade n (%)			
1	4 (6.7)	2 (5.6)	2 (8.3)
2	18 (30.0)	9 (25.0)	9 (37.5)
3*	38 (63.3)	23 (69.4)	13 (54.2)
Surgery n (%)			
Upfront	49 (81.7)	29 (80.6)	20 (83.3)
NACT#	11 (18.3)	7 (19.4)	4 (16.7)
CA-125** median (range)	736.9 (5-8000)	1097.4 (13-4440)	482.9 (5-8000)

ASA: American Society of Anesthesiologists, SD: Standard deviation, BMI: Body mass index, CA: Cancer antigen, FIGO: International Federation of Gynecology and Obstetrics, *Includes G3 tumors and high-risk histology different than Serous, #Neoadjuvant chemotherapy, **UI/mL

Twenty-eight patients (46.7%) had carcinomatosis, in 12 (20%) patients there were isolated implants and 20 (33.3%) patients had no visible peritoneal disease (p=0.001). Overall, 93.0% of patients had optimal cytoreduction (<1 cm). Complete cytoreduction was achieved in 55.5%. Complete cytoreduction was significantly more frequently achieved in patients with recurrent disease than in those with primary disease (79.2% vs 38.9%; p=0.006). Carcinomatosis was significantly associated with a high SCS (93.0% vs 6.3%; p=0.001) and significantly decreased the probability of complete cytoreduction (OR: 0.22, 95% CI, 0.62-0.80; p=0.02).

Pathological prognostic factors

Bowel wall infiltration was found in 83.3% (50/60) of cases. The serosa was involved in 22/50 (38.3%) and muscularis-mucosa in 28/50 (56.0%).

Table 2. Surgical characteristics of the cases

	All (n=60)	Primary (n=36)	Recurrent (n=24)	p
CRA below Douglas pouch				
>7 cm	58 (96.7)	35 (97.2)	23 (95.8)	0.769
<6 cm	2 (3.3)	1 (2.8)	1 (4.2)	
Lymphadenectomy				
No	35 (58.3)	19 (52.8)	16 (66.7)	0.423
Any*	25 (41.7)	17 (47.2)	8 (33.3)	
Omentectomy				
Infracolic	28 (65.1)	24 (67.0)	4 (17.0)	0.002
Infragastric	15 (34.9)	12 (33.3)	3 (12.5)	
Intestinal resection**				
Small bowel	11 (18.3)	6 (16.7)	5 (20.8)	0.467
Large bowel	11 (18.3)	9 (25.0)	2 (8.3)	0.102
Appendectomy	19 (32)	17 (47.2)	2 (8.3)	0.002
Diaphragm stripping/resection	27 (45.0)	24 (66.7)	3 (12.5)	0.001
Others				
Splenectomy	5 (8.3)	5 (14.0)	0 (0.0)	0.057
Partial hepatectomy	2 (3.4)	1 (2.8)	1 (4.2)	NA
Partial pancreatectomy	1 (1.7)	1 (2.8)	0 (0.0)	0.033
Ureterectomy/ cystectomy	6 (10.0)	1 (2.8)	5 (20.8)	
Surgical complexity score#				
Low	8 (13.3)	0 (0.0)	8 (33.3)	0.001
Intermediate	33 (55.0)	18 (50.0)	15 (62.5)	
High	19 (31.7)	18 (50.0)	1 (4.2)	
Peritoneal disease				
No	20 (33.3)	3 (8.3)	17 (70.8)	0.001
Isolated	12 (20.0)	8 (22.2)	4 (16.7)	
Multiple	28 (46.7)	25 (69.4)	3 (12.5)	
Residual disease, cm				
0 (complete resection)	33 (55.0)	14 (38.9)	19 (79.2)	0.006
<1 (optimal)	23 (38.3)	18 (50.0)	5 (20.8)	
>1 (suboptimal)	4 (6.7)	4 (11.1)	0 (0.0)	
Ascites				
No	41 (71.9)	22 (61.1)	19 (90.5)	0.018
Scanty	5 (8.8)	3 (8.3)	2 (9.5)	
Abundant	11 (19.3)	11 (30.6)	0 (0.0)	
Surgery length†	338 (89.3)	344 (87.7)	330 (92.8)	0.566

Percentages in parentheses. †In minutes, mean with SD in parentheses, *Any: Aortic/ pelvic /both ± debulking, **Total number of bowel resection in addition to recto-sigmoid resection, #Aletti et al. (25) (2007), CRA: Colorectal anastomosis.

The mesorectum was found to be infiltrated by tumor (defined as either directly or through lymph node metastases) in 83% (50/60) of the cases. Mesorectal involvement was associated with the depth of bowel infiltration [36.0% (18/50) if serosa was involved, 56.0% (28/50) if muscularis-mucosa was involved, and 8.0% (4/50) when the bowel wall was not involved]. It should be noted that there were four patients with bowel wall infiltration but not mesorectum infiltration and there were another four patients that had mesorectum infiltration but no bowel wall involvement.

Survival

After a median follow-up of 37.5 months (95% CI: 38.3-63.7), the 5-year OS and DFS for the whole series were 30.3% and 23.2%, respectively. Median survival time was 47.2 months (95% CI: 34.8-59.5) and median time to recurrence was 18.6 months (95% CI: 12.8-24.5).

The HRs of prognostic factors assessed for OS and DFS are shown in Table 3. Univariate analysis showed a worse outcome for age >65 years, carcinomatosis, residual disease after surgery, high-risk serous tumor, and mesorectum infiltration. In multivariate analysis, only carcinomatosis was significantly associated with OS whereas no residual tumor and age <65 were associated with DFS.

In spite of we did not find mesorectum involvement statistically significant, it should be noted that when the mesorectum was involved the risk of death was 1.9 times higher (HR: 1.9, 95% CI: 0.82-5.2; p=0.157) than when it was not involved. Median OS was 46.1 months (95% CI: 31.9-60.3) and 79.1 (95% CI: 0-172.6) for patients with and without infiltration of the mesorectum, respectively (p=0.15).

All cases with extra-abdominal disease (liver, spleen, suprarenal, chest or others) occurred in patients with mesorectum infiltration. However, despite this pattern of spread, 5-year OS was similar to that of patients with intra-peritoneal and/or retro-peritoneal recurrences (32.0% vs 29.0%; p=0.80).

Pattern of recurrence

Overall, forty-four (73.3%) patients recurred. Most recurrences were intra-peritoneal (53.3%). It is noticeable that all extra-abdominal (liver, spleen, suprarenal, chest or others) recurrences occurred in patients with mesorectum infiltration. Logistic regression analysis showed that factors related with extra-abdominal recurrence were multiple peritoneal implants [OR: 4.2 (95% CI: 1.02-17.2; p=0.04)] and muscularis-mucosa invasion [OR: 5.4 (95% CI: 1.3-22.4; p<0.01)].

Morbidity

Five major intraoperative complications occurred: three cystotomies, one vascular injury and one severe hemorrhage. The number of units of blood transfused intra-operativley was higher in the group of patients with recurrent disease (3.2 vs 1.9; p=0.03).

According to the Clavien-Dindo classification (26), most patients suffered from mild to moderate complications (85.0 % of type 1-2, 15% type >3), without statistically significant difference between patients with primary and recurrent disease (p=0.773). CRA leak occurred in two patients (3.6%), both in the group with recurrent disease. One had previous pelvic radiotherapy and the second was undergoing her third adjuvant chemotherapy regimen that included bevazizumab.

Logistic regression analysis showed that pleural effusion, diaphragm resection, infragastric omentectomy, appendectomy, units of blood transfused and surgery length increased the probability of major complications during the postoperative period. In multivariate analysis, diaphragm resection had more than five times the risk of major complications (OR: 5.35, 95% CI: 1.40-20.4; p=0.014), while units of blood transfused postoperatively had a 48% higher risk (OR: 1.48, 95% CI: 0.98-2.23; p=0.06) (Table 4).

There was no mortality during the three month follow-up after surgery.

Table 3. Logistic analysis of co-variates related to survival (Cox regression)

OS				**DFS**			
Univariate	**HR**	**95% CI**	**p**	**Univariate**	**HR**	**95% CI**	**p**
Age >65 years	1.80	0.89-3.60	0.035	Age >65 years	1.97	1.01-3.82	0.045
Carcinomatosis*	2.70	1.20-6.10	0.013	Carcinomatosis*	2.40	1.16-5.00	0.019
No RD vs. any	1.65	0.86-3.14	0.118	No RD vs any	1.89	1.03-3.46	0.038
Mesorectum	1.90	0.82-5.20	0.157	Mesorectum	1.90	0.70-4.82	0.177
HR Serous	1.50	0.70-3.20	0.260	HR Serous	1.30	0.70-2.70	0.410
Multivariate				**Multivariate**			
Carcinomatosis*	2.79	1.25-6.25	0.013	No RD vs <1 cm	1.86	1.02-3.43	0.041
				Age >65 years	1.90	1.02-3.70	0.049

*Multiple peritoneal implants. OS: Overall survival, DFS: Disease free survival, HR: Hazard ratio, CI: Confidence interval, RD: Residual disease

Table 4. Factors associated with morbidity with Clavien-Dindo score ≥3

Univariate	OR	95% CI	p
Pleural effusion	4.14	1.06-16.20	0.041
Diaphragm resection	6.73	1.85-24.4	0.004
Infragastric omentectomy	4.08	0.81-20.3	0.086
Appendectomy	3.71	1.13-12.16	0.030
Postoperative BU	1.57	1.07-2.28	0.019
Surgery length (hours)	1.47	1.0-2.18	0.050
Multivariate			
Diaphragm resection	5.35	1.42-20.4	0.014
Postoperative BU	1.48	0.98-2.23	0.06
OR: Odds ratio, CI: Confidence interval, BU: Blood units transfused			

Discussion

Rectosigmoid colon with mesorectum resection is considered a standard surgical management for patients with AOC when there is contiguous extension to pelvic peritoneum and sigmoid colon (1). This approach seems reasonable in view of the high frequency of involvement of the distal sigmoid by the ovarian tumor. However, due to the possible associated morbidity there is still reluctance by some to perform this procedure. Histological bowel and mesorectum lymph node infiltration is described in a very large percentage of cases as factors associated with a poorer survival, including this series (7-10,13-21). Some authors suggest that a close colorectal dissection (CRD) without a partial mesorectum excision or adequate longitudinal margin may leave residual tumor in the mesorectum or in the intestinal wall and this is not consistent with the complete disease resection concept (7-10,13-21). A recent study comparing sigmoidectomy with total mesorectal resection (TMR) and without mesorectal resection for removing focal disease did not find difference in progression free survival and concluded that CRD could be an acceptable alternative (27).

It has been suggested that mesorectal infiltration by OC may influence the natural history of the disease through an alternative metastatic pathway similar to the lymphatic or vascular spread of primary intestinal malignancies (7-17). In our series, we observed that liver, spleen, suprarenal and distant extra-abdominal metastasis exclusively occurred in patients with mesorectal infiltration, which is consistent with this concept. Aletti et al. (12) reported that in the subgroup of patients whose pelvic tumor was completely reduced to no visible residual disease by means of a radical proctosigmoidectomy, OS appeared to be superior to those who just underwent pelvic peritonectomy, further reinforcing this hypothesis. Another, similar study did not find the same results and stressed that it was the amount of residual tumor that was independently associated with survival. Therefore, based on this finding and the lower rate of complications without proctosigmoidectomy, systematic mesorectal excision as part OC cytoreduction surgery is not supported (15).

This radical pelvic surgery should contribute to improve survival if disease throughout the abdomen is removed to the point of optimal cytoreduction, as observed in different studies (12,16,18). We observed in univariate analysis that mesorectal infiltration increased the risk of death. However, in multivariate analysis only carcinomatosis influenced this risk. A Mayo Clinic study focused on the prognostic value of FIGO stage IVB due to mucosal colon invasion in patients with no residual disease after surgery and found no correlation of survival with intestinal wall invasion (28). Our results agree with theirs regarding the presence of carcinomatosis as the main prognostic factor for survival and that survival was not associated with mesorectal involvement or depth of colon wall infiltration.

In our series, the risk of death was 90% higher for patients with any amount of residual disease but it only showed a trend toward statistical significance. This could be explained by the small number of patients and the heterogeneity of the group with recurrent disease. Nevertheless, the risk of recurrence was significantly influenced by residual disease.

The pattern of recurrence after this type of surgery for AOC is not well known. In our series, the most frequent recurrence site was the peritoneum followed by retro-peritoneal lymph nodes and similar findings were reported by Amate et al. (29). Some publications focused on pelvic recurrence as the paradigm to explain the benefit of pelvic disease resection with "en-bloc" rectosigmoidectomy and report a lower rate of pelvic recurrence (7,8,10,18,22). In addition, Scarabelli et al. (7) called attention to distant metastases, specifically hepatic metastases. Distant metastasis was more frequent among patients with deep infiltration of the muscularis of the rectosigmoid, mesorectal lymph node involvement and residual tumor >1 cm.

The analysis of risk factors for the spread pattern in our series is similar to those reported by Scarabelli et al. (7), which are volume of disease (multiple peritoneal implants) and deep infiltration of the bowel being the factors associated with the spread of the disease.

Several recent studies dealing with this surgical approach to AOC conclude that the morbidity and mortality following this procedure is acceptable. We reviewed published series that included at least 50 patients who had undergone a colorectal resection with CRA and in which morbidity had been analyzed (7-20). CRA leak occurred in 3.6% (range, 0% to 9.0%), proximal fecal stream diversion was performed in 18.8% (range, 0% to 58.4%), major complications occurred in 23.3% and death in 1% (range, 0-6%) of the cases, respectively. Recently several

publications have focused on this topic (27,30). The series from Korea (27) compares the rate of CRA leakage according to whether a TMR or a CRD were performed and they showed a higher rate for TMR (5.3 vs 0%). Multivariate analysis showed postoperative hemoglobin as an independent prognostic factor. Lago et al. (30) in a multinational European centers study of 695 patients found a rate of CRA leakage of 6.6% (1.7-12.5%) despite 37% of them having undergone a diverting ileostomy. Multivariate analysis showed several risk factors for anastomotic leak including manual anastomosis and distance of the anastomosis from the anal verge. Results regarding morbidity in our series are similar to the above-mentioned literature review. We had no deaths within the 90 days following surgery.

Study Limitations

The main strength of our study is that the pathological examination and reporting allowed us to analyze the relationship between the depth of infiltration of the rectal wall and/or involvement of the mesorectum and the pattern of recurrence. Whether or not extra-abdominal recurrences impact survival would require analysis of a larger group of patients.

Our study has some weaknesses such as the small number of patients, the heterogeneity of the group of patients with recurrent disease, the lack of specificity with regard to the mesorectal involvement being due to nodal metastasis or infiltration of the mesentery, and the lack of a control group. These weaknesses preclude us from drawing strong conclusions.

Conclusion

Mesorectal and intestinal wall involvement by tumor is frequent in patients with AOC that grossly appears to involve the sigmoid colon, suggesting that performing an “en-bloc” pelvic disease resection may benefit some patients. However, complete or optimal cytoreduction is the main prognostic factor associated with survival. Our data and other authors’ findings suggest that the morbidity and mortality associated with “en-bloc” colorectal resection and anastomosis is acceptable.

Ethics Committee Approval: *Institutional Ethical Review Board approval from Clinica Universidad de Navarra (approval number: 2018.001).*

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Binary grading may be more appropriate for endometrial cancer

Kazibe Koyuncu[1], Duygu Altın[1], Batuhan Turgay[1], Bulut Varlı[1], Bahar Konuralp[2], Yavuz Emre Şükür[1], Salih Taşkın[1], Fırat Ortaç[1]

[1]Department of Obstetrics and Gynecology, Ankara University Faculty of Medicine, Ankara, Turkey
[2]Clinic of Obstetrics and Gynecology, University of Health Sciences Turkey, Tepecik Traning and Research Hospital, İzmir, Turkey

Abstract

Objective: To elucidate the survival consequences of the prognostic factors for endometrial cancer.

Material and Methods: This was a retrospective study of 276 patients diagnosed with endometrial cancer who admitted for staging surgery. The extent of the surgery was determined by clinical staging and preoperative evaluation. The pathology specimens were reassessed by a gynecopathologist. Independent risk factors were revealed for the progression-free survival (PFS), overall survival (OS) and disease-specific survival (DSS) utilizing Kaplan-Meier and "Cox" proportional analysis.

Results: The median follow up of the patients was 50 months. Of the 29 patients who died, 15 (5.43%) died because of endometrial cancer. Multivariate analysis revealed that independent risk factors for OS and PFS were stage (p=0.002, 0.002, respectively) and grade 3 (G3) histology (p=0.013, 0.015, respectively). Positive peritoneal cytology was an independent risk factor for OS (p=0.024), but not for PFS (p=0.050). Stage (p=0.005) was found to be the only independent risk factor for DSS. Patients with G1 and G2 histology had a similar and more favorable prognosis than patients with G3 histology.

Conclusion: Advanced stage, high-grade tumor and the presence of positive peritoneal cytology were ascertained as independent prognostic factors for endometrial cancer. A binary histological grading system could be simpler and as effective as the current three grade system because grade 1 and 2 patients showed similar prognosis. (J Turk Ger Gynecol Assoc 2020; 21: 163-70)

Keywords: Endometrial cancer, prognostic factor, survival, peritoneal cytology, grade

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Introduction

Endometrial cancer is the most commonly diagnosed gynecologic malignancy in the USA and European countries (1). Two different subtypes of endometrial cancer have been defined as their pathogenesis and outcomes differ. Approximately 80% of the patients are diagnosed with type 1 (endometrioid) cancers which are estrogen related, more than 70% of the cases have stage 1 disease at diagnosis, and the five-year overall survival is approximately 83%. Type 2 non-endometrioid cancers are seen in elderly women, recognised at more advanced stages, and outcomes are worse (2,3).

The leading prognostic factor for endometrial cancer survival rates is the stage. Stage 1 patients have 91% overall survival (OS) whereas stage 4 patients have rates of 30% (4). In addition to the stage, many other prognostic factors play an important role for survival, such as age, histologic subtype, lymphovascular space invasion (LVSI), myometrial invasion (MI), histologic grade, and tumor size (4-6). Positive peritoneal cytology was removed from the International Federation of Gynecology and Obstetrics (FIGO) staging system. However, it should be noted that its prognostic significance is still controversial (7-12). In recent years, the presence of LVSI has gained importance

Address for Correspondence: *Kazibe Koyuncu*
e.mail: kazibekkoyuncu@gmail.com ORCID: orcid.org/0000-0001-9070-3962

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(6,13,14). Risk of recurrence and treatment is stratified, based on these prognostic factors (15-17).

This study was carried out to elicit the effects of prognostic factors on different types of survival, such as OS, progression-free survival (PFS) and disease-specific survival (DSS) in patients who were treated with staging surgery for endometrial cancer at a single tertiary institutional center.

Material and Methods

Patients

A retrospective observational study was conducted in a tertiary center. Institutional review board approved the study (IRB approval number: 10-42014, date: June 9th, 2014). Data of 303 patients diagnosed with endometrial cancer and treated between January 2005 and February 2014 at the Department of Gynecologic Oncology were reviewed. Patients with previous or concurrent primary cancers, who were not treated surgically, whose follow-up information was missing and surveillance time less than six months were excluded from this study. Eventually, 276 patients were selected for the study. The pathology specimens of all patients were re-evaluated by an expert gynecopathologist who has worked in this field for more than 30 years to ensure the accuracy of the diagnosis.

Surgical treatment consisted of total abdominal hysterectomy and bilateral salpingo-oophorectomy or bilateral salpingectomy, including pelvic/paraaortic lymph node dissection according to the circumstance, omentectomy and peritoneal cytology assessment. Adjuvant treatment after surgery was decided after each patient was presented and discussed at the tumor board in the light of the guidelines. Adjuvant treatment was administered as radiotherapy (RT) and/or chemotherapy (CT), including cisplatin and/or doxorubicin according to tumor characteristics and practices at the time. External beam RT and/or intravaginal brachytherapy (BT) was administered to the patients for RT. Adjuvant treatments of the patients were given in the same tertiary center, in the medical oncology or radiation oncology centers.

Demographic information, clinicopathologic features and survival status for women diagnosed with endometrial cancer and fulfilling the inclusion criteria were collected from the hospital medical records (95% of the data), national database (1% of the data) and via telephone calls with patients (4% of the data). Data included age, menopausal status, parity, extent of surgery, stage, number of dissected lymph nodes, histological subtype, tumor size, tumor grade, MI, LVSI, cervical tissue involvement, peritoneal cytology, the kind of adjuvant therapy, the appointment date and status of the patient at latest follow-up, date and location of recurrence and time of death, if applicable. Patient's co-morbidities were also documented: 75 (26.4%) of the patients had diabetes mellitus; 125 (45.2%) of them had hypertension; 32 (11.5%) had cardio-vascular disease; and 11 (0.03%) had other malignancy (four breast cancer, four colon cancer, one lung cancer, one multiple myeloma and one osteosarcoma). In order to avoid bias, disease specific and OS rates were calculated separately. For the histological classification and grading, World Health Organization criteria were used (18). Peritoneal cytology samples were obtained by either taking the fluid which is already present in the intra-abdominal cavity or after splashing the intra-abdominal cavity with 100 mL saline. The existence of malignant cells, regardless of the number, was considered to be positive peritoneal cytology.

Patients proceeded to follow up protocol after the treatment, which was every three months after the surgery for the first two years, every six months for the next consecutive three years, and subsequent annual visits were suggested.

OS was described as lifetime between initial surgery to death from any kind of reason, DSS as the lifetime between first surgery to death from disease and PFS as for the time from the initial surgery to the initial recurrence. If patient did not have recurrence or had died then OS, DSS and PFS were determined as the length of time from surgery until the last follow-up visit.

Informed consent was taken from all patients in this study.

Statistical analysis

The normality of distribution was tested by Shapiro-Wilk test. According to the results nonparametric tests were preffered. Continuous data are peresented as median (min-max). Categorical data are presented as frequency (percentage).

Univariate and multivariate analysis were used to determine independent prognostic factors. Cox proportional hazard regression analysis was used to determine hazard ratio (HR) for survivals and the ratio of increased hazard for recurrence and death.

The Kaplan-Meier method was used to establish survival curves for OS, DSS and PFS. The differences between groups were compared using the Log-rank test. Statistical analyses were performed using SPSS software, version 15.0, (IBM Inc., Chicago, IL, USA) and a p-value of less than 0.05 was noted to be statistically significant.

Results

Median (range) age of the 276 patients was 60 (25-86) years. The median follow-up time was 50 (6-141) months. Table 1 shows the demographic and clinical features. Two hundred sixty-four patients (95.7%) had endometrial cancer with endometrioid histology. Twelve patients (4.3%) had non-endometrioid histology, which consisted of seven serous, two mucinous, one clear cell, one mixed and one neuroendocrine tumor.

Surgery included total hysterectomy and bilateral salpingectomy or salpingo-oophorectomy in all cases. In 244

cases (88.4%), pelvic lymphadenectomy was performed, with a median of 26 (3-76) lymph nodes removed. One hundred and twenty-four cases (44.9%) of the patients underwent paraaortic lymphadenectomy with a median of 9 (1-36) lymph nodes removed. Peritoneal washings were obtained from 208 patients (75.4%). Nine patients (4.3%) had positive cytology.

One hundred thirty one patients (47.5%) received adjuvant therapy. Eighteen patients (6.5%) received CT alone, 89 (32.2%) received radiation therapy alone, and chemo-radiation was administered to 24 patients (8.7%). Of the 113 patients who received RT, 68 received BT alone, 17 received external beam radiation therapy alone. Of the remainder, four patients were administered extended field radiation therapy and 24 were administered combined BT and external beam radiation therapy.

Table 1. Characteristics of the endometrial tumors in two hundred seventy-six patients

Characteristics	Patients (n, %)
Grade	
G1	66 (24)
G2	146 (53.1)
G3	63 (22.09)
Stage	
1	231 (83.7)
2	11 (4)
3	28 (10.1)
4	6 (2.2)
Histological subtype	
Endometrioid	264 (95.7)
Non-endometrioid	12 (4.3)
Myometrial invasion	
<½ depth	153 (55.4)
>½ depth	123 (44.6)
Lymphovascular space invasion	
+	105 (38)
-	171 (62)
Tumor size	
<2 cm	76 (33.6)
>2 cm	150 (66.4)
Peritoneal cytology	
Negative	199 (95.7)
Positive	9 (4.3)
Menopausal status	
Premenopausal	42 (15.8)
Postmenopausal	234 (84.8)
Age <50	41 (14.9)
Age ≥50	235 (85.1)
Parity	
Nulligravida	63 (27.6)
1	14 (6.1)
>2	151 (66.3)

A total of 29 patients died, of whom 15 (5.43%) died due to the endometrial cancer. Stage, grade, histologic subtype, LVSI, age, having positive peritoneal cytology and the administration of adjuvant therapy were risk factors regarding OS in univariate analysis. Among the variables stage, grade and positive peritoneal cytology were shown to be independent risk factors in multivariate analysis. Factors evaluated for an association with OS are summarized in Table 2.

The 5-year OS was 92.1% for patients with stage 1 disease, 90% for patients with stage 2 disease, 65.9% for patients with stage 3 disease and 42.9% for patients with stage 4 disease. Figure 1 presents the Kaplan-Meier survival curves of patients based on stages (p=0.002). The 5-year OS was 96.3% for G1 disease, 92.1% for G2 disease and 70.3% for G3 disease. When the patients were recategorized as G1+2 and G3, 5-year OS was found to be 93.4% for G1+2 disease and remained 70.3% for G3 disease. Figure 2 shows the Kaplan-Meier curves of patients according to three- and two-tiered FIGO grades respectively.

The 5-year DSS was 97.7% for stage 1, 100% for stage 2, 74.7% for stage 3 and 42.9% for stage 4 (Figure 3). While stage, grade, histologic subtype, LVSI, MI, positive peritoneal cytology and the administration of adjuvant therapy were risk factors for DSS on univariate analysis, stage remained the only independent variable (p=0.005) associated with poor DSS in multivariate analysis (Table 2).

Thirty patients (10.9%) developed recurrences. Recurrences occurred at a median (range) time of 23 (3-86) months. Distribution of the recurrences' regions were as follows: vaginal apex (n=4), pelvis (n=2), lymph nodes (n=8), abdominal (n=4) and distant (n=4). The remaining nine patients had recurrences in two different areas. Recurrences were seen in 24 (9.1%) of the 264 patients with endometrioid histology and 6 (50%) of the 12 with non-endometrioid histology.

The 5-year PFS was 92.2% for stage 1 EC, 90% for stage 2 EC, 63.9% for stage 3 EC and 34.3% for stage 4 EC (p=0.002) (Figure 1). Stage, grade, histologic subtype, LVSI, age, administration of adjuvant therapy and having positive peritoneal cytology were shown to be significantly related with PFS in univariate analysis. Stage and grade retained independent significance in the multivariate analysis (Table 2). There was no statistically significant difference between the outcomes of grade 1 and 2 patients (p=0.475). The 5-year PFS of patients with grade 1, 2 and 3 tumors was 96.3%, 92.3% and 67.8% respectively (p=0.015) (Figure 2). When grade was recategorized as a binary system, the 5-year PFS was found to be 93.5% for G1+2 disease and remained 67.8% for G3 disease (p=0.015) (Figure 2).

Table 2. Multivariate and univariate analyses of the prognostic factors

	PFS			OS			DSS		
Prognostic factors	**Univariate**	**Multivariate**		**Univariate**	**Multivariate**		**Univariate**	**Multivariate**	
	p	**p**	**OR**	**p**	**p**	**OR**	**p**	**p**	**OR**
LVSI	0.001	0.567	-	0.001	0.553	-	0.002	0.923	-
MI	0.05	-	-	0.057	-	-	0.026	0.143	-
Histology	<0.001	0.443	-	<0.001	0.454	-	<0.001	0.420	-
Grade	<0.001	0.015/0.015*	3.18/3.81*	<0.001	0.013/0.015*	3.346/3.979*	<0.001	0.248/0.264*	-
Adjuvant therapy	<0.001	0.564	-	0.001	0.381	-	<0.001	0.258	-
Cytology	0.009	0.050	-	0.008	**0.024**	**5.8**	<0.001	0.070	-
Stage	<0.001	**0.002**	**2.67**	<0.001	**0.002**	**2.772**	<0.001	0.005	4.905
Tumour size	0.753	-	-	0.722	-	-	0.698	-	-
Age	0.029	0.240	-	0.03	0.257	-	0.905	-	-

*Result if the proposed two-tier grading system was used, PFS: Progression-free survival, OS: Overall survival, DSS: Disease-specific survival, LVSI: Lymphovascular space invasion, MI: Myometrial invasion, OR: Odds ratio

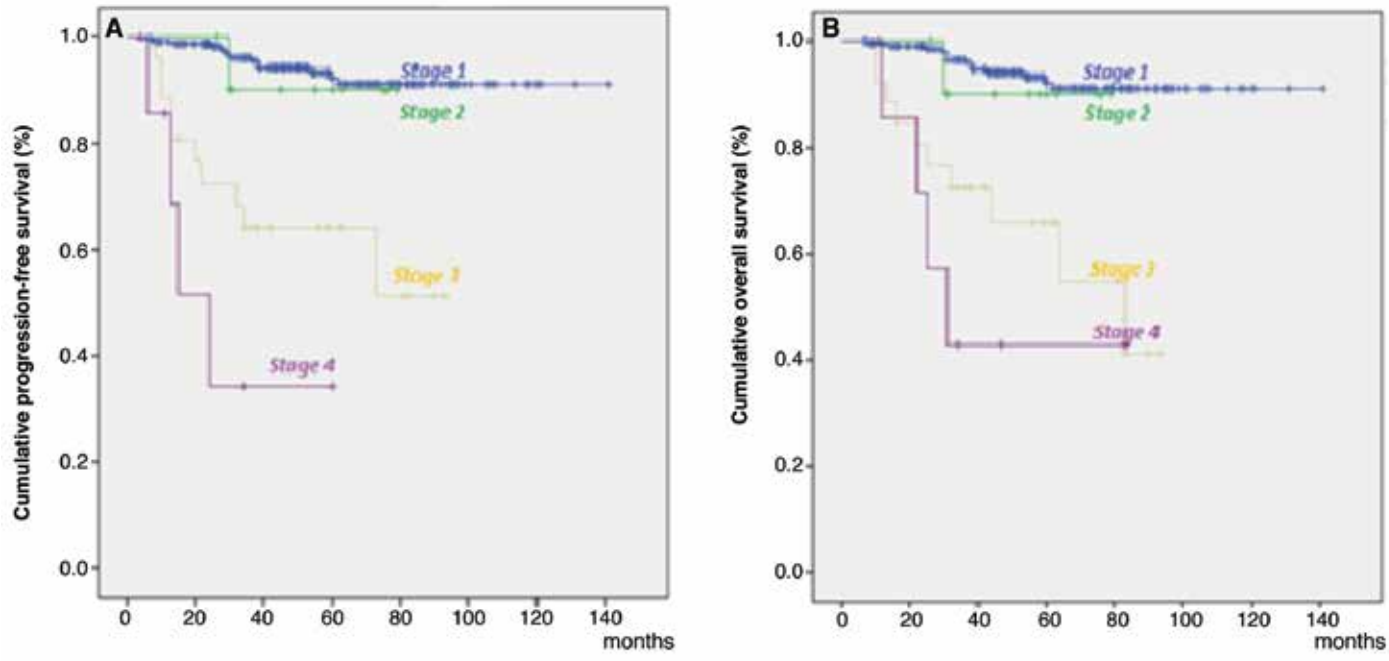

Figure 1. Kaplan-Meier survival curves for the stage. A) Progression-free survival stratified by stage. B) Overall survival stratified by stage

Discussion

Grade has been shown to be an important prognostic factor in many studies (19,20). Consistent with the literature, the grade was found to be an independent factor regarding survival in our study. However, the current FIGO grading system's reproducibility, ease of use and prognostication are being debated (21). Some studies showed that grade 1 and 2 tumors had similar survival rates which were better than grade 3 tumors (22-24). Consistent with this, our results showed that both OS and PFS rates were not statistically different regarding G1 and 2 tumors and better than G3 tumors (p=0.015 and p=0.015, respectively).

Furthermore, grade 2 tumors are not consistent in defining the recurrence risk and necessity for postoperative adjuvant treatment. Colombo et al. (15) described a guideline for "European Society for Medical Oncology" defining prognosis, treatment and follow-up of endometrial cancer which

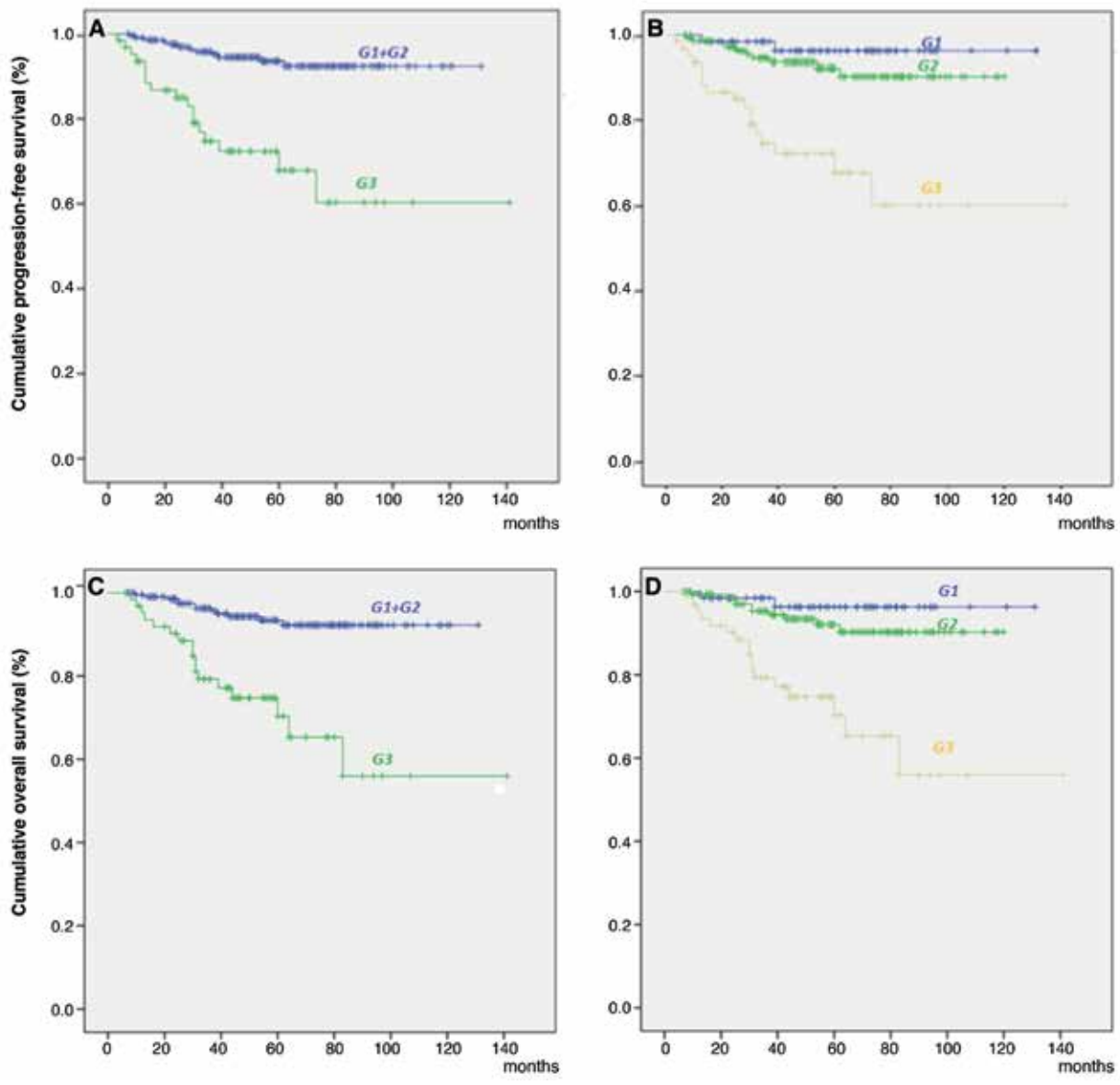

Figure 2. Kaplan-Meier survival curves for prognostic factors. A) Disease-specific survival stratified by two-tiered grade. B) Disease-specific survival stratified by three-tiered grade. C) Overall survival stratified by two-tiered grade. D) Overall survival stratified by three-tiered grade

emphasized that the decision for giving adjuvant therapy does not differ between G1 and G2 patients for stage 1A and B. Studies showed that G3 endometrioid tumors do not differ compared to patients who have papillary serous or clear-cell histology and should be considered and treated as type 2 endometrial cancers (25,26). According to early evidence and our study, it may be more practical and efficient to use a simple binary grading system where G1 and 2 were classified as a single group.

Stage is accepted as the best prognostic factor to predict survival in endometrial cancer as advancing stage is related to a poorer OS and PFS (27). The approximate 5-year survival rates for stage 1, 2, 3 and 4 EC disease are 80-90%, 80%, 50-70% and 20% respectively (3,4). Except for higher survival rates of stage 2 patients with EC and stage 4 patients with EC, survival rates in our study are in accordance with the literature, as there was a significant reduction with advancing stages. An explanation for this finding may be related to the relatively small number of patients with stage 2 (n=11) and 4 (n=6) disease in our population.

Given that peritoneal cytology is not a part of surgical staging, the relationship between survival and peritoneal cytology

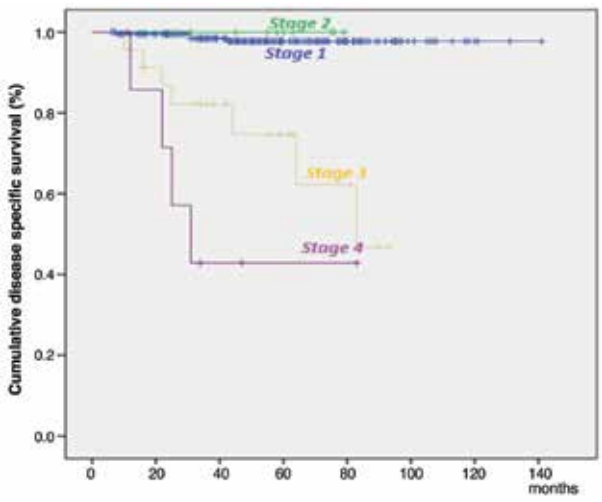

Figure 3. Kaplan-Meier survival curves for stage

particularly is still very controversial in early-stage patients. While some studies showed positive peritoneal cytology is related to high rates of recurrences and poor survival (7,10,11), some found out no relationship between positive peritoneal cytology and survival (28,29). Seagle et al. (30) analyzed data from the National Cancer Database and reported that adjuvant CT provides better survival in patients who were diagnosed as an early endometrioid type of endometrial cancer patients with positive peritoneal cytology. In the literature, it was suggested that having positive peritoneal cytology might be related to worse prognosis in alliance with other adverse prognostic factors (31). In the literature non-endometrioid histology was shown to be related to positive peritoneal cytology (31). In the present study there were only 12 non-endometrioid patients, and thus it was not possible to draw any firm conclusion, due to the low number of patients. Furthermore, in our cohort positive peritoneal cytology incidence was 4.3% and less than in the literature (8,32). This may be related to the low number of high-risk patients. Although positive peritoneal cytology emerged as an independent prognostic factor for OS (p=0.024, HR: 5.8, 95% confidence interval: 4.98-7.01), it did not quite achieve statistical significance in the multivariate analysis (p=0.050) but was also related with poor PFS (p=0.009). Despite not conducting a subgroup analysis, our findings support the suggestion that positive peritoneal cytology adversely affects survival besides grade, irrespective of the given adjuvant therapy.

MI has long been recognized as a prognostic factor (19). Although it did not reach statistical significance, MI deeper than half of the myometrium was associated with shorter OS and PFS (p=0.057, and p=0.05, respectively). For DSS, MI was shown to be a poor prognostic factor but not an independent one (p=0.026). The decrease in the survival rates could be explained by increased lymph node involvement with deeper MI. In previous studies, deep MI was reported to be related to higher rates of nodal involvement (19,33).

LVSI is another well-documented prognostic factor in endometrial cancer. Patients with LVSI have 5.8 times the increased risk of recurrence (34). Guntupalli et al. (13) showed that LVSI had a 95% negative predictive value for nodal disease. In the present study, we also showed that patients with LVSI have poorer survival rates compared to the patients without LVSI. In addition to this, being a prognostic factor, LVSI is also used in the risk stratification systems. Several authors place LVSI positive patients into the high-intermediate risk category and suggest these patients could benefit from adjuvant RT (35-37).

A strength of this study is the uniform management of patients since this study was conducted in a single center. Surgical management of the patients differs between countries and even within countries. In our clinic, full staging with pelvic and para-aortic lymphadenectomy in the 2000s and early 2010s was preferred, which comprised most of the patients in this study and enabled us to know the definite stage of the patients and administration of the adjuvant therapy accurately. After abandoning lymphadenectomy in low-risk patients, some of the advanced staged patients stay under-staged which is another issue that is an ongoing debate. Uterine risk factors and adjuvant treatments are known in detail in this study. Another strength of the study is that patients were monitored for a long time and this has enabled us to better understand risk factors for recurrence.

Study limitations

The limitations of the study are the retrospective design of this research, small numbers of patients in two of the EC grading groups and the small number of patients with positive cytology.

Conclusion

Advanced stage, grade 3 tumor and positive peritoneal cytology were regarded as independent prognostic factors for endometrial cancer. Since grade 1 and 2 tumors show similar prognosis, a binary grading system combining these two grades could be simpler. Removing grade 2 from the current grading system may also improve risk stratification and help to eliminate confusion regarding adjuvant therapy.

Positive peritoneal cytology is not a part of staging, but in several studies, including the present study, the findings showed that positive peritoneal cytology is a poor prognostic factor. Thus, it may be clinically useful for risk stratification to plan adjuvant treatment.

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Informed Consent: *: Informed consent was taken from all the participants.*

Peer-review: *Externally peer-reviewed.*

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The comparison of pegylated liposomal doxorubicin and beta-carotene effects on JAR and JEG-3 choriocarcinoma human cell culture models

Seyit Ahmet Erol[1], Görker Sel[2], Mehmet İbrahim Harma[3], Müge Harma[3], İshak Özel Tekin[4]

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

[2]Clinic of Obstetrics and Gynecology, Zonguldak Bülent Ecevit University Health Practice and Research Hospital, Zonguldak, Turkey

[3]Clinic of Obstetrics and Gynecology, Division of Gynecologic Oncology, Zonguldak Bülent Ecevit University Health Practice and Research Hospital, Zonguldak, Turkey

[4]Clinic of Immunology, Zonguldak Bülent Ecevit University Health Practice and Research Hospital, Zonguldak, Turkey

Abstract

Objective: The aim was to investigate the effectiveness of pegylated liposomal doxorubicin (PLD), beta-carotene, and a combination of PLD and beta-carotene on JAR and JEG-3 human choriocarcinoma (CC) cell lines for the treatment of CC.

Material and Methods: JAR and JEG-3 cells were cultured. PLD and beta-carotene trial groups were determined with different doses (for single drug trial; PLD 1, 2, 5 μg/mL and beta-carotene 1, 5, 10 μg/mL, and for combined drug trial; all PLD doses combined with beta-carotene 5 μg/mL). Drugs were administered to cultures simultaneously, and 72 hours later the cells were detached using trypsin-ethylenediamine tetraacetic acid solution. The percentage of apoptotic cells was determined by flow cytometry after annexin V staining. One set of the supernatant was collected before trypsin application to investigate beta-human chorionic gonadotropin (β-hCG) and hyperglycosylated hCG (H-hCG) levels. Statistical analyses of the apoptotic ratios were performed using Shapiro-Wilk, Kruskal-Wallis and Mann-Whitney U tests.

Results: Apoptosis increased in JAR and JEG-3 cultures after treatment with all doses of PLD ($p<0.05$). A single application of each beta-carotene dose increased apoptosis in JAR cells ($p<0.05$) but had no apoptotic effects on JEG-3 cells. In the PLD and beta-carotene combination group, apoptosis increased in both JAR and JEG-3 cells ($p<0.05$).

Conclusion: To our knowledge, this is the first investigation of the effectiveness of PLD, beta-carotene, and PLD + beta-carotene combination therapy in two different CC cell lines. PLD is a promising chemotherapeutic drug, and beta-carotene can be used as a novel non-chemotherapeutic agent for treatment of CC. Based on the results of this study, vitamin A supplementation may have promise as a preventive measure. However, these data need support from animal experiments and clinical trials. (J Turk Ger Gynecol Assoc 2020; 21: 171-9)

Keywords: Pegylated liposomal doxorubicin, beta-carotene, choriocarcinoma, JAR cell culture, JEG-3 cell culture

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Introduction

Gestational trophoblastic disease (GTD) appears in the fetal chorion and involves a variety of interrelated diseases. The effects of GTD range from benign hydatidiform moles (HM), which usually resolve spontaneously, to life-threatening. GTD usually develops from HM, but it has been observed in aborted, term, and ectopic pregnancies. Choriocarcinoma (CC) is the most aggressive histologic type of GTD (1). The most

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Address for Correspondence: *Seyit Ahmet Erol*

e.mail: gyn.aerol@gmail.com ORCID: orcid.org/0000-0002-2494-4896

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common clinical biomarker for the diagnosis and follow-up of this disease is serum beta-human chorionic gonadotropin (β-hCG). β-hCG follow-up should be carried out in series in terms of the course and behavior of the disease and after the treatment period. However, hCG produced in CC has a larger oligosaccharide side chain than hCG synthesized in normal pregnancy and is known as hyperglycosylated hCG (H-hCG) (2).

There are many medical treatments for CC depending on the stage of the disease. Multi-agent chemotherapy protocol is the preferred treatment method if hCG levels increase during treatment, if metastasis develops, or a resistance develops to sequential single-agent chemotherapy protocol (3). The current evidence-based initial therapy in treatment of high-risk metastatic GTD is the etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine (EMA-CO) protocol. Drug resistance may develop during or after primary chemotherapy in approximately 20% of high risk gestational trophoblastic neoplasia patients. In patients who do not respond to first-line EMA-CO therapy or for those who relapse, the most appropriate second-line therapy is the etoposide, methotrexate, actinomycin D, etoposide, cisplatin (EMA-EP) protocol. However, this protocol is quite toxic (4). The International Society of the Study of Trophoblastic Diseases has reported that different treatment regimens such as paclitaxel/etoposide may be effective in relapsing patients after paclitaxel/cisplatin based combination therapies, but more studies should be conducted in this regard (5).

Doxorubicin is an anthracycline antibiotic which intercalates between base pairs in the DNA helix, thereby preventing DNA replication and ultimately inhibiting protein synthesis (6). Pegylated liposomal doxorubicin (PLD) is a polyethylene glycol-coated form of doxorubicin that has fewer side effects than those of doxorubicin, and it is approved for the treatment of HIV-related Kaposi's sarcoma, metastatic breast cancer, advanced ovarian cancer and multiple myeloma (7).

Beta-carotene is a naturally-occurring retinol (vitamin A) precursor obtained from certain fruits and vegetables with potential antineoplastic and chemopreventive activities. It is particularly protective in chemical carcinogenesis by taking part in the detoxification of peroxide radicals (8). It was reported that the incidence of complete hydatidiform mole decreased due to increase in carotene consumption. Parazinni et al. (9) suggested that low beta-carotene consumption is related to GTD.

The options that can be used against multiple drug resistance in the treatment of CC are limited in the literature. Therefore, the aim of this study was to investigate the effects of PLD and beta-carotene and treatment efficacy on cell culture CC models using JAR and JEG-3 cell cultures in order to provide more effective treatment methods by providing a new perspective in CC treatment.

Material and Methods

JAR and JEG-3 cell culture lines

This study was planned as pre- and post-test study. JAR and JEG-3 cell culture lines were obtained from the American Tissue Type Culture Collection. All cell cultures were maintained and cultured in Roswell Park Memorial Institute (RPMI) - 1640 medium (INTERLAB Laboratory Products, İstanbul, Turkey) supplemented with 10% heat-inactivated fetal calf serum, (DATEKS Technical Systems, Ankara, Turkey) penicillin streptomycin, and L-glutamine (BRK Chemistry and Biotechnology, İzmir, Turkey) in a 98% humidified, 5% CO_2 atmosphere at 37 °C in a Nuve EC 160 CO_2 incubator in 75 cm^2 flasks.

Pegylated liposomal doxorubicin preparation

PLD was purchased from Sigma-Aldrich Chemie GmbH, Germany, prepared in dosages of 1, 2 and 5 μg/mL by diluting in dimethyl sulfoxide (DMSO) (DATEKS Technical Systems, Ankara, Turkey), and diluted in RPMI-1640 to a maximum concentration such that DMSO formed less than 1% of the mixture.

Beta-carotene preparation

Beta-carotene was purchased from Sigma-Aldrich Chemie GmbH, Germany, prepared in dosages of 1, 5 and 10 μg/mL by diluting in DMSO, and diluted by RPMI-1640 to a maximum concentration such that DMSO formed less than 1% of the mixture.

Preparation of chemotherapeutics for tests, β-hCG and H-hCG measurement

The dosages used in the PLD and beta-carotene trial groups were as follows:

Single drug trial: PLD 1, 2 and 5 μg/mL; beta-carotene 1, 5 and 10 μg/mL.

Combined PLD and beta-carotene drug trial: PLD 1, 2 and 5 μg/mL; and beta-carotene 5 μg/mL.

Drugs were administered to the cells simultaneously, and 72 hours after drug administration, the cells were detached from the bottom of plate by using trypsin ethylenediamine tetraacetic acid (EDTA) solution. The degree of apoptosis was determined by flow cytometry (FCM). The supernatant was collected before trypsin application from one set of samples from each experiment and stored at -80 °C frozen to investigate H-hCG levels. H-hCG levels were investigated using an immunoenzymatic method (Sunred Elisa Kit and DXI 600, Beckman Coulter, CA, USA). All tests were repeated six times.

Detection of apoptosis using annexin V

The annexin V-binding assay is one of the most sensitive and widely used techniques to detect and distinguish between early apoptosis and late apoptosis, as well as between apoptosis and necrosis. Annexin V is a protein that binds preferentially to phosphatidylserine, which is located at the outer surface of the cell membrane. This feature allows apoptotic cells to be observed after marking them with a fluorescent agent such as fluorescein isothiocyanate (FITC) (10). The binding ratio of FITC-annexin-V complex to phosphatidylserine at the cell membrane can be measured using FCM.

Ethics committee approval was received for this study from the Ethics Committee of Bülent Ecevit University Faculty of Medicine (approval number: 2014-68-25/03, date: 03/25/2014). No informed consent was obtained due to cell culture study.

Statistical analysis

Statistical analyses of degree of apoptosis were performed with the SPSS, version 19.0 package program. Descriptive statistics related to continuous variables are given as median and range (minimum and maximum values). The conformity of variances to normal distribution was evaluated through the Shapiro-Wilk test. The Kruskal-Wallis test was used in quaternary comparison of doses and the Mann-Whitney U test and the Mann-Whitney U test with Bonferonni correction were used in binary subgroup comparison between doses. In all statistical analysis in the study, comparisons below a p-value of 0.05 were accepted as statistically significant.

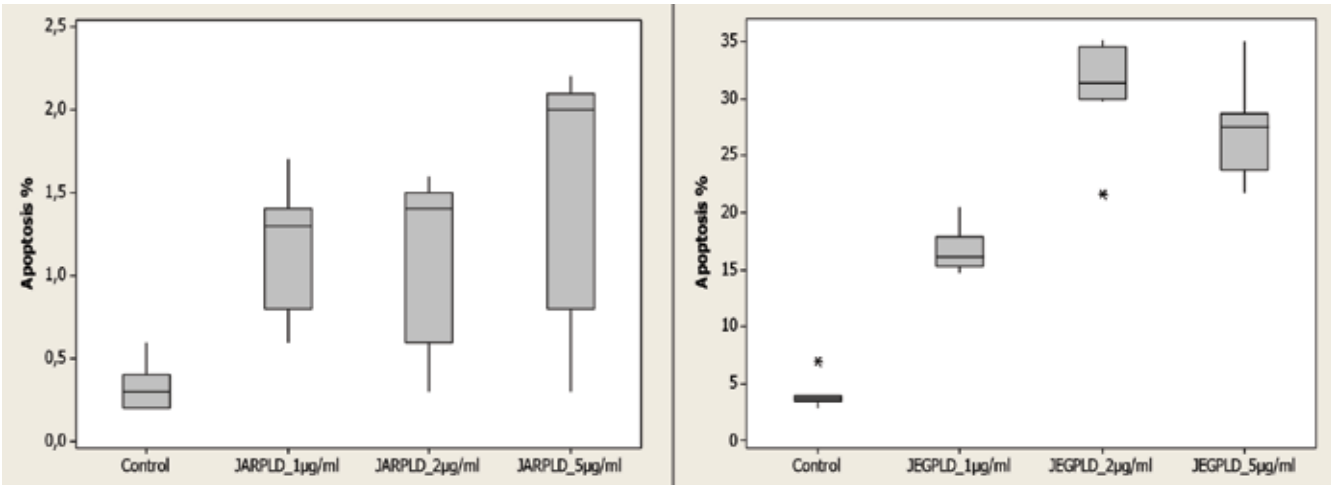
Results

The median apoptotic ratio in the control group, in which only DMSO was used, was 0.3% in the JAR cell culture and was 3.7% in the JEG-3 cell culture. The median apoptotic ratios after the application of 1, 2 and 5 μg/mL PLD were; 1.3, 1.4 and 2% in the JAR cells, respectively. The increase in the apoptotic ratio was statistically significant ($p<0.05$) (Table 1, Graph 1). The median apoptotic ratios after the application of 1, 2 and 5 μg/mL PLD were; 16.1, 31.4 and 27.5% in the JEG-3 cells, respectively. The increase in the apoptotic ratio was also statistically significant ($p<0.05$) (Table 1, Graph 1).

After 72 hours, median β-hCG levels in JAR and JEG-3 cell cultures were respectively, 126 and 118 mIU/mL. Median H-hCG levels in JAR and JEG-3 cell cultures were respectively, 63 and 66 mIU/mL (Table 2). After the application of 1, 2 and 5 μg/mL PLD to JAR cell lines, median β-hCG levels were: 115, 106 and 117 mIU/mL, respectively; median H-hCG levels were: 64, 60 and 64 mIU/mL, respectively (Table 2); in JEG-3 cell lines, median β-hCG levels were: 127, 114 and 118 mIU/mL, respectively and median H-hCG levels were: 63, 61 and 59 mIU/mL, respectively (Table 2). Median β-hCG and H-hCG

Table 1. Ratio of apoptosis (%) on control group and after application of PLD to JAR and JEG-3 cell lines

	Ratio of apoptosis; median (minimum-maximum) in JAR ($p<0.05$)	Ratio of apoptosis; median (minimum-maximum) in JEG-3 ($p<0.05$)
Control	0.3 (0.6-0.2)	3.7 (2.9-7)
PLD 1 μg/mL	1.3 (0.6-1.7)	16.1 (14.7-20.5)
PLD 2 μg/mL	1.4 (0.3-1.6)	31.4 (21.6-35.1)
PLD 5 μg/mL	2 (0.3-2.2)	27.5 (21.8-35)
*Shapiro Wilk, Mann-Whitney U and Kruskal-Wallis tests, PLD: Pegylated liposomal doxorubicin		



Graph 1. Ratio of apoptosis (%) on control group and after application of pegylated liposomal doxorubicin to JAR and JEG-3 cell lines ($p<0.05$)

Shapiro-Wilk, Mann-Whitney U and Kruskal-Wallis tests, PLD: Pegylated liposomal doxorubicin

levels after application of PLD were found to be statistically similar despite increasing PLD dose (p>0.05).

The median apoptotic ratios after the application of 1, 5 and 10 μg/mL beta-carotene were 0.35, 0.6, and 0.8% in the JAR cells, respectively. The increase in the apoptotic ratio was statistically significant (p<0.05) (Table 3, Graph 2). The median apoptotic ratios after the application of 1, 5 and 10 μg/mL beta-carotene were 3.7, 2.5, and 2.9% in the JEG-3 cells, respectively. The increase in the apoptotic ratio was found to be statistically similar despite increasing beta-carotene dose (p>0.05) (Table 3, Graph 2).

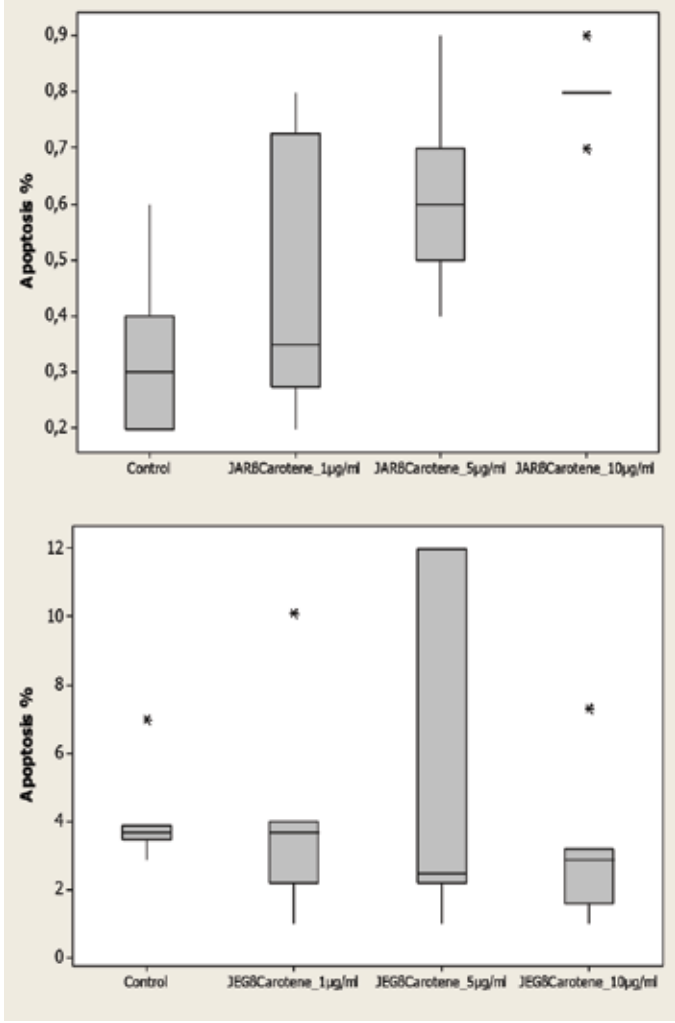
After the application of 1, 5 and 10 μg/mL beta-carotene to JAR cell lines, median β-hCG levels were: 107, 115 and 111 mIU/mL, respectively; median H-hCG levels were: 64, 68, and 56 mIU/mL, respectively (Table 2). In JEG-3 cell lines, median β-hCG levels were: 116, 121 and 115 mIU/mL, respectively and median H-hCG levels were: 63, 63, and 64 mIU/mL, respectively (Table 2). Median β-hCG and H-hCG levels after application of beta-carotene were found to be statistically similar despite increasing beta-carotene dose (p>0.05).

The median ratios of apoptosis were 1.3, 1.4 and 2% after application of 1, 2 and 5 μg/mL PLD in JAR cell cultures, respectively; as mentioned above. With the addition of 5 μg/mL beta-carotene to those PLD doses, and after application of 5 μg/mL beta-carotene combined with 1, 2 and 5 μg/mL PLD, the median apoptotic ratios were 0.4%, 1% and 1%, respectively. This incremental increase of the combined doses were statistically significant in comparision with the control group (p<0.05), but the incremental increase in doses of PLD alone were statistically significant compared to 5 μg/mL beta-

Table 2. H-hCG and β-hCG levels on control group and after application of pegylated liposomal doxorubicin, beta-carotene and combined doses to JAR and JEG-3 cell lines

	H-hCG mIU/mL (p>0.05)	β–hCG mIU/mL (p>0.05)
JAR control	63	126
JAR PLD 1 μg/mL	64	115
JAR PLD 2 μg/mL	60	106
JAR PLD 5 μg/mL	64	117
JAR beta-carotene 1 μg/mL	64	107
JAR beta-carotene 5 μg/mL	68	115
JAR beta-carotene 10 μg/mL	56	111
JAR PLD 1 μg/mL + beta-carotene 5 μg/mL	54	107
JAR PLD 2 μg/mL + beta-carotene 5 μg/mL	63	107
JAR PLD 5 μg/mL + beta-carotene 5 μg/mL	58	111
JEG-3 control	66	118
JEG-3 PLD 1 μg/mL	63	127
JEG-3 PLD 2 μg/mL	61	114
JEG-3 PLD 5 μg/mL	59	118
JEG-3 beta-carotene 1 μg/mL	63	116
JEG-3 beta-carotene 5 μg/mL	63	121
JEG-3 beta-carotene 10 μg/mL	64	115
JEG-3 PLD 1 μg/mL + beta-carotene 5 μg/mL	63	109
JEG-3 PLD 2 μg/mL + beta-carotene 5 μg/mL	59	117
JEG-3 PLD 5 μg/mL + beta-carotene 5 μg/mL	52	111

*Shapiro Wilk, Mann Whitney U and Kruskal-Wallis tests.
PLD: Pegylated liposomal doxorubicin, H-hCG: hyperglycosylated-human chorionic gonadotropin, β-hCG: beta-human chorionic gonadotropin



Graph 2. Ratio of apoptosis (%) on control group and after application of beta-carotene to JAR and JEG-3 cell lines (p<0.05, p>0.05, respectively)

Shapiro-Wilk, Mann-Whitney U and Kruskal-Wallis tests

carotene combined with PLD ($p<0.05$) (Table 4, Graph 3). However combination of PLD and beta-carotene had no effect on β-hCG and H-hCG levels in JAR cell cultures which were between 54-63 IU/mL for H-hCG and 107-111 IU/mL for β-hCG ($p>0.05$) (Table 2).

The median ratios of apoptosis were 16.1, 31.4 and 27.5% after application of 1, 2, and 5 μg/mL PLD in JEG-3 cell cultures, respectively; as mentioned above. With the addition of 5 μg/mL beta-carotene to those PLD doses, and after application of 5 μg/mL beta-carotene combined with 1, 2, and 5 μg/mL PLD, the median apoptotic ratios were 6.1%, 5.7% and 15.5%, respectively. This incremental increase of the combined doses were statistically significant in comparision with the control group ($p<0.05$), but the incremental increase when PLD was used alone were statistically significant in comparision to the combination of PLD at different concentrations and 5 μg/mL beta-carotene ($p<0.05$) (Table 4, Graph 3). After treatment with PLD and beta-carotene in JEG-3 cell cultures, H-hCG and β-hCG levels were between 52-63 IU/mL and 109-117 IU/mL, respectively ($p<0.05$) (Table 2).

Table 3. Ratio of apoptosis (%) on control group and after application of beta-carotene to JAR and JEG-3 cell lines

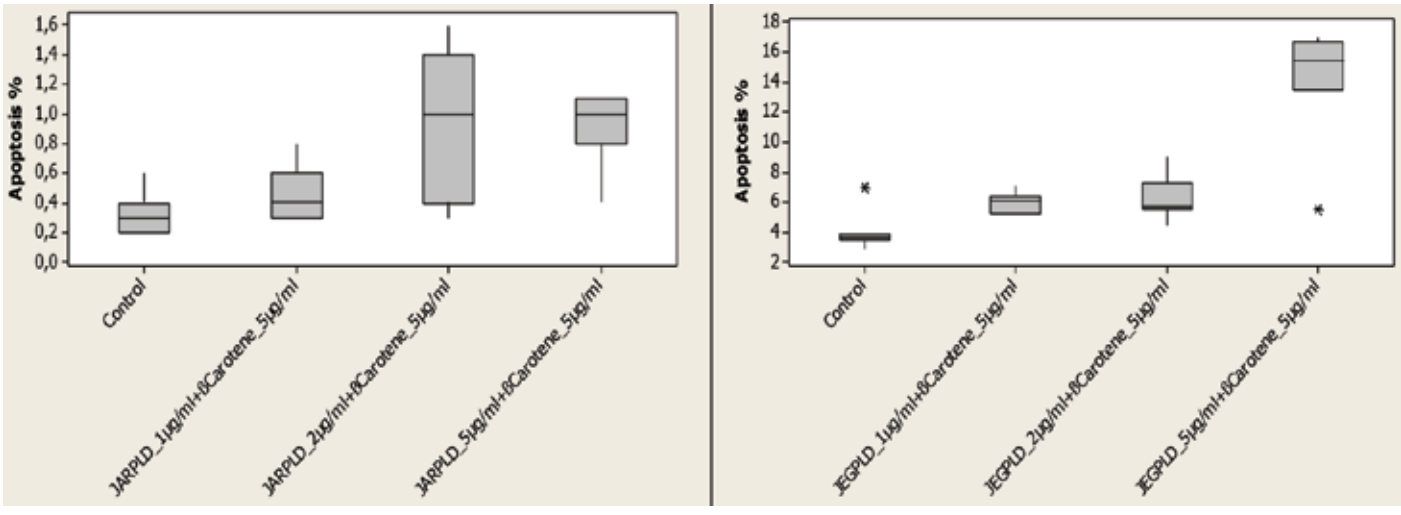
	Ratio of apoptosis; median (minimum-maximum) in JAR ($p<0.05$)	Ratio of apoptosis; median (minimum-maximum) in JEG-3 ($p<0.05$)
Control	0.3 (0.2-0.6)	3.7 (2.9-7)
Beta-carotene 1 μg/mL	0.35 (0.2-0.8)	3.7 (1-10.1)
Beta-carotene 5 μg/mL	0.6 (0.4-0.9)	2.5 (1-12)
Beta-carotene 10 μg/mL	0.8 (0.7-0.9)	2.9 (1-7.3)
*Shapiro Wilk, Mann-Whitney U and Kruskal-Wallis tests, PLD: Pegylated liposomal doxorubicin		

Discussion

Cell culture studies are frequently employed, especially in the evaluation of newer drugs. This is because it enables determining the effects caused by candidate drugs on molecular targets, which are common or are exptected to be effected by those drugs, and predicting the effects that the drug will have on target tissue. Recurrent tumor growth due

Table 4. Ratio of apoptosis (%) on control group and after application of combined doses of pegylated liposomal doxorubicin and beta-carotene to JAR and JEG-3 cell lines

	Ratio of Apoptosis; Median (minimum-maximum) in JAR ($p<0.05$)	Ratio of Apoptosis; Median (minimum-maximum) in JEG-3 ($p>0.05$)
Control	0.3 (0.2-0.6)	3.7 (2.9-7)
PLD 1 μg/mL + beta-carotene 5 μg/mL	0.4 (0.3-0.8)	6.1 (5.2-7.1)
PLD 2 μg/mL + beta-carotene 5 μg/mL	1 (0.3-1.6)	5.7 (4.4-9)
PLD 10 μg/mL + beta-carotene 5 μg/mL	1 (0.4-1.1)	15.5 (5.5-17)
*Shapiro Wilk, Mann-Whitney U and Kruskal-Wallis tests, PLD: Pegylated liposomal doxorubicin		



Graph 3. Ratio of apoptosis (%) on control group and after application of combined doses of pegylated liposomal doxorubicin and beta-carotene to JAR and JEG-3 cell lines ($p<0.05$)

***Shapiro-Wilk, Mann-Whitney U and Kruskal-Wallis tests, PLD: Pegylated liposomal doxorubicin**

to unpredicted changes caused by newly developed cancer drug components on cell phenotype may lead to unexpected results in clinical practice. The use of human monolayer cancer cell lines is fairly common and an important method for understanding the causes of these unexpected results obtained in clinical practice (11). JAR and JEG-3 cells are frequently used in CC cell culture studies, so these cells lines were chosen for our study.

Various drugs have cytotoxic effects on cells through either necrosis or apoptosis or a combination of these. It is a preferred characteristic of drugs used or being developed to be used in cancer treatment that the cytotoxic effects function in apoptotic pathways, as one of the mechanism leading to cancer is disruption of the normal apoptotic mechanism of a pre-cancerous cell, and domination of anti-apoptotic signal pathways of the cell on apoptotic pathways. Thus, an aim of this study was to analyze the apoptotic effect due to the use of PLD and beta-carotene, alone or in combination. In this study, the degree of apoptosis of JAR and JEG-3 cells was identified with FCM.

GTD is an interrelated group of tumors, characterized by abnormal proliferation of placental trophoblasts (12). Benign GTD consists of placental site nodules, exaggerated placental site and hydatidiform mole (complete or partial). There is potential for local invasion and distant metastasis. Malign GTDs are a persistent form of GTD usually CC, which may be grouped into three types: gestational trophoblastic neoplasia (GTN); placental site trophoblastic tumor (PSTT); and epithelioid trophoblastic tumor, which is a variation of PSTT (13).

Complete molar pregnancy risk increases in those with dietary deficiency of vitamin A (carotene) and animal fat, while there is no increase in partial molar pregnancy incidence (9). CC is seen once in every 50.000 pregnancies (14). Advance maternal age, previous molar pregnancy and blood type A carrier increase the risk (15). Patients with stage 4 or stage 2-3 and risk score of >7 are accepted as high risk and they are aggressively treated with multiple agent chemotherapy and/or adjuvant radiotherapy/surgery. Remission and survival rates in high-risk patients have improved with use of the EMA-CO protocol (16). EMA-CO protocol is the first-line treatment, based on recent evidence in metastatic GTD in high-risk patients. The optimum second-line treatment in patients not responding to first-line treatment, defined as a low plateau in measured hCG or relapsing levels of hCG after complete response, is the EMA-EP protocol. Cyclophosphamide and vincristine present in the EMA-CO protocol are replaced by etoposide and cisplatin in EMA-EP protocol (4). At present, the clinical use of PLD in CC appears to be limited and only in selected cases.

All forms of GTD are characterized by β-hCG increase due to the interrelated, heterogeneous structure of GTD, especially arising from trophoblastic epithelium of the placenta, and gestational tissue is responsible for pathogenesis of GTD. HCG is used as a biomarker for diagnosis and follow-up of the disease and response to treatment. H-hCG is a glycosylated variant of β-hCG with a larger side chain produced by invasive cytotrophoblast cells in pregnancy implantation, GTD and CC. An increase in H-hCG levels has been reported in testicular and ovarian germ cell tumors (17). Thus, the effect of beta-carotene and PLD on CC cell culture lines were measured by using both β-hCG and H-hCG together with the ratio of measures of apoptosis. This is the first report of the effects of the combination of beta-carotene and PLD on JAR and JEG-3 and the measurement of β-hCG and H-hCG by immunoenzymatic methods.

Cole et al. (17) showed in their study analyzing the biological function of H-hCG in BeWo, JAR, and JEG-3 cell culture and isolated gravid cytotrophoblast cells that H-hCG values were 841 ng/mL, 126 ng/mL, 165 ng/mL and 2.3 ng/mL, respectively and thus H-hCG was a biological tumor marker indicating cell invasion in active CC. Rubin et al. (18) evaluated the usability of H-hCG in the differentiation of benign parathyroid disease (n=18) and parathyroid cancer (n=8) and reported that H-hCG levels in the patient group with cancer were higher than 3.77 pmol/L which was the maximum concentration for patients with benign primary hyperparathyroid and thus, H-hCG could be a possible tumor marker.

PLD is a long-release formulation consisting of doxorubicin hydrochloride contained in pegylated liposomes. In breast cancer patients with increased cardiac risk PLD is administered and is also used in advanced ovarian cancer cases when platinum-based chemotherapy is unsuccessful and acquired immuno-deficiency syndrome with Kaposi sarcoma. Embedding doxorubicin into liposomes causes changes in pharmacokinetics and biodistribution and is reported to decrease toxic effects (19). Soininen et al. (20) investigated quantitative cellular intake and toxicities of doxorubicin and other liposomal forms with different concentrations (0.5 and 5 μM) in human placental CC cell culture (BeWo). These authors reported that fetal exposure decreased due to the pegylated formulation with lower cellular intake and toxicity of PLD compared to doxorubicin.

Eetezadi et al. (21) evaluated the intratumoral penetration and efficacy of block copolymer micella doxorubicin (BMC-DOX) formulation, with similar toxicity to PLD measured by the effects on microscopic lesions in HEYA8, OV-90 and SKOV3 human ovarian cell cultures, for second-line treatment of recurring ovarian cancer after cytoreduction that BCM-DOX in increasing doses between 2 mg/mL and 7.6 mg/mL provided nine-fold greater monolayer cytotoxicity compared to PLD. These authors suggested in vivo studies were required to confirm their in vitro results.

Hanf et al. (22) evaluated oxidative stress, apoptosis, phosphoproteome and epigenome changes due to doxorubicin in a cardiomyocyte cell culture model of doxorubicin cardiotoxicity. They reported that caspase-3 and fractin, which are markers of apoptosis, and 3-nitrotyrosine and malondiadehyde, which are markers of oxidative stress, increased in a dose-dependent manner following administration of (H9c2) doxorubisin at 24 and 48 hours at concentrations of 1 and 5 μM in cultured rat myoblasts and in addition histone-3 acetylation decreased. They concluded that apoptosis related to oxidative stress caused cell death in this model.

Popadiuk and Power (23) showed that there was a complete response, especially against brain metastasis, when PLD only was administered in 2-3 cycles in two patients with multiple organ metastatis recurring after standard chemotherapy and radiotherapy regimens and concluded that PLD was an active agent in high-risk CC. Essel et al. (24) stated that multiple salvage chemotherapy regimen was effective in patient group with GTN in whom standard treatment regimens had been unsuccessful and that liposomal doxorubicin was an effective treatment regimen.

Saul et al. (25) showed that the presence of folic acid receptors modified the effects of liposomal doxorubicin at doses of 10 μM in KB, C6 glioma cells and E9 cortex cells. Similarly, Lee and Low (26) identified folate-PLD complexes at doses of 10 μM, 20 μM and 50 μM and 20 μM folic acid and stated that these complexes had higher affinity in targeting cancer cells.

Pariente et al. (27) evaluated the in vitro effect of melatonin on the cytotoxic and pro-apoptotic actions of chemotherapeutic agents including cisplatin (CIS), 5-fluorouracil (5-FU) and DOX in cervical cancer HeLa cells. It was shown that melatonin increases the cytotoxic effect of the chemotherapeutic agents, caspase-3 activation in CIS- and 5-FU-challenged cells and also elevated the ratio of the cells which enter mitochondrial apoptosis due to the production of reactive oxygen species. HeLa cell viability was approximately 73.1% after administration of 20 μM doxorubicin alone whereas this decreased to 57.9% when administered in combination with 1 mM melatonin. Doxorubicin alone was found to cause an approximately 12-fold elevation in caspase-3 activity whereas a 17-fold elevation was shown when administered in combination with 1 mM melatonin. The administration of 20 μM CIS alone induced an approximately 53% apoptosis in HeLa cells whereas an apoptosis of 73% was demonstrated when 20 μM CIS and 1 mM melatonin were concomitantly administered. It was concluded that indoleamine can be applied as a potentially strong synergistic agent in the treatment of cervical cancer (27).

Similarly, it has been shown that in vitro melatonin potentiates cytotoxic and apoptotic effects of CIS and particularly 5-FU by stimulating MT3 receptor in the human colorectal adenocarcinoma cells (HT-29) and HeLa cells (28). Cell viability rates in HT-29 and HeLa cells were approximately 30.7% and 22.7% respectively, after administration of 1 mM 5-FU alone, whereas these were approximately 11.1% and 10.7% after combined administration of 1 mM 5-FU and 1 mM melatonin, respectively. Apoptosis rates in HT-29 and HeLa cultures were approximately 29% and 45% after administration of 20 μM CIS alone, whereas these rates were 45% and 50% after administration in combination with 1 mM melatonin, respectively. Apoptosis rates induced by 5-FU alone were approximately 46% and 47% whereas these rates were 71% and 65% after concomitant treatment of 5-FU and 1 mM melatonin, respectively. These increased rates of apoptosis after administration in combination with melatonin was significant ($p<0.05$). A further study reported that melatonin increases the efficacy of CIS and 5-FU in HT-29 cells. Apoptosis rate after administration of 1 mM 5-FU alone was 24.1% whereas apoptosis reached 30% when administered in combination with 1 mM melatonin. It was concluded that melatonin increased the sensitization of HT-29 cells to 5-FU treatment and thus indoleamine could be used as a potential chemosensitizing agent in the treatment of adenocarcinoma (29).

There are studies showing that carotenoids are protective against head, neck, mouth, skin, lung and other malignancies and hematopoietic diseases. Furthermore, increased dietary intake of carotenoids (beta-carotene, alfa-carotene, lycopene, beta cryptoxanthin, lutein and zeaxanthin) was associated with lower oesophagus cancer risk. Most of the in vitro studies recently focused on anti-carcinogenic mechanism of beta-carotene in lung, liver and blood cells. In some animal studies, alfa-carotene demonstrated a higher suppressor activity on liver, lung, skin and colon carcinogenesis compared to beta-carotene (30).

Studies have shown that the molecular protective mechanisms of carotenoids in isolated human cell culture include: (1) stopping the cell cycle in G1/G0 phase by decreasing cycline D1 levels; (2) apoptosis induction downregulating survivin levels; (3) increase in cellular gap junction communication and (4) angiogenic effect through modulation of various cytokines including decreased interleukin-6 (IL-6), IL-1b, tumour necrosis factor alpha and granulocyte-macrophage colony-stimulating factor levels and increased IL-2 and TIMP metallopeptidase inhibitor 1 (TIMP-1) levels (30,31). Chemotherapeutic effects via similar mechanisms have been reported for all-trans retinoic acid (ATRA), another vitamin A analog (32). Beta-carotene is a modified antioxidant and reduces oxidative stress (33). Researches have indicated that oxidative stress plays a critical role in the etiopathogenesis of GTD (34-36).

Dutta et al. (30) showed that alfa and beta-carotene, in 5 and 10 μM doses respectively, synergistically decreased cell proliferation and DNA synthesis in oesophagus epithelial cell

culture (HEE) and squamous cancer cell culture (HESC) when used in combination and identified that early administration could be of benefit in oesophagus cancer treatment, for example in Barret oesophagus, and a lower dose of beta-carotene could be synergistically used with alfa-carotene for protection against oesophagus malignancy.

Wang et al. (37) indicated that minimum beta-carotene concentration for significant cell proliferation of EC9706 cells was 40 μM. However, in our study the minimum beta-carotene concentration for significant decrease in cell proliferation was 1 μg/mL (approximately 1.86 μM). Hurst et al. (38) reported that high doses of beta-carotene caused decreased mitochondrial function through unidentified mechanisms in human K562 erythroleukemic and 28SV4 retinal pigment epithelial cells. They noted that there were insufficient clinical studies evaluating beta-carotene toxic effects on human.

Gloria et al. (39) in a cell culture model of breast cancer identified that use of beta-carotene doses of 0.5, 1, 2.5, 5, and 10 μM had dose-dependent apoptosis and necrosis-enhancing effects in MCF-7, MDA-231 and MDA-235 cell lines. Moreover, Wang et al. (40) showed doxorubicin use at doses starting from as low as 0.5 μM induced apoptosis in PA-1 ovarian teratocarcinoma cells and MCF-7 cells. Osman et al. (41) indicated that doxorubicin at 0.25 μg/mL caused early apoptosis at a rate of 78%, resveratrol 15 μg/mL caused early apoptosis at a rate of 76% and use in combination caused early apoptosis at a rate of 90%. Previously, Sel et al. (42) had reported that ATRA was an effective drug on JAR and JEG-3 cell lines due to decreasing oxidative stress.

To the best of our knowledge this is the first report presenting the effect of beta-carotene in combination with PLD on JAR and JEG-3 CC cells in vitro. When beta-carotene was administered alone and at increasing doses, its apoptotic effects on JAR cell significantly increased. However, its apoptotic effects on JEG-3 cell was not significant. When PLD was administered alone at increasing doses its apoptotic effects on both JAR and JEG-3 cells significantly increased. When increasing concentrations of PLD were combined with a fixed concentration of beta-carotene there was a significant increase in apoptotic effect in both JAR and JEG-3 cells.

Conclusion

This is the first report, as far as we are aware, presenting the effects of beta-carotene and PLD in combination on JAR and JEG-3 human CC cell line models. The effects were assessed by measuring the concentrations of β-hCG and H-hCG. Apoptotic data showed that beta-carotene and PLD combination has a synergistic effect and may be a viable option for treatment of multiple drug resistant human CC. The results of this study suggest that vitamin A supplementation may have a preventive role in human CC in the future. However, these were in vitro studies and the results should be confirmed in animal trials and, if justified, clinical studies, as the effects of the drugs and the effects in combination may differ in in vivo systems.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Bülent Ecevit University Faculty of Medicine (approval number: 2014-68-25/03, date: 03/25/2014).*

Informed Consent: *No informed consent was obtained due to cell culture study.*

Author Contributions: *Surgical and Medical Practices: İ.Ö.T.; Concept: M.İ.H., M.H., İ.Ö.T;, Design: M.İ.H., M.H., Data Collection or Processing: S.A.E., M.İ.H., M.H., İ.Ö.T.; Analysis or Interpretation: S.A.E., G.S., M.İ.H., M.H;, Literature Search: S.A.E., G.S., M.İ.H., M.H.; Writing: S.A.E., G.S., M.İ.H., M.H.*

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Perinatal outcomes of twenty-five human immunodeficiency virus-infected pregnant women: Hacettepe University experience

Ahmet Cağkan İnkaya[1], Gökçen Örgül[2], Nurhayat Halis[2], Şehnaz Alp[1], Ateş Kara[3], Özgür Özyüncü[2], Murat Yurdakok[4], Serhat Ünal[1], M. Sinan Beksaç[2]

[1]Department of Infection Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[2] Department of Obstetrics and Gynecology, Division of Perinatology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[3]Department of Pediatric Infectious Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey
[4]Department of Child Health and Diseases, Hacettepe University Faculty of Medicine, Ankara, Turkey

Abstract

Objective: To evaluate perinatal outcomes in human immunodeficiency virus (HIV) infected pregnant women in Turkey.

Material and Methods: Maternal characteristics, pregnancy complications, laboratory findings including HIV load, CD4 cell count, CD4/CD8 ratio, neonatal features and final HIV status of the baby were retrospectively analyzed.

Results: The sample included 26 singleton pregnancies, from 25 HIV-infected women. The ethnicities were Turkish (n=18), East European (n=4), Asian (n=2) and African (n=2). The majority (76.9%) was aware of their HIV status before becoming pregnant. Four cases (15.3%) were diagnosed during pregnancy and two (7.8%) at the onset of labor. The results for median HIV viral load, CD4 count, and CD4/CD8 ratio at birth were 20 copies/mL (0-34 587), 577/mm^3 (115-977), and 0.7 (0.1-1.9), respectively. The HIV viral load rate was 5.5% in eighteen women taking anti-retroviral treatment. The rates of gestational diabetes mellitus, gestational hypertension, intrauterine growth restriction, and preterm delivery were 3.8%, 3.8%, 7.6%, and 8% (numbers are 1;1;2;2), respectively. The mean gestational week at birth was 38 weeks and mean birthweight is 2972±329 g. Two babies were congenitally infected with HIV (infection rate of 8.3%). There was one needle-related accident during surgery.

Conclusion: Timely diagnosis of HIV infection during pregnancy is important for preventing mother to child transmission. HIV infected women may give birth to HIV negative babies with the help of a multidisciplinary team, composed of perinatology, infectious diseases, and pediatrics specialists. (J Turk Ger Gynecol Assoc 2020; 21: 180-6)

Keywords: HIV, Pregnancy, antenatal care, Turkey

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Introduction

According to estimations in 2015, 36.7 million people were infected with human immunodeficiency virus (HIV), globally (1). Among them, 17.8 and 2.1 million were “women over 15 years of age” and children, respectively. Nearly half of infected women have access to treatment, and 43% of children are receiving treatment (1). Treatment coverage for children is therefore somewhat restricted, which highlights the importance of preventive measures in early childhood, including preventing mother-to-child transmission (MTCT). The number of newly-infected children has decreased by 47%, since 2010. Maternal antiretroviral therapy (ART) is the backbone of MTCT preventive measures but, as of 2016, 24% of pregnant women in need cannot access ART (1).

Over the last 30 years, the HIV landscape was revolutionized by the advent of new class antiretrovirals (ARVs). Currently, HIV-

Address for Correspondence: *Gökçen Örgül*
e.mail: gokcenorgul@gmail.com ORCID: orcid.org/0000-0003-0578-4230

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infected people enjoy a similar life-expectancy and quality of life as their uninfected counterparts. Annually, up to 8800 (95% confidence interval 8400-8800) HIV-infected women give birth in USA (2). An orchestrated team effort is necessary for good reproductive health, family planning preconception health services, and prevention of MTCT (3).

As reported by the Turkish Ministry of Health in 2019, over 20.000 people are living with HIV infection in Turkey (4). Despite the low prevalence of HIV (<0.001%) in Turkey, the number of newly diagnosed cases increases by 452% after 2010 (5). In addition, women constitute 25% of those living with HIV or acquired immune deficiency syndrome (AIDS), known as people living with HIV/AIDS (PLWHA), in Turkey (6).

MTCT was recognized in 1982 as a mode of HIV infection and numerous prevention efforts have been investigated and reported subsequently (7). Pre-conception counselling, antenatal HIV screening, ART, and access to perinatal follow-up are key preventive measures (3,8). As a result of successful implementation, the MTCT rate in the UK decreased from 25.6% in 1993 to <0.5% in 2011, which is considered a major success (8,9). Furthermore, the ACTG076 study confirms that zidovudine (ZDV) monotherapy lowers the risk of MTCT and ART can further decrease that from 10.4% to 1.2% (10,11).

Concerns regarding the teratogenic risk of ART were alleviated by emerging data and, subsequently, maternal ART became the cornerstone of MTCT prevention strategies (12). MTCT in a non-breastfeeding setting will occur during pregnancy, which emphasizes the importance of prenatal care (8). In addition, premature rupture of membranes is associated with increased MTCT risk (13). Use of a planned caesarean section (CS) is found to lower the risk of transmission from 10.5% to 1.8% (14). In addition, maternal viral load at delivery is another major risk factor. If perinatal maternal viral load is below 50 copies/mL, MTCT risk drops below 0.5%, regardless of treatment or delivery mode (15).

Despite the growing problem of HIV infection in Turkey, there is a scarcity of real-world data from Turkish research centers. This study aimed to evaluate the Hacettepe University Hospital Antenatal Care/HIV cohort, in terms of obstetric and perinatal outcomes.

Material and Methods

Hacettepe University Hospital is a tertiary referral center, located in the capital city of the Turkish Republic. The center provides multidisciplinary treatment for HIV-infected people, as well as for high-risk pregnancies. The Hacettepe University Hospital HIV cohort is composed of 636 PLWHA, as of October 2017, who are registered with the Infectious Diseases Clinics of Hacettepe University. Among the cohort, 92 (14.4%) are female. This study consists of all HIV-infected pregnant women who delivered at the hospital between January 2009 and October 2017. Early pregnancy losses before the 22th gestational week were excluded from analysis. During the study period, 26 deliveries occurred, in 25 individual HIV-positive women.

Data was extracted from patient files, as well as from digital records. Maternal age, obstetric history such as gravida, parity, coexisting diseases, and length of hospital stay were recorded for each patient. Main pregnancy complications, such as gestational diabetes mellitus (DM), preeclampsia/gestational hypertension, preterm contractions, and premature preterm ruptures of membranes, were also noted. Laboratory findings regarding HIV virus load, CD4 cell count, CD4/CD8 ratio, and hemoglobin concentration were recorded separately during pregnancy and at birth. Details of the pregnancy follow-up protocol used are given below. Neonatal features, such as birthweight, Apgar scores at first and fifth minute, gestational week at birth, requirement for neonatal intensive care, duration of hospitalization, and final HIV status were analyzed. Antiretroviral prophylaxis with nevirpine (7 mg/kg bid or 200 mg/m^2 bid) was administered to all babies regardless of maternal and neonatal plasma HIV-RNA result for 12 weeks. Antiretroviral treatment was commenced in all babies with positive plasma/cord blood HIV-RNA (detectable plasma HIV-RNA).

For known HIV-positive women, there is an integrated pregnancy follow-up program at the hospital center. PLWHA over eighteen years of age are routinely followed up in the infectious diseases department. All HIV-infected patients willing to conceive undergo extensive pregnancy counselling and, if special help is needed, the divisions of perinatology and andrology are involved. Upon pregnancy, the women are referred to the division of perinatology for regular follow-ups. The antenatal care program is initiated quickly after pregnancy diagnosis, to prevent MTCT for cases involving known HIV infection. The center will also accept newly diagnosed PLWHA, referred from other centers, for pregnancy follow-up. The follow-up is comprised of routine prenatal ultrasonography examinations, combined or triple-test aneuploidy screening tests, glucose challenge test between the 24th to 26th gestational week, and a non-stress test after the 37th gestational week. Further evaluations are completed according to obstetric indications until delivery. A pediatric infectious disease consultation takes place in the last trimester, to further inform parents on postnatal management.

HIV-infected pregnant women are evaluated on admission, as recommended by international guidelines (3). All patients undergo routine testing, including complete blood count, extensive biochemical work-up, virological work-up (viral load, genotypic ART resistance testing), immunological work-up (CD4/CD8 count), and documentation of childhood

immunization and diseases. ART is started before genotypic resistance testing, as recommended by international guidelines. The treatment regime is mainly composed of a Nucleoside reverse transcriptase inhibitor (NRTI) and a protease inhibitor (PI) such as boosted-lopinavir (LPV/r). PI is preferred, as pregnancy outcomes with high genetic barrier Integrase and strand transfer inhibitor (INSTI)-based regimes are obscure and INSTIs were introduced recently in Turkish market. Pregnant women are checked on a monthly basis for HIV viral load and side effects of treatment. If the maternal HIV viral load cannot be suppressed below 200 copies/mL, or viral-rebound occurs during the third trimester, the daily dose of LPV/r is increased by 50% (3x400/100 mg), without checking plasma drug levels. If an HIV-infected woman presents late in the course of pregnancy, or viral load remains high during the last trimester, intensive ART with NRTI, PI and raltegravir is given.

Caesarean section after the 38th gestational week is performed after counselling with parents. Regardless of maternal HIV viral load, a ZDV infusion is given before surgery and continued until cord-clamping. All the newborns were bathed twice and admitted to pediatric wards for close monitoring. Institutionally, breast feeding of PLWHA was clearly forbidden at all times and neonates were fed through formulary compounds. Antiretroviral prophylaxis was started with ZDV syrup. Neonates born to mothers with unsuppressed HIV viral load were further evaluated for combined antiretroviral regimes as dictated by Turkish Guidelines (16).

The study protocol was reviewed and approved by a Local Review Board (Hacettepe University Non-Interventional Clinical Studies Board decision approval number: GO-18/186-29, March 20, 2018).

Statistical analysis

The data was analyzed using the SPSS, version 23 (IBM Inc., Armonk, NY, USA). Qualitative data is presented as percentage and frequency, whereas quantitative data is presented as mean, standard deviation, and number.

Results

During the study period, 26 singleton pregnancies in 25 HIV-infected women were recorded. Maternal characteristics and main findings of the study is given in the Table 1. The total number of cases increased from 2009 to 2017, as shown in Figure 1. Mean maternal age at birth was 27.5±6.6 years. Most participants (n=18, 72%) were of Turkish ethnicity. Of the remaining eight women, three had East European, two had Asian, and two had African origin. A previous abortion was noted in ten (38.4%) of the 26 women, six of whom had a single abortion, three miscarried twice, and one had three previous abortions. Overall, there were twelve primigravid women (46.2%) in the study group.

Six coexisting diseases were noted in the 25 women, including chronic Hepatitis C, major depressive disorder, asthma and tuberculous empyema (n=2;2;1;1). Twenty women (76.9%) knew their HIV status before becoming pregnant and eighteen were already on ART. Two HIV-positive patients refused to take medications during pregnancies. Two women (15.3%) were diagnosed during pregnancy and the remaining two (7.8%) were diagnosed at the onset of labor. ARV was given to the latter four cases immediately after diagnosis. Of the two women presenting at delivery, one delivered by CS under ZDV prophylaxis and the last one did not receive perinatal prophylaxis, due to late admission and urgent CS.

Laboratory test results were collected for the twenty participants. We analyzed the laboratory parameters for all the women, except the two diagnosed at birth. The median HIV viral load, CD4 count, and CD4/CD8 ratio during the first trimester were 2203 copies/mL (0-529,000), 460/mm^3 (26-786), and 0.7 (0.04-1.3), respectively. The results for median HIV viral load, CD4 count, and CD4/CD8 ratio are 20 copies/mL (0-34 587), 577/mm^3 (115-977), and 0.7 (0.1-1.9), respectively in the last trimester.

The blood HIV viral load was under 200 copies/mL at birth in the twenty pregnant women. After excluding the two participants with missing data, four women (16.7%) were found to have an HIV viral load below 20 copies/mL before delivery. Within the study sample, two refused treatment, one was receiving ART, and one had a late diagnosis. The positive HIV viral load rate was 5.5% in the eighteen women receiving ART. There were 23 participants with full data on CD4 count and CD4/CD8 ratio. When participants were classified into groups, according to their CD4 count, seventeen were below 500/mm^3, two were between 350-500/mm^3, two were between 200-350/mm^3, and two were above 200/mm^3. The women were further divided in terms of CD4/CD8 ratio. In regard of CD4/CD8 ratio, eight were

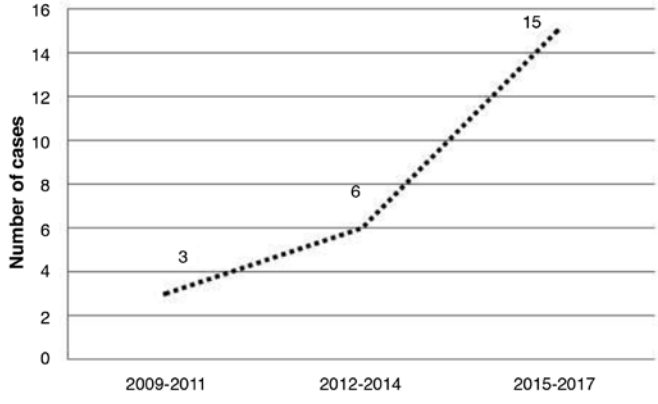

Figure 1. Total number of deliveries among human immunodeficiency virus infected women

Table 1. Maternal characteristics and main findings

Maternal characteristics			
	Mean age (years)		27.5±6.6
	Ethnicity		
		Turkish	18 (72%)
		East European	3 (12%)
		Asian	2 (8%)
		African	2 (8%)
	HIV diagnosis		
		Before conception	20 (76.9%)
		During pregnancy	4 (15.4%)
		At birth	2 (7.7%)
	Treatment		
		Before + during pregnancy	18 (69.2%)
		Refused in pregnancy	2 (7.7%)
		Started in pregnancy	4 (15.4%)
		Only at birth	1 (3.8%)
		None	1 (3.8%)
Laboratory Findings			
	During Pregnancy (n=20)		
		HIV viral load	2203 copies/mL (0-529 000)
		CD4 count	460/mm^3 (26-786)
		CD4/CD8 ratio	0.7 (0.04-1.3)
	At birth (n=24)		
		HIV viral load	20 copies/mL (0-34 587)
		CD4 count	577/mm^3 (115-977)
		CD4/CD8 ratio	0.7 (0.1-1.9)
Pregnancy complications			
	GDM		1 (3.8%)
	GHT		1 (3.8%)
	Preterm delivery		2 (8%)
	IUGR		2 (8%)
Fetal			
	Gestational week		38 (35 0/7-40 1/7)
	Birthweight		2972±329
	Apgar 1st minute		8.4±1.47
	Apgar 5th minute		9.4±1.47

HIV: Human immunodeficiency virus, GDM: Gestational diabetes mellitus, GHT: Gestational hypertension, IUGR: Intrauterine growth restriction

above 1, nine were between 0.5-1, three were between 0.2-0.5, and three were below 0.2.

There were three PLWHA with viral load count below 200 copies/mL. Two of three were late presenters, they presented at term and thus they did not receive any prophylactic treatment. The third woman was on trimethoprim/sulfamethoxazole (1 ds tablet/day) prophylaxis during gestation. However, it was discontinued after pregnancy was diagnosed. Immunization of the PLWHA is of concern. All the patients applying to our center has been evaluated for childhood vaccinations and respective serology results. Immunization included conjugated *pneumococcus*, polysaccharide pneumococcus, seasonal influenza, diphtheria-tetanus-acellular pertussis, mumps-measles-rubella, varicella zoster, hepatitis-B and hepatitis-A

vaccines. Live cell vaccines were contraindicated in pregnant woman. All vaccinations were performed according to Turkish HIV treatment guidelines (16).

After excluding data from one participant with late admission and missing data, the remaining 25 women were evaluated for pregnancy-related complications. Three instances of hospitalization during pregnancy were noted (two with pneumonia and one with gastroenteritis). One woman (3.8%) developed insulin-dependent gestational DM. Additionally, gestational hypertension was present in one (3.8%) woman.

We did not observe a premature rupture of membranes among any of the participants. Four women were hospitalized, due to preterm contractions, and two of those women delivered before the 37th gestational week. As a result, the preterm delivery rate was 8%. Also, intrauterine growth restriction was recorded in only two cases (7.6%).

There were eleven male (42.3%) and fourteen female (53.8%) babies born to the study sample. The mean gestational week at birth was 38 weeks, with a range from 35 0/7 to 40 1/7 weeks. There were two (7.7%) vaginal deliveries within the group and the CS rate was 92.3%. Mean birthweight was 2972±329 g. Mean Apgar scores at first and fifth minute were 8.4 and 9.4, respectively. Neonatal resuscitation in the delivery room was performed for one infant after birth. Furthermore, the HIV status of two babies was not available from patient files. Of the remaining 24, only two were HIV-infected, showing a low MTCT rate of 8.3%. There were no stillbirths, perinatal mortalities, or congenital abnormalities.

One HIV-infected baby was born to a 39 year-old mother, gravida 2 and parity 1, whose HIV status was first detected within the first trimester. The expectant woman had a high viral load (256.000) at the time of diagnosis and was given treatment immediately. The baby's HIV-RNA was 56, CD4 was 266, and CD4/CD8 ratio was 0.4 at birth. The baby was delivered via CS in the 38th gestational week and weighed 3230 g. Records for the other HIV-infected baby show the mother was diagnosed with HIV at delivery. The maternal laboratory findings were HIV-RNA 11.100 copies/mL, CD4 count 562/mm^3, and CD4/CD8 ratio was 0.8. The CS delivery was performed in the 38th gestational week and the 3,470 g fetus was transferred to the neonatal intensive care unit.

Mean hemoglobin decrease after delivery was 1.7±0.9 and there was no need for red blood transfusion for either infant. A surgical site infection developed in one mother (3.8%), which was treated with empiric antibiotics and wound care. There was one needle-stick injury which occurred during delivery, affecting a member of the surgical team, but antiretroviral prophylaxis was deferred as the index-patient had undetectable HIV-RNA.

Discussion

Despite decreasing trends across the world, HIV infection incidence is increasing in Turkey, due to a lack of knowledge and stigma (4,5). Effective interventions must include multidisciplinary teams and involvement of relevant stake holders. This, in turn, necessitates increasing scientific information available at every level. In this study, the obstetric and perinatal outcomes of HIV-infected woman were evaluated in the Hacettepe University Hospital cohort. This is the first scientific report on obstetric outcomes in the HIV-infected Turkish population.

In accordance with increasing HIV incidence in Turkey, the number of HIV-positive pregnancies is also increasing. This may be due to changes in epidemiology and understanding of HIV infection among the population at risk (17). There was a near five-fold increase in HIV-positive pregnancy rates over the last three years.

Contrary to previous reports, our study records intentional pregnancies among the women receiving ART. Unintended pregnancies risk harm to the mother and to the baby. Furthermore, unintended pregnancies among HIV-infected women are associated with delayed antenatal care, poor fetal outcomes, and poor retention of postpartum care (18,19).

HIV-infected sero-discordant or sero-concordant partners are recommended to receive reproductive counselling before conception, including identification of coexisting conditions and risk factors associated with adverse maternal and fetal outcomes (20). Ideally, all sexually active women require screening for HIV infection before considering pregnancy (3). Raffe et al. (8) report 72% of pregnant women know their HIV status before conceiving. Furthermore, in a study of a large British cohort, antenatal HIV serostatus awareness was shown to increase from 24.6% between 2000 and 2006 to 12.5% between 2007 and 2011 (9). The Republic of Turkey Ministry of Health recommends an HIV screening test with the consent of the pregnant woman in new prenatal care management guidelines in 2018 (21). Most centers, though, test all pregnant women in their first trimester and at birth. Our results show the importance of HIV screening during pregnancy in Turkey, as 15.3% of participants are diagnosed during pregnancy and 7.8% are diagnosed at onset of labor.

Risk of MTCT may be affected by many factors, including high maternal viral load, lower CD4 count of the mother, mother with AIDS defining disease, premature rupture of the amniotic membrane, preterm delivery and breastfeeding (22). As maternal viral load is the main determinant of these risk factors, ART delivered during pregnancy is the cornerstone of MTCT elimination strategies. Recent guidelines recommend the use of ARTs, regardless of viral load and immunologic

status, as the preventative effect is present irrespective of these factors. Furthermore, ART drugs can affect the fetus through the placenta and act as pre-exposure prophylaxis (23). Initiation of PI-based ART is associated with preterm deliveries in univariate analysis but not multivariate analyses (24). The main goal of ART during pregnancy is to suppress the virus to an undetectable level at the time of delivery so that MTCT will be an infrequent event. Maintaining a pregnancy-compatible ART is recommended in all women. Women conceiving under ART should be evaluated for possible adverse outcomes of certain antiretroviral drugs. Dolutegravir has been associated with neural tube defects (NTD) in the newborn. However, recent data shows decreased NTD risk when compared to previous report (25,26). However, the debate over antiretroviral associated NTD continues, so that drugs with a better safety profile should be preferred during pregnancy. These include LPV, raltegravir and efavirenz (especially in resource poor settings). In our cohort two MTCT events were seen. The underlying reason for transmission was late diagnosis in one case and high viral load at first trimester and delivery in the second case. High viral load at birth is related with an increased risk of MTCT according to our findings, which is consistent with existing literature (2,9,17).

PI-based treatments, including ritonavir, have been associated with preterm delivery (3). The potential mechanism underlying this effect remains unknown. Our results demonstrate that preterm delivery rate with PI-based treatments was 8%. Despite preterm delivery risk, PI-based ART promises various advantages compared to other regimes. In a resource-poor setting, with little access to genotypic resistance testing, the high genetic barrier of PIs provides an opportunity to administer ART without genotypic resistance test results. Moreover, PIs are potent drugs and lower viral load extremely rapidly (27). As distribution volumes may change during the third trimester, LPV levels are usually checked beforehand (28). We did not have access to therapeutic drug monitoring for ARTs. Therefore, the pregnant women were closely monitored for HIV viral load during the third trimester. In a case of virologic rebound during the third trimester, daily dosing would be increased by 50%, as recommended by Manavi et al. (29).

In addition to the importance of MTCT, HIV-positive pregnancies are vulnerable to several other complications. A previous study showed no increased risk for preeclampsia, preterm birth, or smallness for gestational age, in women receiving treatment (30). A more recent meta-analysis demonstrated a two-fold increase in risk for preterm delivery and low birthweight in HIV-positive pregnancies (31). Our results show a preterm delivery rate of 8% and Intrauterine growth restriction rate of 7.2%. The number of cases is low in this study, so it is not possible to calculate a definitive frequency of prematurity among these cases.

The mode of delivery is dependent on multiple factors in HIV infected pregnant women. Previously, elective CS was recommended to minimize the risk of MTCT in all these pregnancies (15). With the findings of recent studies, vaginal delivery is shown to be safe for neonates if maternal viral load is <1,000 copies/mL. Thus, CS should be performed only for obstetric indications such as placenta previa, previous CS history, malpresentation, fetal distress, and the like (32). The high rate of CS in our cohort indicated that clinicians tended to choose elective CS, most probably due to medicolegal issues.

Pregnancy-induced hypertension and preeclampsia are important causes of maternal morbidity and mortality. Fortunately, studies have reported the risk of preeclampsia was not increasing in HIV-infected women (33,34). We also showed that the frequency of preeclampsia was only 3.8%. GDM is another concern for HIV-positive pregnant women, due to related medication and infection. Previous studies, however, reported the risk of GDM was not increasing in HIV-positive expectant women, as compared to healthy pregnancies (35,36). Our findings are in concordance with the relevant literature.

Study Limitations

The major limitation of the current study is the small number of cases and the single center nature of the cohort. In addition, missing and unreliable data due to the retrospective design of the study was a further limitation.

Conclusion

Timely diagnosis of HIV infection during pregnancy is important for preventing MTCT. HIV infected mothers may give birth to HIV negative babies with the help of multidisciplinary teams, composed of specialists in perinatology, infectious diseases, and pediatrics.

Ethics Committee Approval: *The study protocol was reviewed and approved by a Local Review Board (Hacettepe University Non-Interventional Clinical Studies Board decision GO-18/186-29, March 20, 2018).*

Informed Consent: *Retrospective study*

Peer-review: *Externally peer-reviewed.*

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Does antenatal magnesium sulphate improve hearing function in premature newborns?

Işıl Kasapoğlu[1], Bilge Çetinkaya Demir[1], Mehmet Aral Atalay[1], Adnan Orhan[1], Hilal Özkan[2], Salih Cağrı Çakır[2], Rabia Tütüncü Toker[2], Fikret Kasapoğlu[3], Kemal Özerkan[1]

[1]Department of Obstetrics and Gynecology, Bursa Uludağ University Faculty of Medicine, Bursa, Turkey
[2]Department of Pediatrics, Bursa Uludağ University Faculty of Medicine, Bursa, Turkey
[3]Department of Otorhinolaryngology, Bursa Uludağ University Faculty of Medicine, Bursa, Turkey

Abstract

Objective: To evaluate whether antenatal magnesium sulphate (MgSO4) exposure has a neuroprotective effect against hearing impairment in premature newborns.

Material and Methods: Retrospective cohort study was performed with prematurely (<37 weeks) delivered newborns at a tertiary university hospital. Newborns of 92 women who received MgSO4 infusions (study group) for various indications were compared to newborns of 147 women who did not receive MgSO4 infusions (control group). All eligible premature newborn underwent hearing screening by auditory brainstem response (ABR) testing before being discharged from the hospital.

Results: The fail rate for ABR hearing screening was 3.3% (n=3) in the study group and 10.9% (n=16) in the control group (p=0.034). The rate of concurrent use of betamethasone was higher in the study group (72.8%; n=67) compared to control group (29.2%; n=43) (p<0.001). Other neonatal parameters, such as the number of neonates who were small for gestational age and the rate of microcephaly were similar between the groups (p=0.54, p=0.48, respectively). After adjusting for co-variates including the use of betamethasone and gestational age at delivery, no statistically significant association between antenatal administration of MgSO4 and ABR fail rates were found (p=0.07).

Conclusion: These results do not suggest a significant benefit in terms of hearing impairment in premature newborns when antenatal MgSO4 infusion was given. (J Turk Ger Gynecol Assoc 2020; 21: 187-92)

Keywords: Magnesium sulphate, prematurity, hearing screening, hearing loss, ototoxicity, neuroprotection

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Introduction

Currently, magnesium sulphate (MgSO4) is widely used in obstetric care for various indications to improve obstetric outcomes. These include a reduction in the risk of eclampsia and as a tocolytic agent, although its efficacy as a tocolytic agent is still controversial. A recent suggestion has been that MgSO4 may be neuroprotective for the immature fetal central nervous system and may reduce the incidence of major neurological morbidity, particularly cerebral palsy (CP) in premature newborns (1,2).

The earliest data on the use of MgSO4 for neuroprotection was published in the 1980s and 1990s (3,4). Data were collected from infants who were exposed to MgSO4 in utero. In those cases, perinatal morbidity including intracranial events and CP were observed to be less severe (3,4). According to those preliminary findings, magnesium has been investigated widely for its neuroprotective effects and furthermore, guidelines have been created, especially for antenatal administration of MgSO4 (AAM) in premature deliveries (1). MgSO4 infusions have been proposed to protect the fetal central nervous system in preterm infants and also to

Address for Correspondence: *Işıl Kasapoğlu*
e.mail: kasapogluisil@hotmail.com ORCID: orcid.org/0000-0002-1953-2475

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reduce the rate of preterm births in patients presenting with threatened preterm labor.

Recent advances in intensive care techniques have been associated with improved survival rates in premature infants (5). However, the rate of preterm birth complications has not yet been reduced (6). Currently, prematurity is the leading cause of the central nervous system morbidities (7). Major neurological disorders accompanying prematurity include CP, mental retardation, sensorineural hearing loss (SNHL), and blindness (8,9). In spite of decreased rates of prematurity-related mortality, the incidence of SNHL has remained high, varying from 0.2% to 6.4% (10). Hearing loss, which also impairs speech and language development, is a major disability closely associated with social and physical development of the newborn, and has a significant impact on the quality of life. Congenital hearing loss is a universal health problem and is one of the health measurements which is used to determine health-related quality of life (11). Early recognition and adequate treatment regimens can mitigate adverse outcomes. Hence, newborn hearing screening programmes have been widely introduced and hearing screening is recommended in all infants during the first month after delivery (12).

There is a lack of evidence-based data on the efficacy of AAM in preventing hearing loss in preterm infants. Most previous studies assessing magnesium usage for neuroprotection have evaluated CP as the primary outcome (2), but there are insufficient data on cochlear function. There are some studies which suggest a beneficial effect of magnesium administration on noise induced hearing loss in adults (13). However the mechanism of noise induced hearing loss and the mechanism of congenital hearing loss are different. Considering the widespread use of AAM in obstetric care, the aim of the present study was to assess the potential neuroprotective effect of antenatal MgSO4 on auditory nerve development and sensorineural hearing in premature newborns.

Material and Methods

This study was performed in a tertiary university hospital in Turkey, from January 2015 to January 2017. The study protocol was approved by the Local Research Ethics Committee (Uludağ University Faculty of Medicine Clinical Research Ethics Committee) at the beginning of the study (approval number: 2017-14/12). Each participant was informed about the study and provided their written consent.

All preterm infants (<37th gestational weeks) born within this two year were included in the study. Medical records of study subjects were retrospectively reviewed. Neonates born to mothers who were administered antenatal MgSO4 for at least eight hours before the delivery for various indications as shown below, were enrolled in the study group. Neonates whose mothers did not receive MgSO4 constituted the control group. Among them, newborns who had a history of intrauterine infections, lethal congenital abnormalities, craniofacial anomalies, and who died after birth were excluded from the study.

Antenatal MgSO4 (magnesium sulfate 15%; Biofarma) was administered by continuous intravenous infusion at a rate of 2 grams per hour. Infusion duration was at least 8 hours, following a loading dose of 4.5 grams, as recommended. Indications for MgSO4 included neuroprotection, tocolysis or prophylaxis for eclampsia. Antenatal administration of corticosteroids consisted of two courses of 12 mg betamethasone, when indicated.

The primary outcome measurement was the failure rate on auditory brain stem (ABR) hearing screening in newborns. ABR allows neurophysiologic assessment of brainstem maturation and auditory pathway in newborns. The effect of antenatal MgSO4 infusion was the main variable.

As all of the newborns were premature and had risk factors for hearing loss, all eligible newborns underwent ABR hearing screening during the first month after delivery, before being discharged from the neonatal intensive care unit (NICU). The results of ABR tests were evaluated and compared between the study and control groups. ABR tests were performed using Madsen Accuscreen (Otometrics, Denmark). The ABR consisted of up to 30 dB click stimulus using disposable, hydrogel electrodes and the standard for the device was EN-60645-7, type 2.

Based on the results of screening ABR test, newborns were considered to have “passed the test”, if the stimulus to produce a response did not exceed 30 decibels (dB-NHL) in both ears; if not, they were considered to have “failed the test”, according to the suggested algorithm of hearing screening of newborns.

Maternal characteristics which could affect neonatal outcomes, such as maternal age, parity, use of antenatal corticosteroids, presence of preeclampsia, premature rupture of membranes (PROM), delivery route and neonatal characteristics including gestational age at birth, birth weight, Apgar scores, and umbilical cord arterial pH values were also retrieved. In addition, other neonatal parameters evaluated within the first 28 days, such as microcephaly and the incidence of postnatal morbidities including requirement for mechanical ventilation, neonatal sepsis and exposure to ototoxic agents, meningitis, intraventricular hemorrhage, retinopathy of prematurity, respiratory distress syndrome (RDS), phototherapy requirement, and bronchopulmonary dysplasia were analyzed as composite outcomes. The rate of pathological electroencephalography (EEG), visual evoked potentials and early Denver Developmental Screening test results were also analyzed.

Statistical analysis

The SPSS version 21.0 (IBM Corp., Armonk, NY., USA) was used to analyze study data. The distribution pattern of the data was examined for normality with the Shapiro-Wilk test. Variables were reported as mean ± standard deviation or median (minimum-maximum) values. According to the normality test result, an independent sample t-test or Mann-Whitney U test were used for intergroup comparisons. The chi-square test or Fisher's exact test was used for the comparisons of categorical variables. Binary logistic regression analysis was performed in order to determine independent risk factors that may have affected failure rates in the ABR screening test. The level of significance was set at $\alpha=0.05$.

Results

All infants born before 37th week were evaluated. During the study period 285 eligible women were administered MgSO4 and/or gave birth before 37th week of gestation. Newborns who died after birth (n=37, 12.9%) and whose hearing screening results were not available (n=9, 3.1%) were excluded from the study. A total of 239 newborns were included in further statistical analysis. Mothers of 92 out of 239 newborns (38.5%) received MgSO4 whereas mothers of 147 (61.5%) newborns did not.

The mean maternal age at delivery was similar between the study group and the control group (30.5±5.9 versus 30.7±5.7 years respectively, p=0.83). Cesarean delivery rate was significantly higher in the study group (80.4%) compared to the control group (68.8%) (p=0.048) (Table 1).

Table 1. Demographic and clinical characteristics of the cohort groups

	Study group (n=92)	Control group (n=147)	p
Maternal age (years)	30.5±5.9	30.7±5.7	0.830
Cesarean delivery	74 (80.4%)	99 (68.8%)	**0.048**
Gestational age (weeks)	32 (26-36^{+3})	35 (26^{+5}-36^{+5})	**<0.001**
Incidence of PROM (%)	5.1%	2.4%	0.29
Birth weight (grams)	1.733±586	2.336±668	**<0.001**
Presence of preeclampsia	38 (41.3%)	5 (3.5%)	**<0.001**
Apgar at one minute	7 (1-10)	8 (1-10)	**0.017**
Antenatal administration of betamethasone	67 (72.80%)	43 (29.20%)	**<0.001**
Umbilical arterial pH	7.30 (6.8-7.4)	7.31 (7.0-7.53)	0.230

Data were presented as median (minimum-maximum), mean ± standard deviation and n (%), PROM: Premature rupture of membranes

Demographic and clinical characteristics of the cohort groups are shown in Table 1. The median (range) gestational age at delivery was significantly lower in the study group compared to the control group at 32 (26-36^{+3}) and 35 (26^{+5}-36^{+5}) weeks, respectively (p<0.001) and the mean birth weight was lower in the study group compared to that in the control group (1733±586 g and 2336±668 g, respectively; p<0.001). The incidence of PROM was similar in the two groups (2.4% versus 5.1%, p=0.29). The prevelence of preeclampsia was significantly higher in the study group compared to the control group (41.3% versus 3.4%, p<0.001). Apgar scores at one and five minutes were lower in the newborns in study group than those in control group (p=0.017 and p=0.03, respectively). There was no significant difference between two groups in the umbilical cord pH measurements (p=0.23) (Table 1).

Nineteen newborns failed the ABR screening test, in total. Failure rates significantly differed between the two groups with 3.3% (n=3) in the study group and 10.9% (n=16) in the control group, respectively, failing the test (p=0.034) (Table 2). Seven out of 19 (36.8%) infants who failed in the ABR test had unilateral hearing loss and 12 (63.2%) had bilateral loss.

The rate of antenatal administration of betamethasone (AAB) was significantly higher in the study group (72.8%) compared to the control group (29.2%) (p<0.001). No statistically significant association was found between AAB and the result of ABR hearing screening (p=0.31).

The rates of small for gestation age neonates and the incidence of microcephaly did not significantly differ between the two groups (p=0.54, p=0.48, respectively). Composite neonatal outcome, with the exception of RDS, was similar in the groups (Table 3). Additionally, the rate of pathological EEGs, visual evoked potentials and the results of early Denver Developmental Screening test and being treated in NICU did not significantly differ between the two groups (Table 3).

Adjustments were analyzed using a logistic regression model for AAM, AAB, and gestational age (day) as major variables significantly differ between groups and that could be associated with ABR hearing screening outcomes. In this final model, none of the variables was found to be an independent variable for ABR results (Table 4).

Table 2. Auditory brainstem response hearing screening outcomes with respect to antenatal administration of magnesium sulphate

	Study group	Control group	p
Infants who passed ABR (n=220)	89 (96.7%)	131 (89.1%)	**0.034**
Infants who failed ABR (n=19)	3 (3.3%)	16 (10.9%)	

Data are presented as n (%), ABR: Auditory brainstem response

Table 3. Neonatal parameters and postnatal morbidities in the study and control groups

Variable (n)	Study group (n=92)	Control group (n=147)	p
Being treated in NICU	38 (41.3%)	61 (41.5%)	0.97
Small for gestational age	7 (7.6%)	12 (8.2%)	0.543
Microcephaly	4 (4.3%)	8 (5.4%)	0.48
Intubation	19 (20.7%)	32 (21.8%)	0.48
Sepsis	18 (19.6%)	20 (13.6%)	0.46
Phototherapy requirement	18 (19.6%)	28 (19%)	0.52
Meningitis	3 (3.3%)	4 (2.7%)	0.54
Bronchopulmonary dysplasia	4 (4.3%)	9 (6.1%)	0.39
Intraventricular hemorrhage	6 (6.5%)	9 (6.1%)	0.55
Retinopathy of prematurity	4 (4.3%)	4 (2.7%)	0.37
Respiratory distress syndrome	25 (27.2%)	24 (16.3%)	**0.03**
Pathological electroencephalography finding	2 (2.2%)	3 (2%)	0.63
Pathological visual evoked potentials finding	2 (2.2%)	3 (2%)	0.63
Pathological Denver finding	4 (4.3%)	7 (4.8%)	0.57

Data are presented as n (%), NICU: Neonatal intensive care unit

Table 4. Logistic regression model of variables associated with the auditory brainstem response hearing screening test results

Factor	OR (95 %CI)	p
Antenatal administration of magnesium sulphate	0.23 (0.05-1.19)	0.08
Antenatal administration of betamethasone	1.59 (0.39-6.43)	0.51
Gestational age at delivery (day)	1.01 (0.98-1.04)	0.34
Being treated in NICU	1.52 (0.46-4.99)	0.48

OR: Odds ratio, CI: Confidence interval, NICU: Neonatal intensive care unit

Discussion

The use of MgSO4 is mainly recommended for the prophylaxis of eclampsia and recently, for the protection of fetal central nervous system in preterm labor before 32 weeks (1). The aim of the present study was to evaluate whether AAM exposure had a neuroprotective effect on hearing in premature newborns. Our results showed that the failure rate in the ABR screening test was lower in the group of newborns exposed to antenatal MgSO4 than in the group of neonates who were not exposed to antenatal MgSO4 (p=0.034). Nevertheless, after adjustment for covariates, including betamethasone and gestational age at delivery (day), there was no statistically significant association between AAM and the failure rate in ABR screening (p=0.07).

Magnesium is an essential element for many physiological processes in the body (14). One of these functions is the maintenance of cell membrane polarization by regulating calcium channels (15). In this context, magnesium contributes to cochlear physiology and has a role in hearing process. Despite its known neuroprotective effects and protective effects against CP, data on the effect of magnesium on sensorineural hearing is limited. In a study conducted with guinea pigs, a negative correlation was observed between cochlear magnesium levels and hearing loss. It was demonstrated that magnesium content of the inner ear has a regulatory function and hinders reactive oxygen species formation after noise exposure (16,17). Furthermore, there is evidence of cellular damage due to oxidative stress associated with magnesium deficiency (18). In addition, in adults, the protective effects of oral magnesium supplementation have been demonstrated in hearing loss due to acoustic trauma (13). Most of these studies were conducted with animals or adults and particularly evaluated noise-induced cochlear damage. This evidence prompted the present study to evaluate the possible protective effects of magnesium on sensorineural hearing in premature newborns. Premature newborns were specifically evaluated, because of the higher incidences of SNHL in this population (19), which is related to delayed neurodevelopment (12,19).

In line with previous studies, we found a high percentage of hearing impairment as assessed by the ABR screening test (7.9%) in the whole cohort of premature newborns. Although various mechanisms could be suggested for the development of hearing loss in premature newborns, prematurity alone is one of the established major risk factors (19) as fetal audiological development mainly occurs between 20 and 33 weeks of gestation. Therefore, the risk for labyrinth pathologies of the inner ear is also increased in premature newborns (20). This was the reasoning behind including premature newborns in this study.

Other etiologic factors include hypoxic damage, mechanical ventilation, hemorrhage, and increased levels of bilirubin and administration of ototoxic drugs such as aminoglycoside antibiotics. In a previous study, which investigated risk factors for hearing loss in preterm newborns, it was suggested that the etiology of hearing loss was multifactorial rather than a single factor that has a prominent effect on ototoxicity (21). Thus clinical factors were evaluated in two groups separately in this study. Intergroup comparisons indicated that newborns in the study group who received antenatal MgSO4 had significantly

lower birth weights and lower one minute and five minute Apgar scores. This could be related with the earlier gestational week at birth. However, they performed better in the hearing screening test than newborns who did not received antenatal MgSO4.

A recent Cochrane review, which evaluated the potential neuroprotective effect of antenatal magnesium in preterm newborns, included a limited number of studies (2). Furthermore, most of these studies evaluated mortality, gross motor disability or CP as primary outcomes. Mortality rates did not differ with magnesium pretreatment with a relative risk of 1.04 (95% confidence invertal: 0.92-1.17) (2). In our study population, we also found a very high mortality rate (12.9%), supporting that prematurity was a risk factor. However newborns died after birth, were not included in the further statistical analysis.

To date, there are not enough data to interpret the protective effect of magnesium administration on sensorineural hearing. Only one study, included in the above review, evaluated neurological impairment in 1047 participants (22). In that study, hearing loss was not separately evaluated but was instead accepted as a component of moderate neurological disability. The authors did not report significant difference between groups of patients who received magnesium and who did not, with respect to sensorineural disability (22).

One previous retrospective study evaluated the outcomes of hearing screening for any possible associations with antenatal maternal medications. The maternal medication list did not include magnesium. Corticosteroid use was reported to be associated with a reduced risk for hearing loss (23). It should be noted that in most of the studies which evaluated the neuroprotective effects of magnesium, participants also received corticosteroids, as was the case in the present study. In another recent study, which only evaluated the effects of antenatal steroid administration on hearing function in newborns, no associations were found between corticosteroids and hearing screening results (24). In a further study repeated antenatal administration of steroids rather than a single dose did not add benefit based on ABR evaluations in newborns (25). Moreover in another study, it was indicated that steroid dosage made no difference in hearing function of newborns born after the 34th gestational week (26). In our study population, we also used betamethasone to improve neonatal outcomes. In line with those studies, we did not find any direct association between antenatal betamethasone administration and neonatal ABR hearing screening test results (p=0.31) in univariate, as well as further multivariate analyses.

Preliminary results of this study of AAM indicated improved hearing screening test results in premature newborns in our cohort. However, further multivariate analysis did not support the initial results. Therefore, there is no robust data indicating a clear benefit from AAM in terms of hearing impairment in premature newborns. This study has significant implications for hearing loss in premature newborns as a high-risk population for SNHL and also suggests that AAM is a possible adjuvant therapy that may reduce the incidence of SNHL in this group.

In our study population we detected a higher incidence of impaired hearing based on screening outcomes in neonates born after 34 weeks of gestation compared to preterm infants who had received antenatal magnesium. A possible explanation for this result might be the potential protective effect of magnesium on cochlear functions or on the fetal brain, which might still remain important in neonates born after 34 weeks of gestation. However possible confounders cannot be excluded. Any discrepancy in such association needs to be elucidated with further studies.

Although this study included all premature newborns (<37 weeks) over a period of two years, this is a relatively small study population and the retrospective nature of the study was a further limitation of this study. Additionally, for the newborns who were treated in NICUs before undergoing the ABR test, we could not exclude possible further external confounding factors.

Conclusion

Our results do not suggest a clear and definite benefit from antenatal MgSO4 infusion in respect of hearing impairment in premature newborns. For the usage of MgSO4 as a neuroprotective medication against hearing impairment in premature newborns, further large scale and carefully designed studies are warranted to reach a definite conclusion.

Ethics Committee Approval: *The study protocol was approved by the Local Research Ethics Committee (Uludağ University Faculty of Medicine Clinical Research Ethics Committee) at the beginning of the study (approval number: 2017-14/12).*

Informed Consent: *Each participant was informed about the study and provided their written consent.*

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Vaginal microbiota and human papillomavirus: a systematic review

Despoina Mortaki[1,4], Ioannis D. Gkegkes[2,4], Victoria Psomiadou[3,4], Nikos Blontzos[3,4], Anastasia Prodromidou[3,4], Fotis Lefkopoulos[3,4], Electra Nicolaidou[1,4]

[1]First Department of Dermatology and Venereology, National and Kapodistrian University of Athens School of Medicine "A. Syggros" Hospital for Skin and Venereal Diseases, Athens, Greece

[2]Department of Surgery, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

[3]Clinic of Gynaecological Oncology, Metaxa Cancer Hospital, Piraeus, Greece

[4]Hellenic Society for Lower Genital Tract Disorders, Athens, Greece

Abstract

Accumulating evidence indicates the potential correlation between the vaginal microbioma and the acquisition and persistence of human papillomavirus (HPV) infection. This study aims to demonstrate the potential relationship through a systematic review of the current literature. A search was conducted on the following medical databases: PubMed and Scopus. Nineteen studies met the inclusion criteria and were incorporated in the present review. A total of 12.204 patients and their demographic characteristics were studied. Commercially available DNA tests and polymerase chain reaction (PCR) were used for the detection of different HPV subtypes, while the identification of the microbiomes was performed through specific diagnostic methods and PCR assay. The most frequently encountered species were classified based on their protective or detrimental impact on the progression of HPV infection. The beneficial role of some types of *Lactobacillus (Lactobacillus gasseri, Lactobacillus jensenii, Lactobacillus crispatus)* is generally supported. On the other hand, high microbial diversity and specific microorganisms such as *Sneathia, Anaerococcus tetradius, Peptostreptococcus, Fusobacterium* and *Gardnerella vaginalis* were found to be implicated with higher frequency and severity of disease, potentially resulting in pre-cancerous and cancerous cervical lesions.The role of vaginal microbiota appears to play an as yet not fully understood role in the susceptibility to HPV infection and its natural history. (J Turk Ger Gynecol Assoc 2020; 21: 193-200)

Keywords: Microbiome, microbiota, human papillomavirus, HPV, *Sneathia; Lactoba cillus gasseri, Lactobacillus jensenii, Lactobacillus crispatus*

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Introduction

Sexually transmitted diseases (STDs) are among the most frequent infectious diseases worldwide and are defined as those which include transmission of infectious organisms between sex partners. According to the Centers for Disease Control and Prevention almost 19 million cases are reported as infected each year by more than 20 different STDs (1).

Human papillomavirus (HPV) represents one of the most frequent causes of STDs in women around the world (2). More than 200 different HPV genotypes have been reported and are generally classified into two groups including high and low risk, which is based on the potential risk of developing cancer. To that end, in about 99% of all cervical malignancies one or more of the HPV types classified as high risk (16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59) are identified. Additionally, high risk types have been reported to play an important role in other malignancies such as anal, oropharyngeal, vulvar, and penile cancers. The genotype distribution, as well as the genome of each HPV, are considered critical for disease prevention, prognosis, and treatment (3,4).

Address for Correspondence: *Ioannis D. Gkegkes*
e.mail: ioannisgkegkes@gmail.com ORCID: orcid.org/0000-0001-8637-5679

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The viral types of HPV can be easily transmitted from one person to another via skin and mucous membranes, which makes the possibility of infection from HPV relatively common. Nevertheless, the majority of infections are subclinical and transient, as they are suppressed by immunocompetent immune systems (5). Therefore, even though the incidence of HPV infection is common throughout life (>80% in sexually active people), the incidence of HPV- related diseases is lower (6).

Cervical cytology and HPV tests are widely used for cervical cancer screening and thus early detection of the underlying disease. Co-infections by multiple HPV types are likely to occur in more than 30% of HPV patients (2). Information regarding HPV co-infections can be an essential part of determining treatment options and therapy. Molecular diagnostic systems are capable of detecting more than 40 distinct HPV types, particularly those correlated to high grade dysplasia (HSIL). The human microbiome refers to the sum of microorganisms that may reside in various parts of the human body (eukaryotic, archae, bacteria and viruses), their genetic information and how they interact with the host environment (7). We now have a lot of data regarding the mapping of microbiota in several sites of human body, especially the gut, due to the development in sequencing technology. During the last years, there is emerging evidence that the vaginal microbiota may play a crucial role in HPV carcinogenesis (8) and is related to protection against dysbiosis and HPV infection (9,10). In healthy women of reproductive age, vaginal pH is primarily regulated by lactic acid producing bacteria such as *Lactobacillus* species. In women, whose vaginal microbiota is not lactobacilli-dominated, anti-bacterial defensive mechanisms are decreased (11). Alterations in vaginal microbiota, such as bacterial vaginosis and vaginal infections, are usually correlated to respective changes in vaginal pH. In this setting a decrease in vaginal pH has been related with decreased risk of infections, such as *Chlamydia trachomatis*, trichomoniasis, and urinary tract infections. In the vaginal environment, five major community state types (CST) were described by Ravel et al. (12) (CST I-V), who studied the vaginal microbiota of 396 asymptomatic women and characterized the found species in five groups based on their genes. In a healthy environment, the following microorganisms are recognized; CST I, II, III, V and are dominated by *Lactobacillus crispatus (L. crispatus), Lactobacillus gasseri (L. gasseri), L. iners and Lactobacillus jensenii (L. jensenii)*. respectively. In contrast, CST IV is characterized by depletion of lactobacilli and increased diversity of anaerobic bacteria, such as *Atopobium* (12). Nevertheless, there are many questions yet to be answered concerning the relationship between the vaginal microbiota and how it correlates with the HPV natural history.

The aim of the present study was to present the current knowledge and to evaluate the correlation between microbiome and HPV.

Data sources

An extensive systematic search was performed in both PubMed and Scopus. All databases were searched up to February 25, 2019. The search strategy used in both databases included the combination of the key words: (microbiome or microbiota) and (HPV or human papillomavirus). The references of relevant articles were also hand-searched, for additional studies.

Study selection criteria

Studies reporting data on the association of microbiota and HPV were included in this systematic review. Abstracts in scientific conferences, editorials, and reviews as well as animal studies were not included in the study. Studies published in languages other than English, Dutch, German, Greek, Italian or Spanish were not taken into consideration.

Selected studies

A total of 78 and 291 articles were retrieved from PubMed and Scopus respectively. From those studies, 16 studies were identified as eligible for inclusion in this review. No additional studies were identified through hand-searching of references. The included studies are graphically presented in Figure 1 (flow diagram).

Techniques

From the eligible articles, the techniques that were used for HPV detection and the identification of species present in the microbiome were DNA tests, Sequencing and polymerase chain reaction (PCR) amplification.

Human papillomavirus detection

HPV detection and genotyping was performed either with commercially available DNA tests, such as Roche Linear Array HPV Genotyping test and Digene Hybrid Capture II DNA test, or through an assay of PCR using specific primers such as MY09/MY11 or GP5+/GP6+. A range of 15-49 HPV types was identified, including predominantly high-risk HPV types with or without low risk subtypes (13,14).

Vaginal microbiota

For the detection of vaginal microbiota, the initial assessment included diagnostic methods such as microscopic evaluation, Gram stain test, microbiological cultures and measurement of

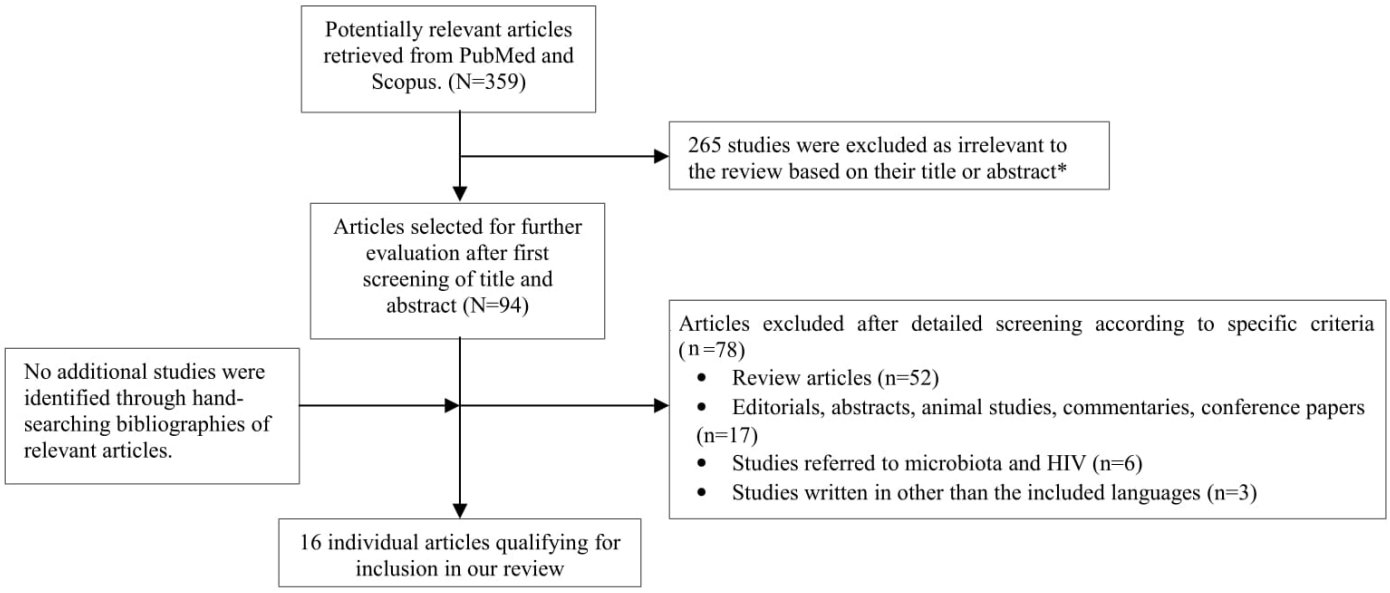

Figure 1. Flow diagram of the selection process of articles included in the review

HIV: Human immunodeficiency virus

****The majority of the studies were retrieved in both databases***

vaginal pH. Amsel criteria were used for diagnosing bacterial vaginosis (15). PCR amplification of V1-V5 hypervariable region of the 16S rRNA genes was used in 12 studies. PCR results were analyzed in accordance with databases such as BLAST, QIIME and Illumina MiSeq in order to identify specific species (Table 1).

Study characteristics

Sixteen studies, including a total of 12.204 patients, have been published in the literature with regard to the association between vaginal microbiota and HPV infection (16-34).

According to the searched literature, participants were categorized based on several epidemiologic criteria which included age, race/ethnicity, educational status, marital status, time of first intercourse, lifetime number of sexual partners, number of live births, use of hormonal contraception or antibiotics/probiotics, prevalence of smoking, alcohol use, and menstrual phase.

Concerning specific population characteristics, the correlation between HPV infection and vaginal microbiota has been studied in human immunodeficiency virus (HIV) infected patients (24,28), while Lee et al. (29) studied a twin cohort, as well as their siblings and mothers (18). A total of 824 individuals were reported to be female sex workers (17,29). Moreover, Kero et al. (31) enrolled 329 asymptomatic, pregnant women in the third trimester of their pregnancy and they studied the potential impact of vaginal microbiota on the outcome of known HPV infection during a 72-month follow-up. Brotman et al. (22) conducted a study of 32 patients, in which they assessed 937 self-collected samples before and after vaginal douching cessation.

The demographic details of the populations under study are shown in Table 2.

Types of vaginal microbiota

The detected microbiotas in these studies included several types of microorganisms: *L. iners* classified as CSTIII (13 studies, 72.22%); *L. crispatus* classified as CSTI (8 studies, 44.44%); CSTIV-B which represents anaerobic microbiomes combined with reduced *Lactobacillus* (5 studies, 27.77%); *Megasphaera*, *G. vaginalis* and *L. jensenii* which is classified as CSTV (4 studies, 22.22%); *Sneathia*, *L. gasseri* classified as CSTII as well as CSTIV-A which represents *Peptoniphilus, Anaerococcus, Corynebacterium, Finegoldia* and *Prevotella* (3 studies, 16.66 %); *Prevotella, L. vaginalis, Phylum protebacteria, Lactobacillus reuteri (L. reuteri)* and other members of Pseudomonas family (2 studies, 12.5%); and in one study each (6.25%) *Dialister, L. formicalis, Fusobacterium, Lactobacillus gallinarum* and *Lactobacillus salivarus* (found only among South African women).

Vaginal microbiota association with HPV and CIN

Interestingly, it was found that women with HPV infection had a higher diversity and a lower proportion of *Lactobacillus*. A

Table 1. Summary of the techniques applied in the included studies

First author, year	Patients included	HPV identification method	Detection method of microbioma
Dols et al. (16), 2012	100	INNO-LiPA® method	Microarray technology
Rodriguez-Cerdeira et al. (17), 2012	208	Digene Hybrid Capture® II DNA Test hrHPV probe B cocktail	Microscopically, microbiological culture
Clarke et al. (18), 2012	9,165	Hybrid Capture® Tube test PCR: MY09/MY11 L1 primer with AmpliTaq Gold polymerase	Vaginal pH measurement with pHydrion strip Detection of *C. trachomatis* using a *C. trachomatis* PCR-DEJA assay
Gao et al. (19), 2013	70	PCR: 1) MY09/MY11 and then 2)GP5+/ GP6+ primers	V2-V3 region of the 16S rRNA genes amplified by PCR BLAST database searches to identify bacterial species
Lee et al. (20), 2013	68	PCR: 1) MY09/MY11 2) GP5+/GP6+ primers	V2-V3 region of the 16S rRNA genes amplified by PCR Data analyzed using QIIME
Borgdorff et al. (21), 2014	174	Cervical cytology	Microarray containing 461 DNA hybridization probes targeting microorganisms and 164 positive (16S conserved regions) and negative controls
Brotman et al. (22), 2014	32	Roche Linear Array® HPV Genotyping test	Pyrosequencing V1-V2 hypervariable regions of 16S rRNA genes using primers barcoded 27F and 338R
Oh et al. (23), 2015	120	Digene Hybrid Capture® II DNA test	V1-V3 region of the 16S rRNA genes amplified by PCR Data analyzed using EzTaxon-e and BLASTn diversity indices calculated using Mothur program
Dareng et al. (24), 2015	278	Roche Linear Array® HPV Genotyping test	V4 region of the 16S rRNA genes amplified by PCR (primers: 515F, 806R) Data analyzed using Illumina® MiSeq
Mitra et al. (25), 2015	169	Abbott RealTime High Risk hrHPV® assay on Abbott M2000® platform	V1-V2 region of the 16S rRNA genes amplified by PCR Data analyzed in Mothur program using Illumina® MiSeq
Audirac-Chalifour et al. (26), 2016	32	HPV testing technique: Seegene Anyplex™ II HPV HR Detection assay NGS technique: 16S rRNA gene regions: V3-V4	High throughput sequencing of 16S rDNA amplicons and classification in community state types
Piyathilake et al. (27), 2016	430	Roche Linear Array® HPV Genotyping test	V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq
Reimers et al. (28), 2016	64	PCR: MY09/MY11/HMB01 L1 primer	V1-V2 region of the 16S rRNA genes amplified by PCR (primers 27F,338R) Data analyzed using QIIME
Lee et al. (29) 2016	616	Riatol HPV test	Microscopically, candida:10% KOH method bacterial vaginosis: Nugent criteria
Adebamowo et al. (30), 2017	194	SPF25/LiPA10 for hrHPV genotypes	V3-V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq
Kero et al. (31), 2017	329	PCR: 1) MY09/MY11 2)GP5+/GP6+ primers, genotyping withMultimetrix® kit	Hay/Ison criteria used for defining bacterial vaginosis as grade 1, 2 or 3
Di Paola et al. (32), 2017	72	Hybrid Capture 2 assay, followed by genotyping by amplifying the target DNA with PGMY09/11 and HLA primers	V3-V5 region of the 16S rRNA genes amplified by PCR, diversity measured using OTUs, Quantitative Real Time PCR of sialidase-encoding gene from Gardenella vaginalis
Shannon et al. (33), 2017	59	Roche Linear Array® HPV Genotyping test	V3-V4 region of the 16S rRNA genes amplified by PCR Data analyzed using Illumina® MiSeq, coinfection diagnostics: microscopically, Nugent criteria, nuclei acid amplification test, HerpeSelect gG-1 ang gG-2 ELISA
Di Pietro et al. (34), 2018	35	Cervical cytology	V3-V4 region of the 16S rRNA genes amplified by PCR, Data analysed using Illumina® MiSeq

hrHPV: High risk HPV, PCR: Polymerase chain reaction, ELISA: Enzyme-linked Immunosorbent Assay, OTU: Operational taxonomic unit, HLA: Human leukocyte antigen, rRNA: Ribosomal RNA, QIIME: Quantitative Insights Into Microbial Ecology, BLASTn: Basic Local Alignment Search Tool of Nucleotide-nucleotide, NGS: Next-generation sequencing, HPV: Human papillomavirus, KOH: Potassium hydroxide

Table 2. Demographics of the populations in the included studies

First author, year	Patients included	Mean age (in yrs)	Ethnicity/race (%)	Specific population characteristics
Rodriguez-Cerdeira et al. (17), 2012	208	27	Spanish	Female sex workers
Clarke et al. (18), 2012	9,165	41.8	Costa Rica	NM
Gao et al. (19), 2013	70	HPV (+): 37.8 HPV (-): 37	Chinese	NM
Lee et al. (20), 2013	68	Siblings:43 Mothers:65	Korean	Twins and their families
Brotman et al. (22), 2014	32†	37	African American (50) Caucasian (40)	Vaginal douching cessation study
Oh et al. (23), 2015	120*	range: 18-65	Korean	NM
Dareng et al. (24), 2015	278	HPV (+): 34.2 HPV (-): 37.9	Nigerian	Included pts with HIV
Mitra et al. (25), 2015	169	31	Caucasian (83)	NM
Piyathilake et al. (27), 2016	430	26.1	Non-Hispanic black (53)	NM
Reimers et al. (28), 2016	64	32.1	African American	Included pts with HIV
Lee et al. (29) 2016	616	28	Western Kenyan	Female sex workers
Adebamowo et al. (30), 2017	194	38	Nigerian	NM
Kero et al. (31), 2017	329	NM	Turkish	Pregnant, asymptomatic women
Di Paola et al. (32), 2017	55§	Range: 26-64	Italian/Caucasian	NM
Shannon et al. (33), 2017	59	HPV (+):33 HPV (-):37.5	African/Caribbean	NM
Di Pietro et al. (34), 2018	35	Healthy: 34.7 *C. trachomatis*: 28 HPV + *C. trachomatis*: 29.3 HPV: 35.8	Italian	NM

yrs: Years, NM: Not mentioned, HPV: Human papillomavirus, HIV: Human immunodeficiency virus, pts: Patients, *70 with CIN versus 50 controls, †Has been received 937 samples, §55 HPV (+) versus 17 controls

lower prevalence of *L. iners* and *L. crispatus* was also observed (20). Dols et al. (16) also reported a shift of the composition of the vaginal lactobacilli in HPV (+) women as well as a significantly reduced prevalence of *L. crispatus*. Other common microorganisms among HPV (+) patients were *L. gasseri* and *Gardnerella vaginalis* (17).

Additionally, patients who were eventually diagnosed with a cervical intraepithelial neoplasia (CIN) also had a high diversity of their vaginal microbiota (25) and they were usually colonised by *Sneathia*, while in women with invasive cervical cancer, *Fusobacterium* was the most common type of microorganism (26). Interestingly, Piyathilake et al. (27) found an abundance of *Lactobacillus* and *L. reuteri* specifically in women with CIN II.

In contrast, in HPV (-) women, *L. crispatus*/CSTI and *L. gasseri*/CSTII were the most common species (26). *L. crispatus* was related by Reimers et al. (28) and Borgdorff et al. (21) to decreased prevalence of oncogenic HPV types, while Darend identified prevalent high risk HPV infections among women with a decreased population of *Lactobacillus* and an increased abundance of anaerobes, particularly of the genera *Prevotella* and *Leptotrichia* (19,22).

Vaginal microbiota and HPV remission

The relationship between vaginal microbiota and HPV remission was highlighted by Brotman et al. (22). CSTIII was the classification group with the fastest remission, while CST IV-B was the one with the slowest. This was also confirmed by Di Paola et al. (32), who suggested CSTIV-B to be a risk factor for HPV persistence.

Transition to HPV and severity of infection

Brotman et al. (22) compared the CSTs among women HPV (-) who later became HPV (+) and reported that CSTIV-A was related to higher transition to HPV (+) status than CSTI.

Concerning the severity of the HPV infection, Mitra et al. (25) reported CSTIV and CSTV to be associated with high severity when CSTI was associated with low severity. CSTIII was related by Piyathilake et al. (27) to high severity CIN lesions.

Vaginal microbiota, HIV and HPV infections

Five of the above mentioned studies have shown an association between vaginal microbiota, HPV and HIV infection. *L. crispatus* was found to have an advantageous effect on the HPV infection evolution in both HIV-infected and uninfected women and in general *L. crispatus* was found to be a protective factor against HIV, high risk HPV and Herpes Simplex type 2, as it was found in high abundance in uninfected women while, in contrast, infected women had a reduced prevalence. In both HIV and HPV infections, a comparable shift in the composition of the *Lactobacillus* flora was identified.

Vaginal microbiota, ethnicity and HPV infection

Another factor that strongly affects the vaginal microbiota seems to be ethnicity. The studies included in this review refer to women of all ethnicities: European/Caucasian, Asian, Latin-American and African. It has been found that Afro-Caribbean women have a fourfold higher risk of suffering from a vaginal dysbiosis or high microbiota diversity, which indicates that the most common type of microbiota among them is CST IV, in comparison with European/Caucasian and African women. However, the prevalence of HPV and the rate of more severe lesions was not proportionately higher (35).

Microbiological markers of HPV infection

It is remarkable, that among all the microbiota, Fusobacteria including *Sneathia*, were identified as a possible microbiological marker correlated with HPV infection, as was shown by Lee et al. (29). However, the relation between HPV infection and the coexistence with other types of vaginal microbiota appears to be either protective, or to predispose to HPV infection. In addition, the evolution of HPV infection is in direct correlation with the species or genus of the vaginal microbiota dominating the vaginal environment. Specifically, some types of *Lactobacillus* including *L. gasseri, L. jensenii* and *L. crispatus* seem to protect from HPV infection while on the contrary other microorganisms, especially *Sneathia, Anaerococcus tetradius, Peptostreptococcus, Fusobacterium, Gardnerella vaginalis* and *L. iners*, often together with a low abundance of the other types of *Lactobacillus* and other factors such as smoking and lack of barrier contraception or low estrogen levels, not only lead to elevated rates of HPV infection, but also to higher disease severity and lower HPV remission. This suggests that some of the microbiota species may be used as a disease marker or even as a therapeutic mean against HPV. The effect of the vaginal microbiota on the evolution of an HPV infection is described in Table 3.

Discussion

A systematic review of the literature was conducted with the aim of examining if vaginal microbiota composition patterns can be related to HPV infection and intraepithelial lesions. This study indicates a significant correlation among vaginal microbiota and HPV infection in that certain microbial species appear to play a protective role against HPV infection while others predispose to either the progression or the remission of the disease.

Alterations in vaginal microbiota have been associated with a variety of complications, either obstetric or gynecologic, such as tubal factor infertility, spontaneous abortion, intrauterine fetal demise, premature rupture of membranes, pre-term labor and delivery, intrauterine growth restriction, endometritis, postpartum infection, chorioamnionitis, ectopic pregnancy and pelvic inflammatory disease (35). Some of these conditions may cause chronic and severe congenital tract infections, which have been associated with vaginal flora alterations (36). Despite the advances in molecular elucidation of the vaginal microbiota, the exact pathophysiologic pathway has not yet clearly identified.

Table 3. Protective and burdening parameters of vaginal microbiota regarding human papillomavirus infection/persistence.

Protective	Predisposing
L. gasseri *L. jensenii* *L. crispatus* Barrier contraception Estrogen levels → protection for dysbiosis	*Sneathia* spp. Reduction of *Lactobacillus* *Anaerococcus tetradius* *Peptostreptococcus* *Fusobacterium* Gardnerella vaginae *L. iners* + unclassified *Lactobacillus* CST IV Vaginal dysbiosis
CST: Community state type	

Microbiota analysis of HPV positive patients and patients with CIN revealed significant alterations compared to that present in women without either HPV positivity or CIN. Certain microbiotic species found in the vagina have been associated with increased risk of infection from HPV and can serve as a critical predictive and prognostic markers for the early detection of those pathologies. For example, a significant proportion of *Lactobacillus* species have been documented to be protective against HPV whereas *Sneathia* species can negatively affect the evolution of HPV. This is in accordance with respective studies in the field which showed that decrease in *Lactobacillus* species in vaginal flora, had significant impact on eubiosis and led to increase in concentration of pathogenic anaerobic bacteria such as *Gardnerella* and *Sneathia* (37). Furthermore, remission and severity of HPV infection were additionally influenced by the presence of certain microbiotic species in the vaginal environment. Moreover, there appears to be a strong association between dysbiosis protection and HPV infection, even though the pathophysiology of this association is not yet fully understood. It has been proposed that these mechanisms not only encompass the patient's defensive function but also her past immunological response against HPV (38). The potential impact of dysbiosis on the immune system could explain the susceptibility of those women to HIV infections (39).

The association between ethnicity and HPV with regards to the vaginal microbiota has not yet been clearly determined. This review also revealed that Afro-Caribbean ethnicity was associated with alterations in vaginal flora. However, HPV incidence was not significantly different in Afro-Caribbean women with dysbiosis. On the contrary, according to a recent meta-analysis, African women presented with higher rates of HPV infection compared to other ethnicities (40). Nonetheless, research in these populations is still limited and further studies are needed so as to elucidate the association among dysbiosis and HPV infection in African populations. Another factor that should be addressed is that flora alterations are responsible not only for increased susceptibility to HPV infections, but are also present in cancerous and pre-cancerous cervical disorders (8). More specifically, vaginal dysbiosis has been associated with more rapid disease progression and more advanced disease stages (25).

Several limitations can be found in such a newly studied field. First and foremost, the number of studies and thus the number of patients included is limited, which serves to highlight the innovation of this approach. In terms of search strategy used for this review, it could be considered unduly limited due to the exclusion of abstracts, review articles, conference papers, editorials, animal studies, and commentaries. The retrospective nature of the available studies could also be highlighted. Large prospective randomized controlled trials are necessary to clarify the possible correlation of vaginal microbiota with HPV and related pathologies.

Conclusion

Recently available data suggest a potential association between the vaginal microbioma and HPV infection. Specifically, (i) highly diverse vaginal flora, (ii) identification of specific species such as *Sneathia*, (iii) low concentration of *Lactobacillus* and the subsequent vaginal dysbiosis were found to affect the incidence, persistence and severity of HPV infection. The aforementioned parameters should be subjected to further investigation via multicentre trials in order to be validated as robust independent risk factors.

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A comparison of the risk of cesarean section in gestational diabetes mellitus patients supplemented antenatally with vitamin D containing supplements versus placebo: A systematic review and meta-analysis of double-blinded randomized controlled trials

Sumanta Saha[1], Sujata Saha[2]

[1]National Institute for Research in Tuberculosis, India
[2]Mankar College, India

Abstract

The aim of this study was to study the role of vitamin D containing supplements in the risk of cesarean section (CS), a common complication in gestational diabetes mellitus (GDM) patients. An additional objective was to assess the risk of developing pre-eclampsia, preterm delivery, macrosomia, and polyhydramnios in these participants. Various electronic databases were searched for double-blinded parallel-arm randomized controlled trials that reported the incidence of CS in adult, non-insulin treated GDM patients who received vitamin D and placebo in different treatment arms, respectively. Next, each eligible trial's risk of bias was assessed, and the effects of the above interventions on the respective outcomes were compared meta-analytically across the trials. This review included five Iranian trials sourcing data from nearly 380 participants. The risk of bias in the trials was primarily low. In contrast to the placebo group, the risk of CS [risk ratio (RR): 0.61, p=0.002, 95% confidence interval (CI): 0.44,0.83; I^2=0%, p-value of Cochrane's Q: 0.373) and macrosomia (RR: 0.31, p=0.006, 95% CI: 0.13,0.72; I^2=0%, p-value of Cochrane's Q: 0.935] was less in the vitamin D supplemented group. The remaining outcomes did not differ between the intervention groups. The antenatal use of vitamin D containing supplements in non-insulin treated GDM patients might reduce the risk of CS and macrosomia. (J Turk Ger Gynecol Assoc 2020; 21: 201-12)

Keywords: Diabetes, gestational, vitamin D, cesarean section, fetal macrosomia, pre-eclampsia, premature birth, polyhydramnios

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Introduction

Gestational diabetes mellitus (GDM) is a glucose intolerance to any degree occurring at the start of pregnancy or first recognized during gestation (1). It is diagnosed between 24-28 weeks of gestation using screening tests with a 50 gram and 1-hour glucose challenge test (1). It is classified as either A1GDM or A2GDM, depending on whether it is managed with dietary therapy or medication, respectively (1). The chief medication used to treat GDM if diet and exercise therapy fails is insulin (1). Glyburide and metformin, two oral hypoglycemic agents with the potential to cross the placenta, are also used to treat GDM frequently. However, such use of these medications is not approved by the U.S. Food and Drug Administration due to inadequate safety information (1,2). Unlike type 1 and type 2 diabetes, newer drugs such as sodium-glucose linked transporter 2 inhibitors, remain poorly studied in GDM patients (3-5).

Address for Correspondence: *Sumanta Saha*
e.mail: sumanta.saha@uq.net.au ORCID: orcid.org/0000-0003-0996-8846

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GDM can cause both neonatal complications including macrosomia, neonatal hypoglycemia, shoulder dystocia, and hyperbilirubinemia and maternal complications (1,6). One of the chief maternal complications of GDM is cesarean section (CS), in which the fetus is delivered surgically by incising the abdomen and uterus of the parturient (1,7-9). The prevalence of CS is high in GDM patients (32-44%), and it is more common than in parturients with no glucose intolerance (7,10-15). The indication for CS is determined by the obstetric need of the GDM mother and includes indications such as pre-eclampsia, macrosomia, excessive fetal growth (fetal weight more than 4500 gm), and past obstetric history, for example previous history of childbirth by CS (7,8,16-18). CS increases the risk of wound hematoma, anesthetic complications, major puerperal infection, and severe hemorrhage which may result in hysterectomy (19). Moreover, women undergoing planned vaginal delivery are less likely to have severe morbidity or mortality compared to those delivered by CS on an emergency basis (19).

To minimize these surgical risks, it is important to identify new pragmatic treatment options that can decrease the incidence of CS in GDM patients. In this regard, the plausible clinical role of antenatal vitamin D supplementation in GDM patients is a novel area to explore, as suggested by recent vitamin D-related research. Existing studies suggest a possible association between vitamin D deficiency and GDM (20-24). Moreover, GDM prevalence tends to decrease on prenatal supplementation of vitamin D (25,26). Besides, maintaining the recommended optimum vitamin D status during pregnancy might be protective against CS, although the mechanism remains unclear (27-29). When vitamin D is complemented in GDM patients, it facilitates better glycemic control when measured by a decrease in fasting plasma glucose and/or insulin and improvement in homeostasis model of assessment-insulin resistance (20-24,30,31). All these vitamin D related findings in pregnancy and GDM formed the rationale for undertaking this study; to explore the risk of CS in (antenatal) vitamin D supplemented GDM patients.

The intervention

Vitamin D is a fat-soluble hormone (32). It is available from diet and supplements in two physiologically inactive forms - D2 (ergocalciferol) and D3 (cholecalciferol) (33,34). Vitamin D3 is additionally synthesized in the skin on exposure to the sun (33). The active form of vitamin D, calcitriol 1,25-(dihydroxyvitamin) 2D, is produced on hydroxylation of vitamin D2 and D3 successively in the liver and kidneys (33,35). This active form plays a role in the physiology of pregnancy via the vitamin D receptors in uteroplacental tissue (33,35).

Recently, different clinical trials have tested the health effects of antenatal vitamin D supplementation in GDM patients. However, the route of vitamin D administration [parenteral (36) versus oral (37-40)], dosing, and the accompanying supplements (when used) varied among such trials. Some trials in pregnant women have used vitamin D as a sole supplement, (36-38) while others used it with co-supplements such as magnesium, zinc, or calcium (39,40). A trial that tested the role of intramuscular administration of vitamin D in GDM patients, used it as a single injection of 300.000 IU (36). In clinical trials that prescribed oral vitamin D, GDM patients were advised to take it at a dose of 50.000 IU, 2-3 weeks apart for 3-8 weeks (38,40). Other such trials asked GDM patients to take 200-500 IU of oral vitamin D twice daily for 6-16 weeks (37,41).

What this review adds?

In GDM patients, the contemporary evidence of the effect of antenatal vitamin D supplementation on CS, and other obstetric outcomes are based on the evidence of clinical trials, like those reviewed in this paper. However, to the best of our knowledge, there has been no previous attempt to synthesize the overall rigor of such evidence by systematic review and meta-analysis. Therefore, this paper reviews this poorly evidenced area of GDM literature and synthesizes new evidence based on the existing highest quality of epidemiological studies (i.e., double-blinded randomized clinical trials). In addition, as this study involved GDM mothers who were not on insulin treatment, the latter's therapeutic effects are unlikely to bias this findings of this study.

Aim

This study aimed to compare the risk of CS between non-insulin treated GDM patients supplemented antenatally with vitamin D containing supplements and placebo. The auxiliary objective was to compare the risk of macrosomia, polyhydramnios, pre-eclampsia, and pre-term delivery among these treatment groups.

Material and Methods

Inclusion criteria: 1. Study design: Parallel-arm (any number of arms) double-blinded randomized controlled clinical trials of any duration were eligible. 2. Participant: The eligible participants were adult (18 years or older) females diagnosed with GDM by American Diabetes Association criteria (42,43), between 24-28 weeks of their concurrent pregnancy who received the intervention of interest before initiation of insulin therapy. 3. Intervention compared: The above-described trials should compare the following interventions - vitamin D (in D2 or D3 form or both; as a sole supplement or adjunct to any other supplements) with placebo. Vitamin D supplementation was accepted irrespective of its dose and route of administration;

oral or intramuscular. 4. Outcome: The trials must report the frequency of CS observed in each of the studied treatment groups, post-intervention.

Exclusion criteria: 1. Study design: Differing from that described in the inclusion criteria, which included observational study designs, single-arm interventional studies, and cross-over trials. 2. Participants: With diabetes of any other type except GDM or those diagnosed previously with GDM were excluded from this review.

The secondary outcomes of interest were the risk of macrosomia, polyhydramnios, pre-eclampsia, and pre-term delivery. However, these did not contribute to the inclusion criteria. This review follows the PRISMA (44) reporting guideline and does not have a pre-published protocol.

The search for eligible trials was conducted in electronic databases (PubMed, Embase, and Scopus) with no restriction to date or language. The following search strategy was used in PubMed: "vitamin D" or calciferol OR "vitamin D2" or ergocalciferol or "vitamin D3" or cholecalciferol or cholecalciferol (MeSH) or "ergocalciferols" (MeSH) AND "diabetes, gestational" (MeSH) and "gestational diabetes" or GDM. The search was restricted to clinical trials by using the filters "Clinical Trial" and "Randomized Controlled Trial." Identical search terms were used for searching the other databases. The last date of database search was 07 February, 2020.

The papers identified by the electronic database search were skimmed for trials matching this review's eligibility criteria. Publications were read in full text when they seemed to match these criteria or in circumstances where a decision of their inclusion or exclusion was not possible by reading the titles and abstracts only. Besides the above, an auxiliary search was conducted in the references of the papers that were included in this review.

Then the following data were extracted from the included trials: author information (first author's last name and year of publication), study design (randomization, blinding, if placebo-controlled, single or multicentric, funding, ethical clearance, trial ID), participants (diagnosis, gestational age of GDM diagnosis, number randomized, mean age, participant consent, trial nation), interventions (intervention/s received by each of the trial arms), and outcomes. Using the appropriate tool from the Cochrane Collaboration, the risk of selection bias in the trials (based on random sequence generation and concealment of participant allocation), performance bias, detection bias, attrition bias, reporting bias and miscellaneous bias were assessed and categorized as high risk, low risk, and unclear risk (45).

The first author conducted the database search and retrieved the eligible trials and their data. The co-author subsequently rechecked it. The risk of bias in the respective trials was assessed by each author independently, and then the findings were cross referenced and matched. The authors resolved disputes in their opinion at all stages of this review by discussion.

The intervention effects on the outcomes were compared across the trials by the random-effect model meta-analysis (DerSimonian and Laird) method, and the summary effect was determined in risk ratios (RR). Despite the relative homogeneity of the participant characteristics and study design, a random-effect model was used since the vitamin D supplement adjuncts used between the trails were not identical. To determine the effects of vitamin D as a chief supplement, in trials that used it in multiple treatment arms, we chose one that included a fewer number of vitamin D adjuncts. For meta-analyses, when an outcome occurred in one of the intervention arms of a trial only, 0.5 was added to each cell of the 2x2 table. Heterogeneity was assessed using the p-value of Cochranes Q (statistical significance determined at $p<0.1$) in conjunction with I^2 statistics (0-40%, 30-60%, 50-90%, and 75-100% represented less, moderate, substantial, and considerable heterogeneity, respectively) (45). Funnel plots were used to visually assess publication bias.

Finally, sensitivity analyses were performed, in which the meta-analysis for the respective outcomes was iterated using a fixed-effect model (inverse-variance method) and also by excluding a study each time (using both fixed-effect and random effect model). At $p<0.05$ and 95% confidence interval, results were considered statistically significant. The Stata statistical software (StataCorp, College Station, Texas, USA; version 16) was used to perform statistical analyses.

Results

The initial electronic search returned 836 citations. After excluding the duplicates, the titles and abstracts of 757 papers were read. For 16 studies, full-text reading ensued. Finally, five trials meeting the eligibility criteria of this review were included for the risk of bias assessment and quantitative analysis (Figure 1) (46-50). These trials were published between 2015-19, were primarily single centered (47-51) except one (46), and based on about 380 GDM patients from Iran. The average age of these participants was approximately between 28-32 years (46-50).

Two of these trials (48,50) tested vitamin D as a sole supplement in one of their treatment arms (48). In the intervention arms of the remaining trials, vitamin D was co-supplemented with another supplement including probiotics, magnesium, calcium, and zinc (46,47,49). All trials had a placebo arm (46-50). Each trial reported both the primary and secondary outcomes (46-50).

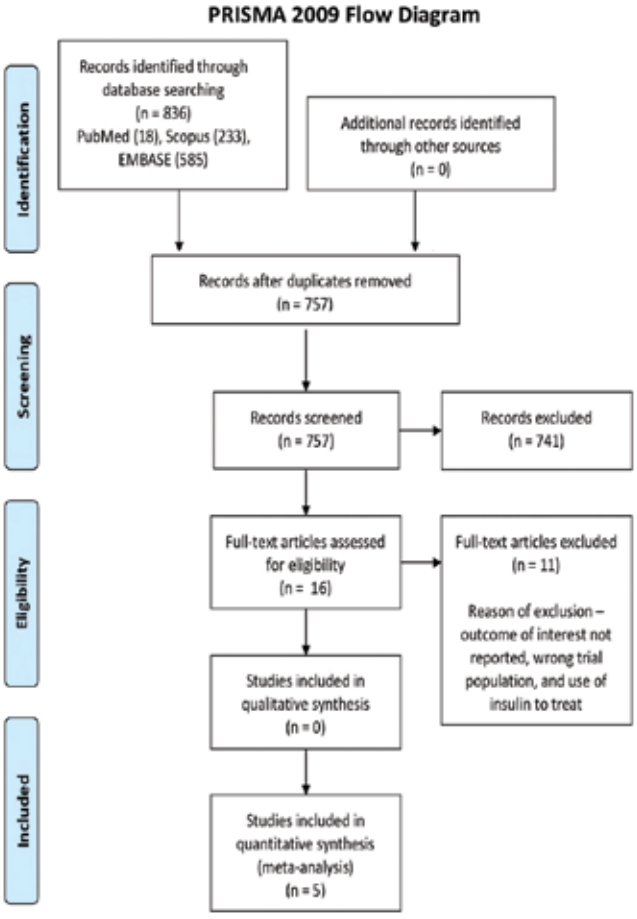

Figure 1. PRISMA 2009 Flow Diagram [From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097]

Regarding the appraisal of the studies, overall the trials are at a low risk of bias except for unclear risk of allocation concealment in four trials (46,47,49,50) and performance bias in one trial (47). Table 1 presents the salient features and the risk of bias assessment of the reviewed trials (46-50).

Upon meta-analysis, GDM patients receiving vitamin D containing supplements had a lower risk of experiencing CS (RR: 0.61, p=0.002, 95% confidence interval (CI): 0.44,0.83; I^2=0%, p-value of Cochrane's Q: 0.373) and macrosomia (RR: 0.31, p=0.006, 95% CI: 0.13,0.72; I^2=0%, p-value of Cochrane's Q: 0.935) than the placebo recipients. The risk of the remaining outcomes did not vary between the compared interventions. Overall, for all outcomes, statistical heterogeneity was classified as less, that is between 0-40% (45). The forest plots (Figure 2-6) depict the outcome data along with their effect sizes.

On visual inspection, the funnel plots (not shown) were not suggestive of any publication bias. Sensitivity analysis results were almost identical to the preliminary analyses (Table 2).

Table 1. Salient features of reviewed papers and risk of bias assessment

Study: Asemi et al. (50)						
Design	**Participants**	**Interventions compared**	**Reported outcomes**			
Randomized Double-blind Placebo-controlled Single centered Funding information: Provided Ethical clearance: Obtained Trial ID: IRCT2013051156 23N7	Diagnosis: GDM Gestational age of GDM diagnosis: 24-28 week Recruited 18-40 years old Randomized (n=50) Mean age: 30.9 years Consent: Obtained. Country: Iran	Two interventions: 1. Vitamin D: 50.000 IU vitamin D3 pearl twice during the trial period (at baseline and day 21), 2. Placebo: Twice (at baseline and day 21). Duration of intervention: 6 weeks.	1. Caesarean section 2. Macrosomia 3. Polyhydramnios 4. Pre-eclampsia 5. Pre-term delivery			
Risk of bias assessment (45)						
Random sequence generation (selection bias)	**Allocation concealment (selection bias)**	**Blinding of participants and personnel (performance bias) All outcomes**	**Blinding of outcome assessment (detection bias) All outcomes**	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk	Unclear risk Comment: Precise mechanism of concealment not clear.	Low risk Comments: Investigators and participants were not aware of the intervention participants received.	Low risk	Low risk	Low risk	Low risk

Table 1. contiuned

Study: Jamilian et al. (47)						
Design		**Participants**		**Interventions compared**		**Reported outcomes**
Randomized Double-blind Placebo-controlled Single centered Funding information: Provided Ethical clearance: Obtained Trial ID: IRCT201706075623N119		Diagnosis: GDM Gestational age of GDM diagnosis: 24-28 week Recruited 18-40 years old Randomized (n=90) Mean age: 30 years Consent: Obtained. Country: Iran		Three interventions: 1. Probiotic: $8x10^9$ CFU/g, 2. Vitamin D3: every 2 weeks plus $8x10^9$ CFU/g probiotic, 3. Placebo. Duration of intervention: 6 weeks.		1. Caesarean section 2. Macrosomia 3. Polyhydramnios 4. Pre-eclampsia 5. Pre-term delivery
Risk of bias assessment (45)						
Random sequence generation (selection bias)	**Allocation concealment (selection bias)**	**Blinding of participants and personnel (performance bias) All outcomes**	**Blinding of outcome assessment (detection bias) All outcomes**	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk	Unclear risk Comment: Precise mechanism of concealment not clear.	Unclear risk Comment: It is not clear how study personnel were blinded.	Low risk	Low risk	Low risk	Low risk
Study: Jamilian et al. (49)						
Design		**Participants**		**Interventions compared**		**Reported outcomes**
Randomized Double-blind Placebo-controlled Single centered Funding information: Provided Ethical clearance: Obtained Trial ID: IRCT201704225623N109		Diagnosis: GDM Gestational age of GDM diagnosis: 24-28 week Recruited 18-40 years old Randomized (n=60) Mean age: 28.4 years Consent: Obtained. Country: Iran		Two interventions: 1. Vitamin D: 200 IU along with 100 mg magnesium, 4 mg zinc, 400 mg calcium twice daily, 2. Placebo. Duration of intervention: 6 weeks.		1. Caesarean section 2. Macrosomia 3. Polyhydramnios 4. Pre-eclampsia 5. Pre-term delivery
Risk of bias assessment (45)						
Random sequence generation (selection bias)	**Allocation concealment (selection bias)**	**Blinding of participants and personnel (performance bias) All outcomes**	**Blinding of outcome assessment (detection bias) All outcomes**	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk	Unclear risk Comment: Precise mechanism of concealment not clear.	Low risk	Low risk	Low risk	Low risk	Low risk

Table 1. contiuned

Study: Karamali et al. (46)						
Design		**Participants**		**Interventions compared**		**Reported outcomes**
Randomized Double-blind Placebo-controlled Multicentric Funding information: Provided Ethical clearance: Obtained Trial ID: IRCT201407115623N23		Diagnosis: GDM Gestational age of GDM diagnosis: 24-28 week Recruited 18-40 years old Randomized (n=60) Mean age: 30.15 years Consent: Obtained. Country: Iran		Two interventions: 1. Vitamin D3: 50000 IU at baseline and day 21 along with 1000 mg calcium carbonate daily, 2. Placebo: two placebos-one for vitamin D at baseline and day 21 and one for calcium everyday. Duration of intervention: 6 weeks.		1. Caesarean section 2. Macrosomia 3. Polyhydramnios 4. Pre-eclampsia 5. Pre-term delivery
Risk of bias assessment (45)						
Random sequence generation (selection bias)	**Allocation concealment (selection bias)**	**Blinding of participants and personnel (performance bias) All outcomes**	**Blinding of outcome assessment (detection bias) All outcomes**	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk	Unclear risk Comment: Precise mechanism of concealment not clear.	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Razavi et al. (48)						
Design		**Participants**		**Interventions compared**		**Reported outcomes**
Randomized Double-blind Placebo-controlled Single centric (51) Funding information: Provided Ethical clearance: Obtained Trial ID: IRCT201701305623N106		Diagnosis: GDM Gestational age of GDM diagnosis: 24-28 week Recruited 18-40 years old Randomized (n=120) Mean age: 29.67 years Consent: Obtained. Country: Iran		Four interventions: 1. Vitamin D: 50.000 IU two weekly and placebo for omega-3 fatty acids two times a day, 2. Vitamin D: 50.000 IU two weekly plus 1,000 mg omega-3 fatty acids two times a day, 3. 1,000 mg omega-3 fatty acids two times a day and placebo for vitamin D two weekly, 4. Placebo. Duration of intervention: 6 weeks.		1. Caesarean section 2. Macrosomia 3. Polyhydramnios 4. Pre-eclampsia 5. Pre-term delivery
Risk of bias assessment (45)						
Random sequence generation (selection bias)	**Allocation concealment (selection bias)**	**Blinding of participants and personnel (performance bias) All outcomes**	**Blinding of outcome assessment (detection bias) All outcomes**	**Incomplete outcome data (attrition bias) All outcomes**	**Selective reporting (reporting bias)**	**Other bias**
Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
GDM: Gestational diabetes mellitus, CFU: Colony forming units						

Table 2. Sensitivity analysis (by dropping a trial in each meta-analytic iteration)

Outcome	Dropped study		RR (95% CI)		P	Heterogeneity	
	Author	Year	RE model	FE model		I^2 statistics (%)	p-value of Cochrane's Q
Caesarean section	Asemi et al. (50)	2015	0.53 (0.36, 0.78)	0.53 (0.36, 0.78)	0.001*	0%	0.470
	Jamilian et al. (47)	2019	0.61 (0.41, 0.90)	0.62 (0.44, 0.87)	0.012*	19%	0.295
	Jamilian et al. (49)	2019	0.62 (0.44, 0.88)	0.63 (0.45, 0.87)	0.008*	12.6%	0.329
	Karamali et al. (46)	2016	0.69 (0.48, 0.97)	0.69 (0.48, 0.97)	0.033*	0%	0.708
	Razavi et al. (48)	2017	0.57 (0.40, 0.80)	0.57 (0.40, 0.80)	0.001*	0%	0.395
Pre-term delivery	Asemi et al. (50)	2015	0.65 (0.15, 2.73)	0.65 (0.15, 2.73)	0.552	0%	0.698
	Jamilian et al. (47)	2019	0.66 (0.16, 2.79)	0.66 (0.16, 2.79)	0.572	0%	0.711
	Jamilian et al. (49)	2019	0.65 (0.15, 2.75)	0.65 (0.15, 2.75)	0.559	0%	0.703
	Karamali et al. (46)	2016	0.33 (0.07, 1.61)	0.33 (0.07, 1.61)	0.170	0%	1.000
	Razavi et al. (48)	2017	0.65 (0.15, 2.75)	0.65 (0.15, 2.75)	0.559	0%	0.703
Pre-eclampsia	Asemi et al. (50)	2015	0.60 (0.25, 1.45)	0.60 (0.25, 1.45)	0.258	0%	0.816
	Jamilian et al. (47)	2019	0.70 (0.25, 1.92)	0.70 (0.25, 1.92)	0.482	0%	0.893
	Jamilian et al. (49)	2019	0.55 (0.21, 1.47)	0.55 (0.21, 1.47)	0.233	0%	0.799
	Karamali et al. (46)	2016	0.60 (0.25, 1.46)	0.60 (0.25, 1.46)	0.261	0%	0.820
	Razavi et al. (48)	2017	0.45 (0.16, 1.25)	0.45 (0.16, 1.25)	0.127	0%	0.957
Polyhydramnios	Asemi et al. (50)	2015	0.48 (0.18, 1.26)	0.48 (0.18, 1.26)	0.136	0%	0.740
	Jamilian et al. (47)	2019	0.39 (0.13, 1.19)	0.39 (0.13, 1.19)	0.099	0%	0.557
	Jamilian et al. (49)	2019	0.40 (0.15, 1.09)	0.40 (0.15, 1.09)	0.072	0%	0.557
	Karamali et al. (46)	2016	0.49 (0.18, 1.37)	0.49 (0.18, 1.37)	0.175	0%	0.677
	Razavi et al. (48)	2017	0.32 (0.11, 0.90)	0.32 (0.11, 0.90)	0.032*	0%	0.795
Macrosomia	Asemi et al. (50)	2015	0.30 (0.12, 0.75)	0.30 (0.12, 0.75)	0.010*	0%	0.847
	Jamilian et al. (47)	2019	0.28 (0.10, 0.78)	0.28 (0.10, 0.78)	0.014*	0%	0.865
	Jamilian et al. (49)	2019	0.33 (0.13, 0.85)	0.33 (0.13, 0.85)	0.021*	0%	0.889
	Karamali et al. (46)	2016	0.34 (0.14, 0.82)	0.34 (0.14, 0.82)	0.017*	0%	0.959
	Razavi et al. (48)	2017	0.27 (0.10, 0.75)	0.27 (0.10, 0.75)	0.012*	0%	0.882

*P<0.05, CI: Confidence interval, RE: Random-effect, FE: Fixed-effect

Discussion

To summarize, five recent double-blinded randomized controlled Iranian trials (comprising about 380 GDM patients) compared the obstetric risk of CS, macrosomia, polyhydramnios, pre-eclampsia, and pre-term delivery between the prenatal recipients of vitamin D and placebo. The risk of bias in the trials was predominantly low with occasional unclear risk of bias components (46-50). The meta-analyses suggested that in GDM patients, antenatal vitamin D containing supplement recipients had a reduced risk of CS and macrosomia than those who took a placebo.

The evidence quality of CS and macrosomia was graded using the GRADE approach [GRADE Working Group (2004)] (52). Due to the unclear risk of bias present in some of the trials, the evidence was downgraded by one level to moderate-quality evidence.

The scope of contrasting the findings of this review with the existing literature is limited, due to its conceptual novelty. In this regard, there is a recent review by Cochrane collaboration comparing obstetric outcomes between the vitamin D (as a sole or complementary supplement) and placebo receiving pregnant females (27). It found no major difference in the risk of CS between these intervention groups (27). However, unlike this review, the Cochrane collaboration review (27) was not specific to the GDM subpopulation.

The implications of this review are discussed here. First, healthcare professionals caring for GDM patients might find this review of worth to expand their existing knowledge in this context. Next, research in this milieu may help to

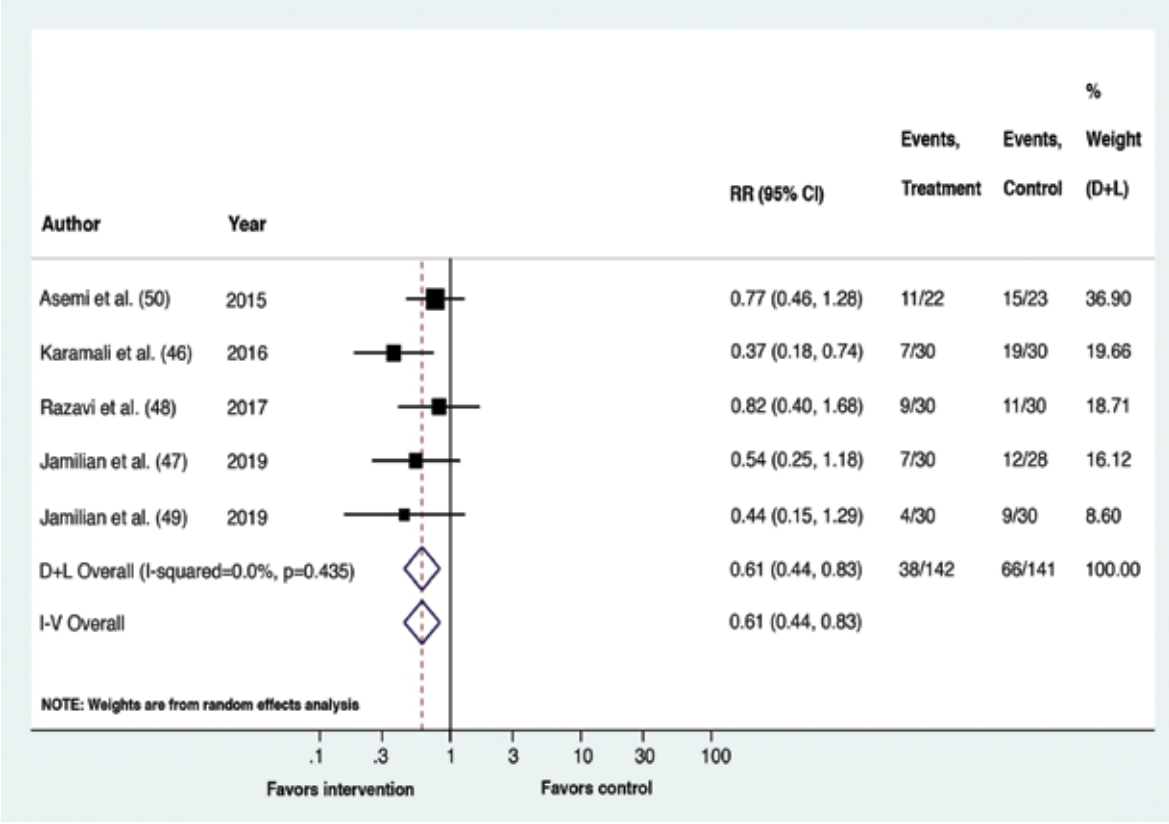

Figure 2. Forest plot: Comparison between vitamin D supplemented group and placebo for the outcome cesarean section

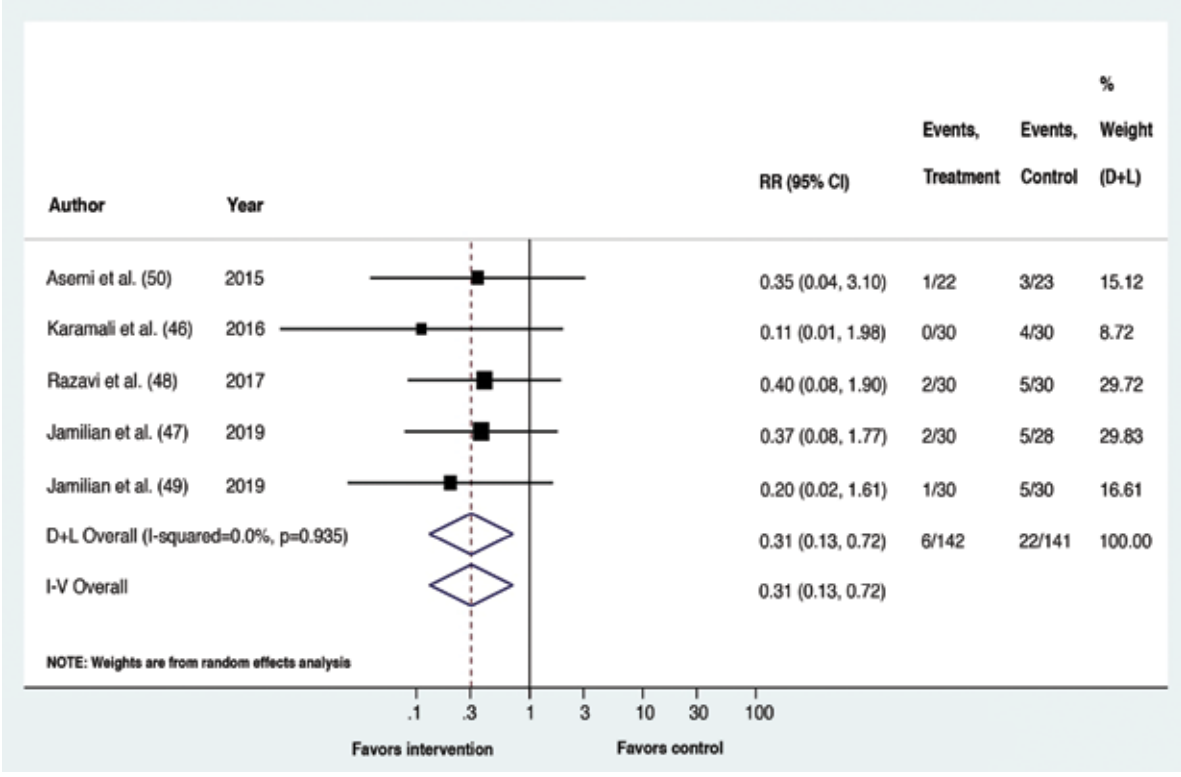

Figure 3. Forest plot: Comparison between vitamin D supplemented group and placebo for the outcome macrosomia

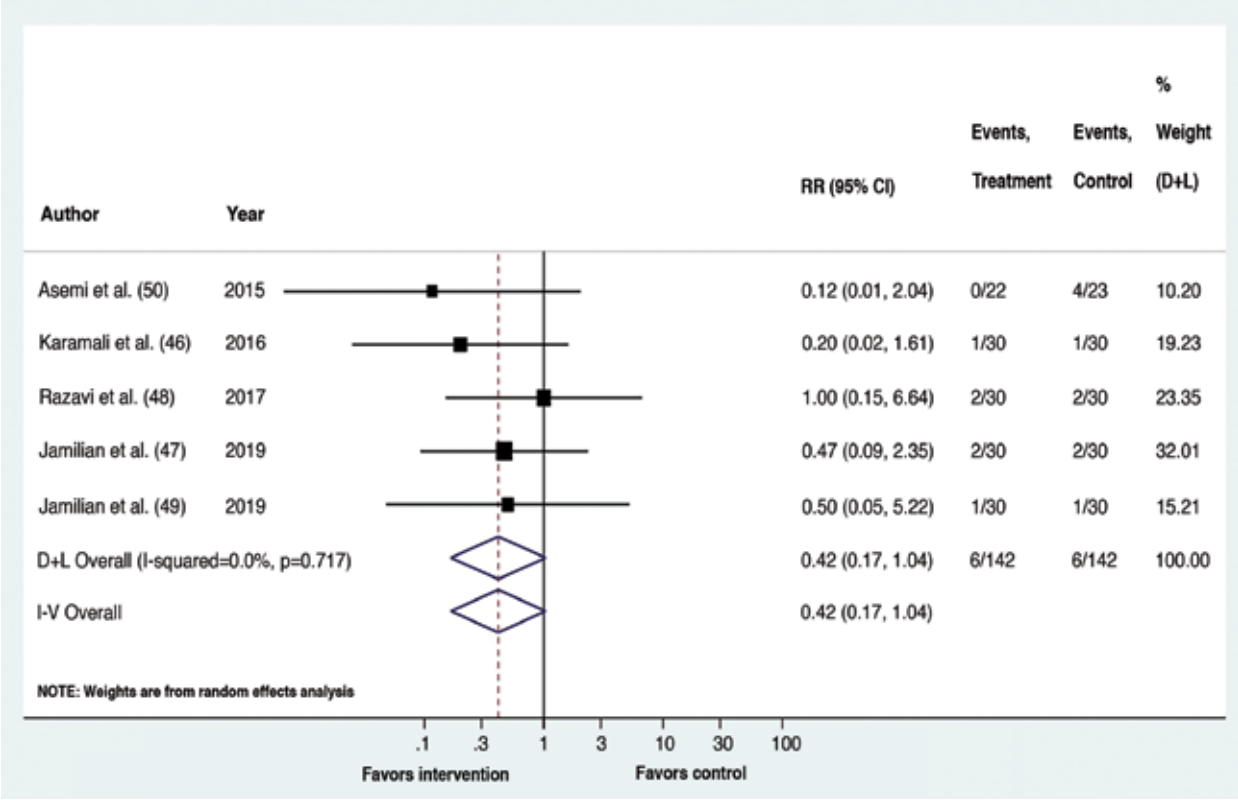

Figure 4. Forest plot: Comparison between vitamin D supplemented group and placebo for the outcome polyhydramnios

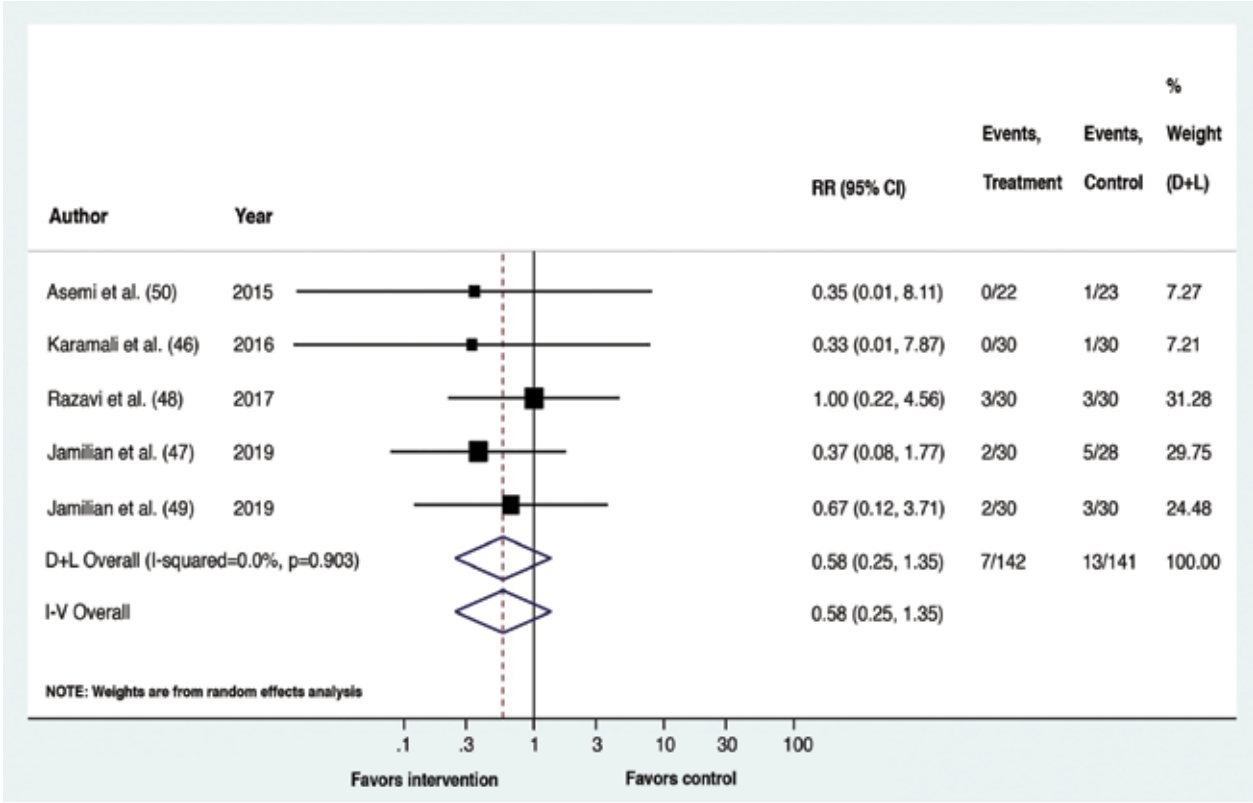

Figure 5. Forest plot: Comparison between vitamin D supplemented group and placebo for the outcome pre-eclampsia

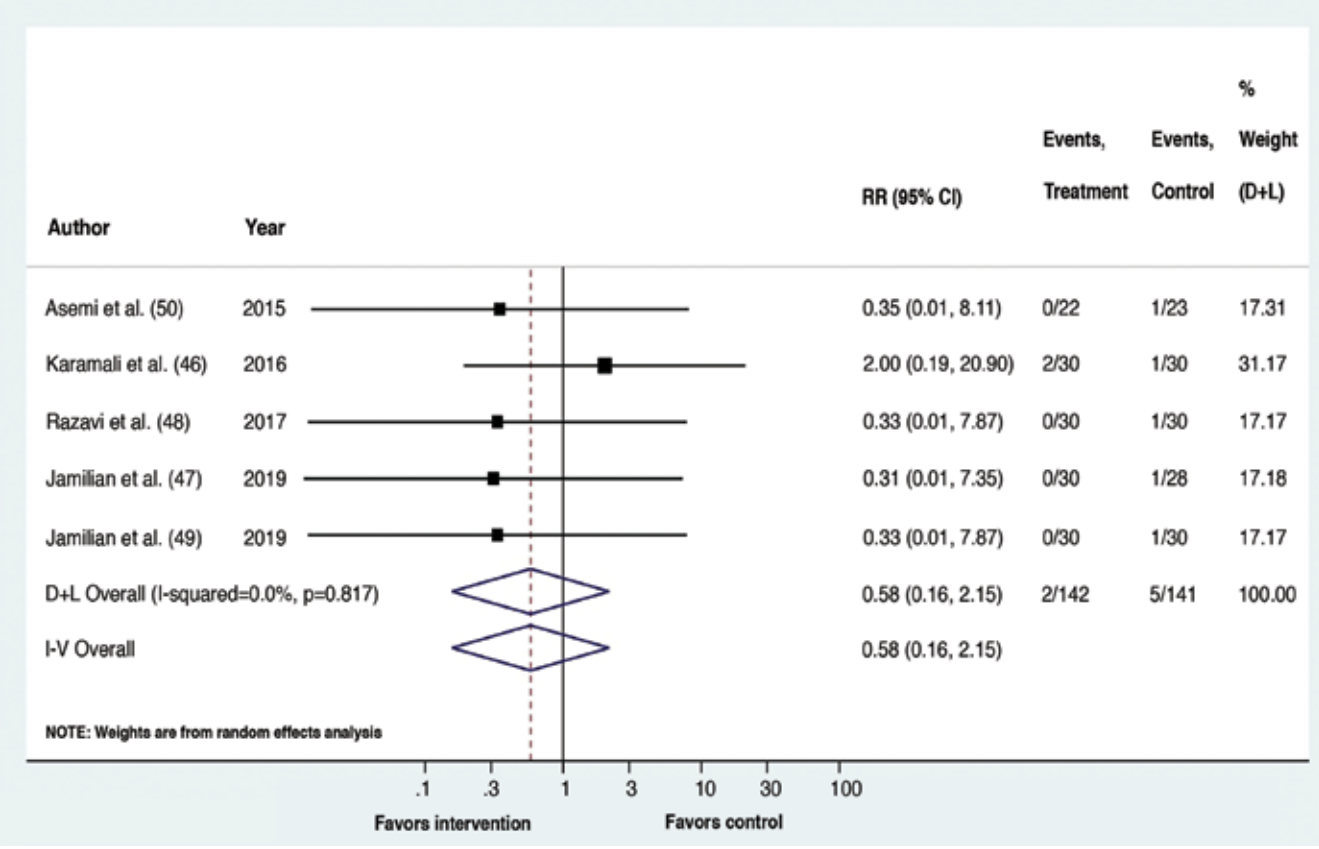

Figure 6. Forest plot: Comparison between vitamin D supplemented group and placebo for the outcome pre-term delivery

inform public health policy about endorsing prenatal vitamin D supplementation in GDM patients. The lower risks of macrosomia and CS due to vitamin D supplementation may encourage future researchers to investigate if there is a causal relationship between these. Moreover, future researchers from nations other than Iran may also consider researching this context to test if these paper's findings are externally valid or not.

The following are the strengths of this review. First, this is perhaps the first systematic review that attempted to synthesize evidence in this study's context. Second, the findings of this review are likely to be rigorous as it utilized evidence from double blinded randomized controlled trials, the highest level of epidemiological evidence. Third, this review is expected to be more comprehensive as its database search method was not restricted to any date or language. Lastly, the meta-analysis findings regarding CS and macrosomia are likely to be robust due to their similarity with the sensitivity analysis.

Despite these strengths, there are certain limitations of this paper. At the review level, the number of trials investigating the context was relatively few, which might have compromised the external validity of this meta-analysis. At the outcome level, by including intervention arms of trials that tested vitamin D along with other nutritional adjuncts, it is difficult to conclude if the observed effects were influenced by the latter. At the study level, the weaknesses were the unclear risk of bias (46,47,49,50), single centric study design (47-50), and relatively small sample size (46-50). Additionally, as all trials were Iran-based (46-50), the findings are unlikely to be generalizable to the global population.

Conclusion

The contemporary evidence in non-insulin treated GDM patients from Iran suggests that antenatal vitamin D containing supplements decreases the risk of CS and macrosomia, compared to placebo. However, to increase the external validity of these findings, methodologically rigorous trials from different parts of the globe might be useful in the future. Furthermore, future trials may use vitamin D as the sole supplement to specifically identify its effects on obstetric outcomes in GDM patients.

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What is your diagnosis?

A 33 year-old, gravida three with two previous living issues was referred to our institute at 31 weeks period of gestation (POG) with an ultrasound finding of polyhydramnios. The patient lived in a remote hilly area of the state and she did not seek any antenatal care in the first or second trimesters. On account of polyhydramnios, we performed a detailed anatomical survey of the fetus by 2-dimensional (2D) ultrasonography, and we were confronted by the following subtle abnormalities: brachycephaly (Figure 1) with borderline increased cephalic index (85.3%); prominent and easy visualization of the eye balls and eyelids suggestive of orbital proptosis (Figure 2, 3); mildly depressed nasal bridge with a beaked nose (Figure 4). Binocular and interocular distances were normal. All four limbs were normal. No other structural defect could be identified in the fetus. Maximum vertical amniotic fluid pocket was only 6 cm (1).

The husband reached the hospital only a day later. Interestingly the father also exhibited similar dysmorphic facial features. The father reported that the couple's two older children, a boy and a girl, looked like him too. He had a broad head with exophthalmos, widely separated and deviated eye balls, beaked nose and mid-facial hypoplasia. His intelligence was normal and there was no associated structural defect in any other body part.

Figure 1. Ultrasound image (2-dimensional) of fetal head showing brachycephaly with cephalic index more than 85% [Biparietal diameter (BPD): 8.11 cm, Occipito-frontal diameter (OFD): 9.5 cm, Cephalic index (BPD/OFD) x100: 85.4%]

BPD: Biparietal diameter

Figure 2. Ultrasound image (2-dimensional) of coronal view of fetal face demonstrating easily visible eyeballs and palpebrae, suggestive of orbital proptosis

Address for Correspondence: *Latika Chawla*
e.mail: latika.c@rediffmail.com ORCID: orcid.org/0000-0001-8220-2105

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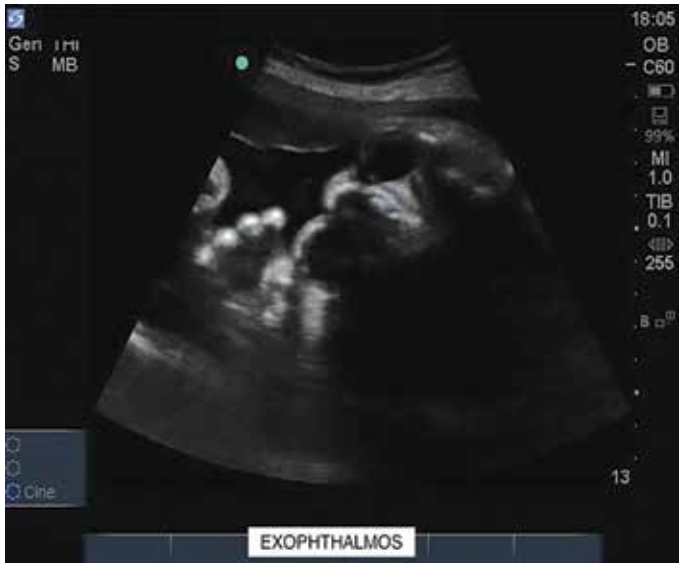

Figure 3. Ultrasound image (2-dimensional) of transverse section of fetal face demonstrating bulging eyeballs, suggestive of orbital proptosis

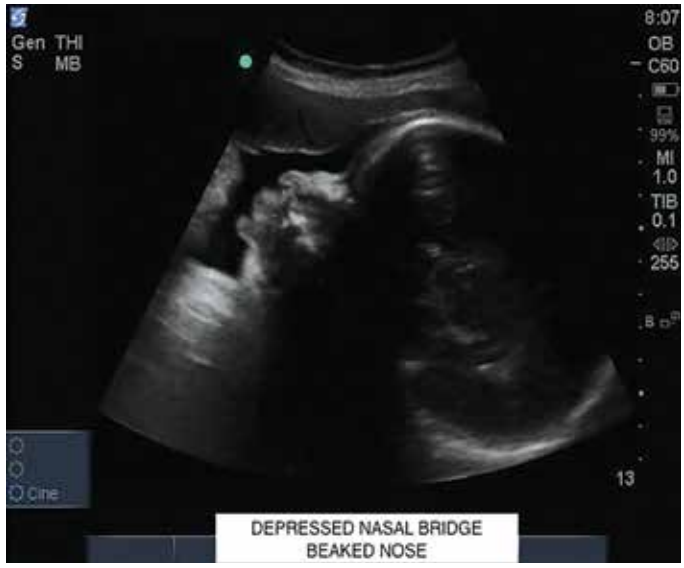

Figure 4. Ultrasound image (2-dimensional) of fetal facial profile showing mildly depressed nasal bridge with a beaked nose

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Answer

The patient underwent a spontaneous normal vaginal delivery at 38 weeks and delivered a male baby of 3.1 kilograms. Baby (Figure 5) was born with all previously mentioned subtle dysmorphic features. Baby and father were evaluated by the geneticist at the institute. The phenotypic features of father, two previous children, this baby and the suggestive inheritance pattern all pointed towards a diagnosis of Crouzon syndrome. The family did not opt for genetic analysis due to financial constraints.

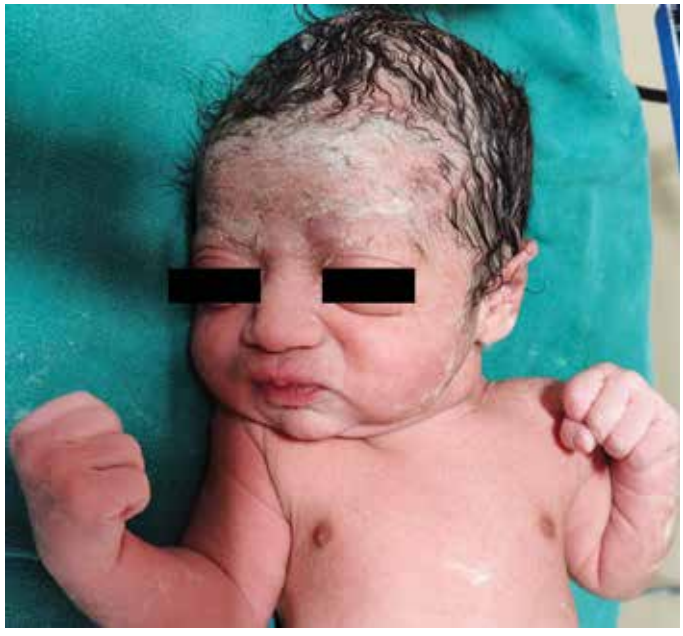

Figure 5. Subtle dysmorphic features of the neonate including a broad head, orbital proptosis, depressed nasal bridge, and beaked nose consistent with typical phenotype of Crouzon syndrome

Crouzon syndrome is a rare disorder with an incidence of 15-16 cases per million live births (2). It is an autosomal dominant disorder, although sporadic cases have also been reported. It occurs due to mutations in the *fibroblast growth factor receptor 2* gene located on chromosome 10.

Facial dysmorphism and related abnormalities in craniosynostosis depend upon which and how many of the cranial sutures fuse prematurely. Even after premature fusion of the sutures, which may occur in utero or after birth, the brain continues to grow along the plane of the remaining open sutures and the pressure gives an abnormal appearance and shape to the skull (3-5). Crouzon syndrome is most commonly associated with bi-coronal synostosis that gives the typical brachycephaly. Other cranial sutures may fuse as well. Abnormal premature fusion of various sutures of the base of the skull and face is associated with mid-facial hypoplasia and beaked nose (psitticorhinia). Retrusion of lateral and inferior orbital margins results in shallow orbits leading to proptosis and exotropia (6). Structural abnormalities of the ear, such as narrowing and stenosis of the ear canal, may lead to hearing

loss. Arnold Chiari malformation is reported to be common in patients of Crouzon syndrome (3).

Affected babies should be followed up for development of ventriculomegaly and increased intracranial pressure. Choanal atresia and abnormalities of the upper airway may lead to life threatening respiratory distress at birth. Since it is an autosomal dominant disorder with variable expression, the phenotype may be variable, even in members of the same family, with certain members having more prominent features and others having subtle dysmorphism, as was in our case. The baby born in our case had most of the typical characteristic features (brachycephaly, proptosis, exophoria and beaked nose) however there was no hypertelorism or mid-facial hypoplasia. Even the exophthalmos was very subtle.

Prenatal diagnosis of cranio-synostotic syndromes has been deemed difficult, especially when gross abnormalities of fetal head are not present (7). Even though Crouzon syndrome is the most common of the cranio-synostotic syndromes, its prenatal diagnosis is extremely challenging and has rarely been reported previously, as the skull abnormalities may be very mild and there is lack of associated limb abnormalities (7). To the best of our knowledge there are only 6 reports of prenatal diagnosis of Crouzon syndrome by ultrasonography in the current literature. Prenatal diagnosis of Crouzon syndrome was first made in 1989 by Menashe et al. (8) in a 35 weeks POG fetus where exophthalmos was the only facial abnormality detected on ultrasound. Leo et al. (9) diagnosed Crouzon syndrome in a 16 weeks POG fetus with increased binocular and interocular diameters. In 1993, Gollin et al. (10) identified a fetus with Crouzon syndrome at 23 weeks with cloverleaf skull, exophthalmos, hypertelorism with mild ventriculomegaly. The same year, Escobar et al. (11) reported prenatal diagnosis of another fetus with Crouzon syndrome at 20 weeks POG and with a positive family history. In 2002, Miller et al. (12) published a retrospective study where they reported prenatal diagnosis of two cases, at 20 and 22 weeks by noting brachycephaly and hypertelorism. Nørgaard et al. (13) in 2011 reported prenatal diagnosis of Crouzon syndrome in a 35-week fetus using both 2D and 3D ultrasonography.

It is important to identify cranio-synostotic syndromes prenatally for the following reasons. Firstly, if diagnosis made is within the legal limit, parents may be offered an option of a medical termination of pregnancy. Secondly, parents should be prepared for the birth of the child with special needs. Pre-natal identification of such syndromes will facilitate in utero transfer/ referral of the mother to a tertiary care center equipped with facilities for multidisciplinary management of such syndromic babies, including neonatology, geneticist, neurosurgeon, and oro-maxillofacial surgeons. Finally, with prior notice a team can be ready for immediate management of possible respiratory compromise at birth in such affected babies.

Latika Chawla[1], Aditi Jindal[1], Prashant Kumar Verma[2], Anupama Bahadur[1], Shashi Prateek[1]

[1]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Rishikesh, India
[2]Department of Pediatrics, Division of Genetics, All India Institute of Medical Sciences, Rishikesh, India

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A novel technique for determining the axis of the fetal heart: Clock position method

To the Editor,

Determination of the fetal heart axis via ultrasonography is crucial as some cardiovascular diseases can only be diagnosed according to the accuracy of fetal heart position. There are reliable methods for ultrasonographic assessment of the left/right axis by using fetal stomach or gallbladder as a landmark. However, if there is a malposition of an indicator organ, it may be difficult to distinguish the left side from the right side. For this reason, these methods sometimes require more experienced physicians to obtain reliable results. For inexperienced clinicians this can be more difficult because both understanding the position of fetus and ultrasound probe orientation can be confusing. There is a need for a practical technique that can be used easily and reliably by perinatologists or obstetricians.

We suggest an easy method for assessment of the fetal heart axis. In our method, the physician should be seated on the right side of the patient, hold the probe with the right hand and perform a transabdominal examination. The left side of the probe should be on the left side of the ultrasound screen. The fetus should be scanned in a transverse plane. The thoracic cavity is imagined as a watch dial and the fetal spine refers the 12 o'clock position. If the fetus is in breech presentation, the axis of the heart should be approximately at the 7 o'clock position (Figure 1a) and similarly, if the fetus is in cephalic presentation, the axis of the heart should be approximately at the 5 o'clock position (Figure 1b). If the fetus is in a transverse lie, indicator of the probe should point to patient's head for sagittal imaging and the fetal structure nearest to clinician (in other words closest part of the fetus to maternal right side) is accepted as the presenting part.

Other methods have been described to assess of the fetal heart position. Cordes et al. (1) described a technique in 1994. The left and right side of the fetus was distinguished depending on parameters such as position of fetus and mother, ultrasound probe orientation and video screen. Although being accurate and reliable, it is not easy to learn and perform for all practitioners. Bronshtein et al. (2) suggested a simple technique to determine fetal situs. They used forearm, hand and thumb for orientation. In this method, right hand for transabdominal examination and left hand for transvaginal examination was used. The dorsal side of the forearm referred to the fetal back and thumb showed the fetal left side. It is more user-friendly than Cordes et al.'s (1) technique but clinicians

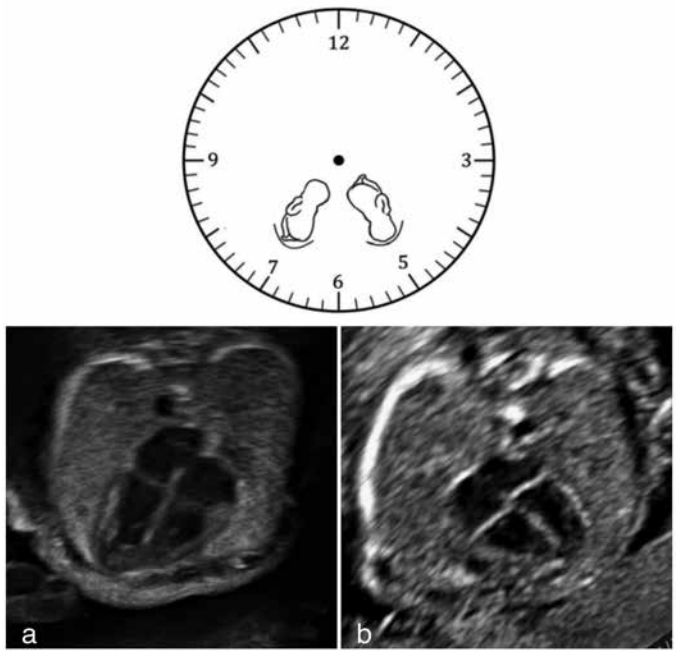

Figure 1. Clock position method to determine fetal heart axis. The spine always refers to the 12 o'clock position (a) Breech presentation; the axis of the heart at the 7 o'clock position. (b) Cephalic presentation; the axis of the heart at the 5 o'clock position

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Address for Correspondence: *Fatih Aktoz*
e.mail: fatihaktoz@gmail.com ORCID: orcid.org/0000-0003-3210-849X

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can be confused when they choose the appropriate hand to use for evaluation. The method that we described can be used by any clinician. It does not require calculation. Thus, it requires less time compared to others. In addition, our method is not affected by fetal movements. In conclusion, we believe that this technique may be a good option for clinicians at every level of experience.

Sezgin Dursun[1], Fatih Aktoz[2]

[1]Private Clinic, Ağrı, Turkey
[2]Clinic of Obstetrics and Gynecology, Ağrı State Hospital, Ağrı, Turkey

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Why and how to proceed to an ultrasound-guided transvaginal drainage of tubo-ovarian abscesses (with demonstrative video)?

Erdogan Nohuz, Géry Lamblin, Clémentine Sciard, Mélodie Mathé, Karine Lebail-Carval, Gautier Chêne

Department of Gynecological Surgery, Hôpital Femme Mère Enfant, HFME, Hospices civils de Lyon, Bron, France

Abstract

The treatment of the acute phase of complicated tubo-ovarian abscesses relies on antibiotics associated with surgical management in case of visible abscess, poor clinical tolerance and resistance to medical treatment. Transvaginal, ultrasound-guided, puncture drainage can be considered as an alternative to initial laparoscopy and has multiple advantages over the latter: same success rate, less invasive nature, simple and fast to perform, easy to access, better tolerated by the patient, decreased hospitalization time and less cost. This video article describes and standardizes the essential steps to perform a transvaginal adnexal abscess drainage with a step-by-step explanation of the technique in logical sequences, making the procedure ergonomic and easy to learn. Thus, as part of minimally invasive approach, this technique is henceforth suggested as an effective alternative and signifies a first-line procedure that can promote a therapeutic de-escalation strategy. (J Turk Ger Gynecol Assoc 2020; 21: 218-20)

Keywords: Abscess/surgery, pyosalpingitis, ultrasound-guided, acute salpingitis, adnexal abscess

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Introduction

The treatment of the acute phase of complicated tubo-ovarian abscesses relies on antibiotics associated with surgical management in case of visible abscess, poor clinical tolerance and resistance to medical treatment (1). The French College of Gynecologists and Obstetricians recommended in 2012 that abscesses greater than 3 cm should be drained by interventional radiology, preferably transvaginally, or laparoscopically, because of the risk of serious complications in the absence of drainage (2). Transvaginal ultrasound-guided drainage in women without signs of acute abdomen or peritonitis and who are clinically stable, should henceforth be considered as an effective alternative, in our opinion. In our experience the transvaginal approach combined with antibiotic therapy is efficient for treatment of tubo-ovarian abscess. This combination promotes the effectiveness of antibiotics, thereby reducing the need for surgical treatment and thus avoiding the potential risks associated with general anesthesia and surgery. In addition this technique contributes to improve the clinical outcome and consequently, to decrease costs and morbidity (3,4). This video article aims to describe and standardize the essential steps to perform a transvaginal adnexal abscess drainage with a step-by-step explanation of the technique in logical sequences, making the procedure easy to learn, ergonomic and safe.

Technique of ultrasound-guided puncture-drainage

The patient, as welle as the anesthetic and surgical team, should be warned that the puncture may, in certain rare situations, fail and therefore require laparoscopy. In addition, iterative puncture may exceptionally be necessary due to the persistence of residual tubo-ovarian abscess.

Address for Correspondence: *Erdoğan Nohuz*
e.mail: enohuz@yahoo.fr ORCID: orcid.org/0000-0002-8577-0682

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This technique offers a direct route from the vaginal wall into areas where tubo-ovarian abscesses are usually situated. The procedure is performed under neuroleptanalgesia, local or general anesthesia after the initiation of broad-spectrum antibiotics. Thus, it can be considered immediately once the abscess has been visually identified, or be delayed from a few hours to a few days depending on the clinical context, to "cool" the lesions and allow antibiotic coverage of the drainage procedure. This involves tri-antibiotic therapy with cephalosporin, cycline and metronidazole, in the absence of allergy, initially administered intravenously (5).

The patient is placed in a gynecological position. Vaginal cleaning with povidone-iodine preparation is performed to ensure a sterile field. The bladder should be positively identified using ultrasound which is facilitated by absence of prior urinary catheterization makes it possible. This is important to avoid bladder injury and to have a fixed anatomical landmark. If necessary, partial bladder emptying will limit the risk of bladder injury. Palpation of the abscess should be attempted which aids in orientation of the ultrasound probe during the puncture' step. An endovaginal ultrasound probe (6 to 10 MHz frequency), covered with a sterile probe cover and gel, is equipped with a guide for follicular punctures (Figure 1a). Ultrasound scanning is then performed to identify the abscess and prepare its puncture (Figure 1b). Once the abscess is visualized, which must be located immediately in contact with the vaginal wall in order to avoid any digestive transfixion, a puncture is performed under permanent ultrasound control (Figure 2,3 and Video 1). We use a 17 gauge/1.5 mm puncture-aspiration bevel needle, the distal end of which is echogenic, to follow the path and facilitate the procedure. A syringe is screwed to the tube connected to the needle. The use of an automatic follicular suction pump is ergonomic and facilitates aspiration. If one is dealing with a collection of thick consistency, it may sometimes be useful to inject saline. A catheter can be left in place to permit further drainage. However, this possibility carries the risk of the catheter becoming displaced. There is no evidence as to the benefit of leaving a catheter in situ and thus we do not recommend this. At the end of the puncture-drainage, a speculum examination of the vagina and an antiseptic cleaning ends the procedure. The sample is sent for bacteriological analysis in order to possibly adjust antibiotic treatment to identified organisms and sensitivities.

The limits of this technique lie in the inability to identify a possible adnexal malignant lesion and the impossibility of evaluating the tubal state in patients with a desire for pregnancy, although a laparoscopy performed a few months

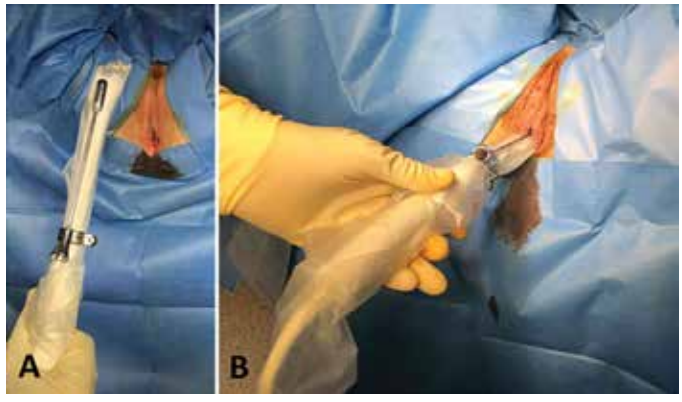

Figure 1. Ultrasound scanning to identify the collection and prepare its puncture. The endovaginal ultrasound probe is covered with a sterile probe cover and gel and equipped with a guide for follicular puncture (A). Ultrasound scanning permits to identify the collection to be evacuated and located immediately in contact with the vaginal wall (B).

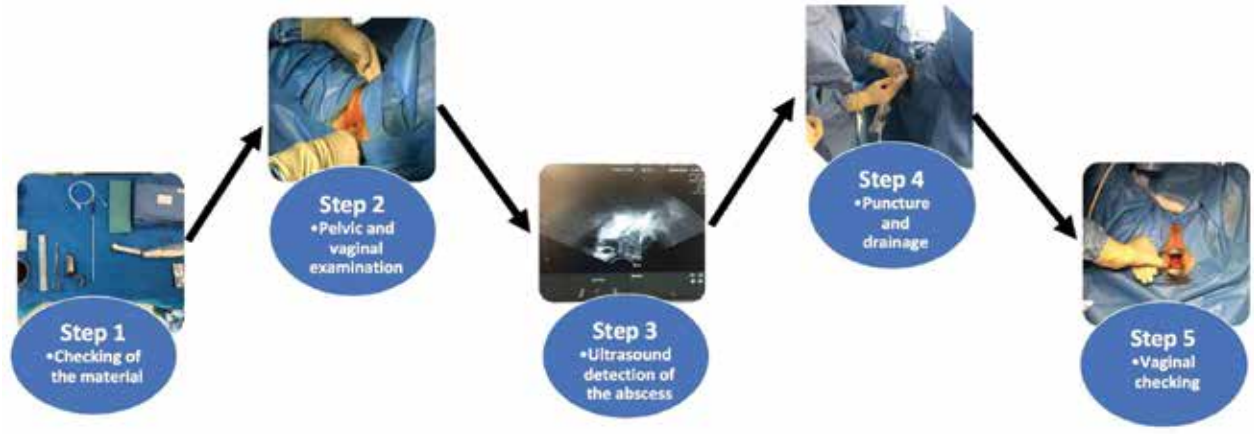

Figure 2. Main steps to perform an ultrasound-guided transvaginal puncture with aspirative drainage. (Step 1) Checking of the material. An endovaginal transducer with an attached biopsy guidance system and needle with a connection tube and syringe are used. (Step 2) Physical examination (vaginal palpation). (Step 3) Ultrasound detection of the abscess and setting of the shooting window. (Step 4) Transvaginal puncture next to the abscess, and drainage-aspiration under ultrasound control. (Step 5) Vaginal checking and cleaning. The aspirated fluid is sent for microbiological analysis.

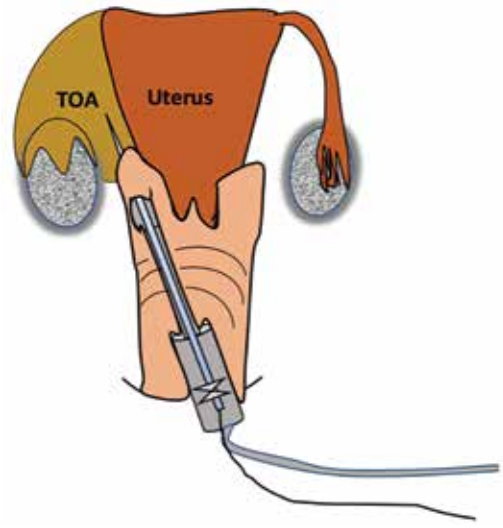

Figure 3. Diagram showing puncture-drainage. Note the proximity of the vaginal cul-de-sac with the tubo-ovarian abscess

TOA: Tubo-ovarian abscess

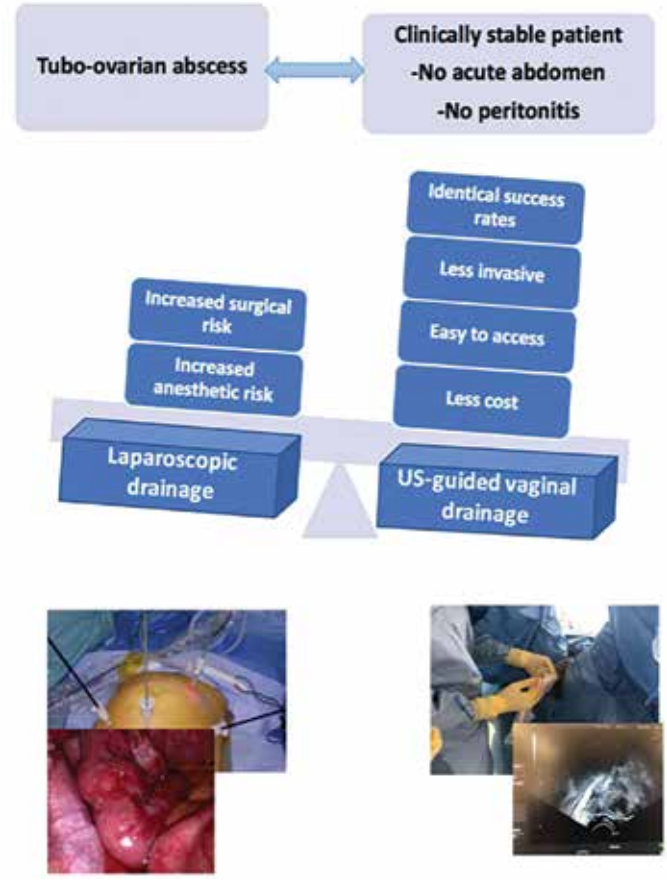

Figure 4. Graphical abstract. Comparison between laparoscopic and transvaginal approaches of adnexal abscesses

US: Ultrasound

later and under better surgical conditions, allows the treatment of possible sequelae such as adhesions and/or tubal stenosis. Moreover, this approach has not yet been compared to that of laparoscopy by clinical study. Thus, a French multicenter randomized trial is currently under way. The PACTOL study aims to demonstrate that the transvaginal method is no less effective than laparoscopy to treat tubo-ovarian abscesses. This study should be able to answer questions that are still outstanding concerning the use of this technique such as impact on chronic pelvic pain and future fertility (5).

Conclusion

Ultrasound-guided, transvaginal drainage is an alternative to initial laparoscopic drainage of tubo-ovarian abscesses. We believe that it should replace laparoscopic drainage because of multiple advantages. These include identical success rate and being less invasive. Laparoscopy or laparotomy may in some cases prove to be complex and a source of intestinal wounds, whether it be a conservative surgery or an excisional procedure. In addition transvaginal drainage is simple and fast to perform, normally taking only 15-20 minimum, is easy to access, is better tolerated by the patient, and results in decreased hospitalization time and less cost (Figure 4, graphical abstract). Thus, as part of a minimally invasive approach, it represents a first-line procedure that can promote the therapeutic de-escalation strategy.

Video 1. https://www.doi.org/10.4274/jtgga.galenos.2019.2019.0131. video1

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

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