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Turkish Journal of Obstetrics and Gynecology is the official peer-reviewed publication of the Turkish Society of Obstetrics and Gynecology and is published quarterly on March, June, September and December.

The target audience of Turkish Journal of Obstetrics and Gynecology includes gynecologists, obstetricians, urogynecologists, reproductive medicine specialists, gynecological oncologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of obstetrics and gynecology. The aim of Turkish Journal of Obstetrics and Gynecology is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

It is an independent peer-reviewed international journal printed in English language. Manuscripts are reviewed in accordance with "double-blind peer review" process for both referees and authors.

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PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

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

STROBE statement—checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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Reports of animal experiments must state that the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed, as well as specific national laws where applicable.

The editors reserve the right to reject manuscripts that do not comply with the above mentioned requirements. The author will be held responsible for false statements or for failure to fulfill the above mentioned requirements.

Authors must provide statement on the absence of conflict of interests between authors and provide authorship contributions and declare if any financial/metarial support.

Manuscript Structure

All manuscripts must be submitted as Microsoft Word (.doc or .docx) files. All manuscript pages (including references, tables, and figure legends) must be double-spaced. Use a standard, 12-point typeface such as Times New Roman. Top, bottom, and side margins should be set at 1 inch. Authors must include the following in the manuscript file:

Title Page

A separate title page should list;

- The manuscript title, which should contain no more than a total of 100 characters (counting letters and spaces) and should not be declarative; do not use abbreviations or commercial names in the title.

- A short title of no more than 50 characters, including spaces, for use as a running foot.

- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

- Corresponding author's name, address, telephone (including the mobile phone number), fax numbers and e-mail address (the corresponding author will be responsible for all correspondence and other matters relating to the manuscript).

Precis

The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

'Using a 45 point questionnaire, we have evaluated the trend of Robotic surgery training in the gynecologic surgery fellowship programs across the nation'.

Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· **Objective:** Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

· **Material and Methods:** Study design, participants, outcome measures, and in the case of a negative study, statistical power.

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· **Results:** Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· **Conclusion:** Directly supported by data, along with clinical implications.

Authors from Turkey or Turkish speaking countries are expected to submit a Turkish abstract including subheadings such as "Amaç, Materyal ve Metod, Bulgular, Sonuç". The abstract of Authors whose native language is not Turkish will be provided free of charge translation services into Turkish language.

A structured abstract is not required with review articles and case reports.

Key Words

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

Turkish abstracts should have key words "Anahtar Kelimeler" picked from www.atifdizini.com under "Turkey Bilim Terimleri" link.

Several types of articles can be submitted for publication in Turkish Journal of Obstetrics and Gynecology: Original research, case reports, systematic reviews, current commentaries, procedures and instruments, and letters. Stated word counts and page limits were shown in Table 1. Copyright transfer forms, the cover letter, and figures do not contribute to the page limits.

Table 1. Manuscript length at a glance

Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	2,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

Introduction

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

Material and Methods

Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

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

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion

Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

Study Limitations

Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

Conclusion

The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

Conflict of Interest

Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research.

The main text of case reports should be structured with the following subheadings: Introduction, Case Presentation, Discussion, Study Limitations, Conclusion and References.

References

References are numbered (Arabic numerals) consecutively in the order in which they appear in the text (note that references should not appear in the abstract) and listed double-spaced at the end of the manuscript. The preferred method for identifying citations in the text is

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Use references found published in peer-reviewed publications that are generally accessible. Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references. Papers accepted by peer-reviewed publications but not yet published ("in press") are not acceptable as references.

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Examples

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Book chapter; Ayhan A, Yenen MC, Dede M, Dursun P, Gultekin M.How to Manage Pre-Invasive Cervical Diseases? An Overview. In: Ayhan A, Gultekin M, Dursun P, editors. Textbook of Gyneaecological Oncology. Ankara, Turkey: Gunes Publishing; 2010.p 28-32.

Book; Arici A; Seli E. In Arici A and Seli E (edts). Non-invasive Management of Gynecologic Disorders. London: Informa Healthcare; 2008.

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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LETTER FROM THE PRESIDENT

Dear Colleagues,

It is a great pleasure to say a few words in the hot summer time to you. As TSOG we are going to do our General Council in November. Our branches are doing their elections nowadays. They have to finish till at the end of August. Local meetings are in summer break.

We are going to do our congress at 5-9 October 2016 in Antalya, Kaya Palazzo Hotel. In our website you can find the details. Please find sponsor for the congress. In this congress we wil have a Hall for oral Presentations which is essential for the new "Associate Professorship Examination" rules. We will give scholarship to our assistans for 50 people to attend the congress. We have the investigation prize for vaginal progesteron. We are making an ultrasonogrophy photos competition you can apply by the name "Anı Yakala" project.

I hope we will meet in the congress in Antalya.

Cansun Demir, Prof. MD
President of TSOG

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

The quality and standarts of medical education in general is a hot topic nowadays. Being aware of the needs of our readers and members of the Turkish Society of Obstetrics and Gynecology we launched several projects. For setting higher standarts for residency programs and sub-specialities like Gynecological Oncology and Perinatology our Society is working with European Board and College of Obstetrics and Gynecology and certification of specialists and clinics are going on. Targeting the residence of Obstetrics and Gynecology "Resident School" workshops are taking place every six months where 16 topics are discussed with about thirty 3rd or 4th year residents, recorded and casted freely in the society web page. Another program has been launched named "100 consultants lecturing 100 topics" on March 2016. In this project leading academicians are lecturing on topics for 50 minutes in a comprehensive informative style from definition to treatment which is recorded live and then released in the society web page. Another newly released project is "TSOG Academy" which focuses to different aspects of a disease or treatment lectured by four or five experts, recorded live and than released in society web page which is followed by online quiz at the end where you can get credits if you answer most of the questions correctly. Upcoming projects include experts teaching surgical and diagnostic methods.

New innovative cadaver courses were supported by our Society on Urogynecology and Obstetric Surgery. In this series of courses two of them focused on Pelvic floor in a three day workshop covering theoretical surgical anatomy of the Pelvis followed by cadaver studies on the second day and hands on Surgeries on 29 patients on the third day. Obstetric Surgery course focused on prevention and management of obstetric hemorrhages. Emergency obstetric response team concept which was first launched in Kocaeli region in 2009 than followed by Anatolian Side of İstanbul in 2015 shared their experience in the workshop. After theoretical education on the first day, cadaver and model course was conducted on Pelvic anatomy, sequential devascularisation of the pelvis and the uterus, different uterine packing sutures on the second day together with video surgery session where experts showed the videos of complex obstetric surgeries and told what they have done why they have done. About 200 specialists were educated by 60 lecturers and experts on the topics.

Our journal as being the publisher of recent developments and research on Obstetrics and Gynecology will launch a series of education materials as pictorials starting from September 2016 issue. We call for papers conducted in the area of Obstetrics and Gynecology education.

Best wishes,

Eray Çalışkan, Editor

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2016;13:56-61

The relationship between estradiol-progesterone alterations after ovulation trigger and treatment success in intrauterine insemination cycles

Ovülasyon tetiklenmesi sonrası östrojen-progesteron değişimi ile intrauterin inseminasyon sikluslarının başarısı arasındaki ilişki

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Abstract

Objective: To assess the relationship between the estrogen-progesterone alterations before and after ovulation trigger and treatment success in intrauterine insemination (IUI) cycles.

Materials and Methods: Two hundred fifty-one women with infertility underwent ovulation induction followed by IUI. For all subjects, estradiol and progesterone concentrations were evaluated on the trigger and IUI day. The results were analyzed to assess the relationship between hormone levels and positive pregnancy test.

Results: There were 34 women with a positive pregnancy test following controlled ovarian stimulation and IUI cycle. Estradiol and progesterone levels on the trigger day and the day of IUI were compared within groups with and without positive pregnancy tests. The comparison revealed significantly increased levels of progesterone after trigger in both groups; however, although there were estradiol level drops in both groups, the drop in the group with negative pregnancy tests was statistically significant.

Conclusion: Significant drops in estradiol concentrations after ovulation trigger are associated with IUI cycle treatment failure.

Keywords: Estradiol, progesterone, intrauterine insemination, ovulation induction

Öz

Amaç: Bu çalışmanın amacı ovülasyon tetiklenmesi öncesi ve sonrası östrojen-progesteron düzey değişimi ile intrauterin inseminasyon (IUI) sikluslarının başarısı arasındaki ilişkiyi değerlendirmek.

Gereç ve Yöntemler: İki yüz elli bir hastaya ovülasyon indüksiyonu sonrası IUI uygulandı. Tüm hastalarda östradiol ve progesteron düzeyleri tetikleme ve IUI günü değerlendirildi. Hormon sonuçları ve pozitif gebelik testi ilişkisi için sonuçlar değerlendirildi.

Bulgular: Kontrollü ovaryen stimülasyon ve intrauterine inseminasyon sonrası 34 hastada gebelik testi pozitif elde edildi. Tetikleme ve intrauterine inseminasyon günü bakılan östradiol ve progesteron düzeyleri gebelik testi pozitif ve negatif olan gruplar arasında karşılaştırıldı. Her iki grupta da tetikleme sonrası progesteron düzeyleri anlamlı olarak arttı. Diğer taraftan her iki grupta da östradiol düzeyleri düştü, fakat gebelik testi negatif olan grupta düşüş istatistiksel anlamlı izlendi.

Sonuç: Ovülasyon tetiklemesi sonrası östradiol düzeyindeki anlamlı düşüş IUI siklus tedavi başarısızlığı ile ilişkilidir.

Anahtar Kelimeler: Östradiol, progesteron, intrauterin inseminasyon, ovülasyon indüksiyonu

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PRECIS: Significant drops in estradiol concentrations after ovulation trigger are associated with intrauterine insemination cycle treatment failure.

Introduction

Monofollicular development should be the main goal in intrauterine insemination (IUI) cycles. Controlled ovarian stimulation (COH) was found associated with supraphysiologic estradiol levels and might affect endometrial implantation. Also, some data showed increased success rates with high peak estradiol levels, which were presumed to be indirect evidence for oocyte quality, whereas some studies showed poorer outcomes due to the detrimental effect of high estrogen on endometrial receptivity[1-4]. Although we do not expect to observe estradiol in concentrations so high in IUI cycles that they would be detrimental to endometrial receptivity, it may be used as a reflection of oocyte quality. Progesterone is thought to be the dominant hormone during the luteal phase of the cycle and the endometrial window of implantation is mainly regulated by progesterone and progesterone- induced gene regulations; this effect is strictly regulated. Data showed that increased mid luteal serum progesterone levels were not associated with a higher clinical pregnancy rate in women who underwent COH with IUI. However, a lower mid luteal progesterone level was proposed to be a predictor for treatment failure[5]. Other data from in vitro fertilization (IVF) cycles showed a detrimental effect of increased progesterone concentrations (>2.0 ng/mL) before ovulatiovn trigger on oocyte quality and therefore embryo quality[6]. In another study, a 10% reduction in estradiol concentrations after ovulation trigger was associated with 40-50% lower clinical pregnancy and live birth rates in IVF cycles[7]. Although serum estradiol concentrations are one of the main parameters in the assessment of the response to controlled ovarian stimulation, the predictive value of estradiol levels before or after ovulation trigger is still not known. Some data showed a poor predictive value of serum estradiol concentration alone on the day of recombinant-HCG in IVF outcomes[8]. A recently published study proposed the use of the post-recombinant-human chorionic gonadotropin (HCG) estradiol level as an additional component to predict the outcome of an IVF cycle just before oocyte pick-up. The authors indicated the necessity for further studies to clarify the underlying mechanisms that might result in a decrease in postrecombinant-HCG estradiol levels, so that physicians may be able modify following IVF cycles accordingly[7]. The aim of this study was to assess the relationship between estrogen-progesterone alterations after the ovulation trigger and treatment success in IUI cycles.

Materials and Methods

In this cross-sectional study, we included 251 IUI cycles performed in the infertility clinic of Zeynep Kamil Women and Children's Health Training and Research Hospital between 2012 and 2014. This study was approved by the Institutional Review Board of the Zeynep Kamil Women and Children's Health Training and Research Hospital. All participants gave signed informed consent. All couples had attempted to conceive for at least one year prior to undergoing COH+IUIs. A self-administered questionnaire was used to collect data about demographic, menstrual, and obstetric characteristics. The study population comprised all couples who were candidates for COH+IUI. Indications for IUI included subfertile male infertility, polycystic ovary syndrome, mild or minimal endometriosis or unexplained infertility and various ovulatory disorders. Subfertile male infertility was defined as per the criteria outlined by Molinaro et al.[9]. The Initial evaluation included the cycle's day 3 hormone profile, and tubal patency as determined using hysterosalpingogram and/or laparoscopy. Exclusion criteria were hydrosalpinx, anatomic abnormalities, infection, and systemic disease before intervention.

Ovarian Stimulation Protocol

Transvaginal ultrasonography was performed for each participant on day 3 of the menstrual cycle and daily 75-100 IU recombinant FSH (Gonal-F; Merck Serono, İstanbul, Turkey; and Puregon; MSD, İstanbul, Turkey) injection was started. The ovarian response and endometrial thickness was started to be assessed by transvaginal ultrasound starting from the 5th day of stimulation. If the leading follicle's diameter was <10 mm on the 8th day of stimulation, the dose of gonadotropin was increased by 50%. The gonadotropin dose remained the same until the day of recombinant-HCG trigger after the leading follicle reached 12 mm in diameter. Cycles were triggered with 250 µcg recombinant-HCG (Ovitrelle; Merck Serono, İstanbul, Turkey) when the dominant follicle became 18 mm in diameter. Cycles were cancelled if there were more than three dominant follicles and/or estradiol levels>1500 pg/mL to prevent ovarian hyperstimulation syndrome and multiple pregnancies. IUI was performed 36 h after recombinant-HCG administration with a disposable IUI catheter (Embryon; Rocket Medical, Washington, Tyne and Wear, U.K.) by two of the authors. The patient was recommended to rest in a supine position for 15 min after the procedure. Luteal phase progesterone support was started following insemination and continued until a pregnancy test was performed. Luteal phase progesterone support was administered in the form of 90 mg (8%) vaginal gel (Crinone, Merck Serono; İstanbul, Turkey). β-HCG was tested on the 15th day of the post insemination day sample. Luteal phase support was continued until 12 weeks of gestation.

Results

There were 34 (13.5%) women with a positive pregnancy test following controlled ovarian stimulation and IUI cycle.

Estradiol and progesterone levels on the trigger day and the day of IUI were compared within groups with and without positive pregnancy test. The comparison revealed significantly increased levels of progesterone after trigger in both groups; however, although there were drops in estradiol levels, the drop in the group with a negative pregnancy test was statistically significant (Tables 1 and 2). The groups were compared in terms of some demographic and hormonal concentrations, the results of which are summarized in Table 3. Estradiol/progesterone at trigger, estradiol/progesterone at IUI, progesterone/estradiol at trigger, and progesterone/estradiol at IUI, all these ratios failed to predict treatment success (p>0.05, Figure 1).

Discussion

In this study, we assessed the effect of estrogen and progesterone alterations before and after ovulation trigger on IUI cycle outcomes. Our data revealed that a significant drop in estradiol levels after ovulation trigger leads to unfavorable results in IUI cycles. A progesterone rise was not found to have a significant impact on cycle outcome and progesterone levels on trigger day were not significant predictors of cycle outcome. According to our literature search, although there are some data for IVF/intracytoplasmic sperm injection (ICSI) cycles, hormonal alterations during the periovulatory period were not investigated in IUI cycles in detail.

Consistent with our result, previous study on 1712 IVF cycles revealed similar results and indicated estradiol drop >10% after trigger was associated with lower pregnancy rates[7]. The mean estradiol drop was 21% in the group with negative implantation and was 11% in successful cycles in our study population.

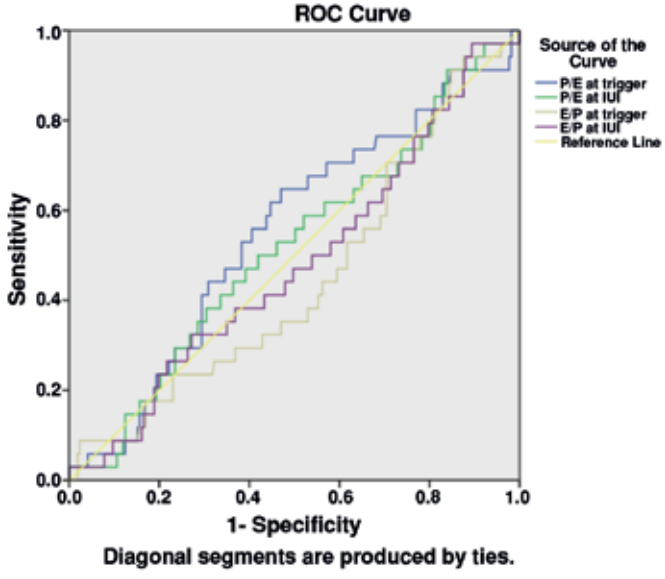

Figure 1. Receiver operating characteristic curve of different ratios to predict positive pregnancy test

ROC: Receiver operating characteristic, IUI: Intrauterine insemination

A study showed a significant association between serum estradiol level on trigger day with the pregnancy rates following ovarian stimulation and intrauterine insemination[10]. Our data analyses revealed no relationship between the estradiol level on the trigger day and pregnancy rates.

Endometrial thickness measurement is the most commonly used parameter to have an indirect idea about endometrial receptivity; an optimal endometrial thickness is required for favorable outcome. However, the use of endometrial thickness alone was found to have high negative predictive value but low positive predictive value with low specificity[11]. It is well known that endometrial development requires the combined effect of estrogen and progesterone. This combination effect should be in balance, previous data showed significant predictive value of progesterone/estradiol ratio at the periovulatory phase in estimating the efficacy of the ovulation induction in IUI cycles[12]; however, our data showed no association between the progesterone/estradiol ratio either at the trigger or on the day of IUI, which indicates that endometrial receptivity is not under a dominant effect of any of these hormones.

Estradiol supplementation has been used to ameliorate endometrial receptivity in IUI cycles. A study on this issue assessed the effect of estradiol supplementation in cycles with luteal phase serum estradiol drop by more than 50% over a 48-

Table 1. Hormone concentrations on the trigger and intrauterine insemination day in the positive pregnancy test group

	Mean	n	Standard deviation	p
Progesterone at trigger (ng/mL)	1.2	34	2.9	p<0.001
Progesterone at IUI (ng/mL)	2.9	34	5.1	
Estradiol at trigger (pg/mL)	468.9	34	345.7	p>0.05
Estradiol at IUI (pg/mL)	413.1	34	363.6	

IUI: Intrauterine insemination

Table 2. Hormone concentrations on the trigger and intrauterine insemination day in the group with negative pregnancy tests

	Mean	n	Standard deviation	p
Progesterone at trigger (ng/mL)	0.7	217	1.7	p<0.001
Progesterone at IUI (ng/mL)	2.4	217	3.7	
Estradiol at trigger (pg/mL)	428.6	217	320.4	p<0.001
Estradiol at IUI (pg/mL)	319.6	217	285.2	

IUI: Intrauterine insemination

hour period within 10 days of recombinant-HCG administration, and the data showed that estradiol supplementation resulted in higher rates of pregnancy (12.6 vs. 20.9%); the difference was more prominent when data were analyzed for patients aged >35 years[13]. Consistent with our results, that study also showed a critical role of estradiol during luteal phase. In our study population, ovulation was triggered by recombinant-HCG in all patients. There is evidence about the effect of recombinant-HCG on ovarian endocrine function, a study showed that higher doses of recombinant-HCG administration promoted the secretion of both estradiol and androgens into the follicular fluid, with a shift toward a more androgenic milieu[14]. This shows the ovarian endocrine response to recombinant-HCG exposure, which indicates a formation of androgenic state in the ovary. Accordingly, one would expect to observe increased serum sex hormone levels after ovulation trigger; however, our data showed decreased estradiol levels both in the pregnant and non-pregnancy groups, and higher decrements resulted in failure of IUI cycle.

Besides the effect of estrogen on the endometrial receptivity, a premature increase in progesterone concentrations in stimulated cycles was found to have a negative impact on pregnancy rates. Although the exact cause of this progesterone concentration elevation is not clear, it was suggested that overstimulation may lead to increased progesterone concentrations at the end of the follicular phase. Furthermore, this premature progesterone elevation was associated with altered gene expression and also reduced endometrial receptivity[15]. In our study, we did not see a significant predictive value of progesterone on the trigger day, the mean values were comparable between the groups (0.8 vs. 1.2, p>0.05). A recent study suggested freezing all embryos in IVF/ICSI cycles if the progesterone level was >1.5 on the trigger day[16]. A modest elevation of progesterone was observed in our study, this was thought to be due to the mild stimulation protocols specific for COH+IUI cycle.

Previous study analyzed the additive value of progesterone level determination 24 hours after recombinant-HCG administration and revealed an improved predictive value compared with a single measurement on the day of recombinant-HCG administration, the authors concluded that the high progesterone levels on both days resulted in low implantation rates compared with normal levels in IVF/ICSI cycles (22% vs. 36%)[17]. There is also some evidence that basal progesterone levels may be used to predict premature progesterone elevation in IVF/ICSI cycles[18]. In our data, post trigger progesterone levels obtained from the laboratory analyses at 36 hours after trigger were not found to affect cycle outcome.

The estradiol/progesterone ratio on the day of embryo transfer has been used to predict implantation in ICSI cycles. A study on this issue indicated that this ratio was predictive for ICSI success when combined with embryo quality, endometrial thickness, and estradiol levels, and higher ratios were found associated with favorable results[19]. This study emphasized the role of estrogen during the luteal phase of the cycle stressing that higher ratios were found to be predictive for desirable outcome. Progesterone plays an important role during the luteal phase for decidualization changes and progression of pregnancy. Premature progesterone elevation is observed in 5 to 30% of treatments despite the use of GnRH analogs in assisted reproduction technique (ART) cycles[20-22]. Some studies revealed favorable outcomes in cycles with elevated progesterone/estradiol ratios with higher oocyte collection and normal pregnancy rates[20,23,24]. In contrast, other data showed low ovarian reserve and reduced oocyte retrieval in patients with high ratios[25].

There is no consensus at to whether embryos should be transferred in women with a premature rise in progesterone.

Table 3. Comparison of groups with and without positive pregnancy test and demographic and hormonal characteristics

	Implantation	n	Mean	Standard deviation	
Age (years)	Negative	217	30.1	5.2	
	Positive	34	28.1	4.4	NS
Progesterone at trigger (ng/mL)	Negative	217	0.7	1.7	
	Positive	34	1.2	2.9	NS
Estradiol at trigger (pg/mL)	Negative	217	428.6	320.4	
	Positive	34	468.9	345.7	NS
Estradiol at IUI (pg/mL)	Negative	217	319.6	285.3	
	Positive	34	413.1	363.6	NS
Progesterone at IUI (ng/mL)	Negative	217	2.4	3.8	
	Positive	34	2.9	5.1	NS
Endometrial thickness (mm)	Negative	217	8.4	1.7	
	Positive	34	8.2	1.5	NS

IUI: Intrauterine insemination, NS: Not significant

There are also no data in the literature regarding optimal stimulation protocols to avoid premature progesterone rises.

We know about the detrimental effect of premature rise in progesterone levels on the ART cycles. However, a study reported a significant correlation between increased progesterone and high estradiol levels and no detrimental effect on the cycle outcome[26]. In fact, unfavorable effects of stimulation have been proposed to be observed in the early luteal phase of the cycle and these effects were thought to be corrected during the late luteal phase[27]. Consistent with this argument, Elgindy et al.[28] documented different implantation rates between cleavage stage embryo transfer and blastocysts transfer. The authors claimed that the adverse effect of the progesterone/estradiol ratio in stimulated cycles was compensated for by a day 5 embryo transfer[28]. The authors of a review on the regulation of steroid production and its function within the corpus luteum concluded that oxytocin and prostaglandin F alpha were found to stimulate estradiol and progesterone release and estradiol itself further stimulated progesterone release. Furthermore, it was also reported that during luteolysis, invading macrophages secrete tumor necrosis factor, which inhibits the luteotropic effects of estradiol and disrupts the intraluteal circuit[29]. These data partially explain why we experienced lower rates of pregnancy in women with high estradiol drop after ovulation trigger; an average estrogenic effect is necessary for optimal corpus luteum function. The proposed underlying mechanisms of insufficient function of the corpus luteum included "supraphysiologic estradiol level, decreased luteinizing hormone level, inhibition of the corpus luteum, and asynchronization of estradiol and progesterone"[30,31]. A Meta-analyses on estrogen plus progesterone replacement during luteal phase of the cycle showed higher rates of clinical pregnancy compared with progesterone alone in women undergoing IVF[32]. According to this and data from our study, an average but not supraphysiologic estrogen function seems to be mandatory during the luteal phase of ovulation induction cycles; significant drops in the estradiol concentrations after ovulation trigger are associated with IUI cycle treatment failure.

Ethics

Ethics Committee Approval: The study were approved by the Local Ethics Committee. Zeynep Kamil women and children's health training and research hospital, Informed Consent: Consent form was filled out by all participants.

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Cansu İpekçi, Concept: Tayfun Kutlu, Design: Birsen Konukçu, Data Collection or Processing: Birsen Konukçu, Cansu İpekçi, Analysis or Interpretation: Enis Özkaya, İlhan Şanverdi, Literature Search: Belgin Devranoğlu, Yavuz Şahin, Writing: Enis Özkaya, Tayfun Kutlu, Ateş Karateke.

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The value of urea, creatinine, prolactin, and beta sub-unit of human chorionic gonadotropin of vaginal fluid in the diagnosis of premature preterm rupture of membranes in pregnancy

Gebelikte prematüre preterm membran rüptürü tanısında vajinal sıvıda üre, kreatinin, prolaktin ve beta-insan koryonik gonadotropinin değeri

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Abstract

Objective: To evaluate the effectiveness of urea, creatinine, prolactin, and the beta sub-unit of human chorionic gonadotropin (β-hCG) of vaginal fluid in the diagnosis premature preterm rupture of membranes (PROM).

Materials and Methods: In this observational study, 160 pregnant women with gestational age of 28 to 40 weeks were divided into two equal groups: investigation (documented PROM) and control (intact membrane) groups. Five cubic centimeters of normal saline was poured into the vagina of all participants and the liquid was extracted after a few minutes using a syringe. The liquid was sent to a laboratory for examination. Data were analyzed using a t-test.

Results: The volume of urea, creatinine, prolactin, and β-hCG was significantly different in the two groups ($p<0.001$). Based on receiver operating characteristic curve and cut-off point, sensitivity, specificity, positive and negative predictive values of β-hCG for detecting PROM were 87.5%, 86%, 86.4%, and 87.3%, respectively. Also, the same factors for urea in detecting PROM were 79.7%, 82.5%, 81.8%, and 80.4%, respectively. Creatinine had 74.6% sensitivity, 85% specificity, and 83% and 77.2% positive and negative predictive values for detecting PROM. Finally, prolactin had 87.5% sensitivity, 90% specificity, and 90% positive and 88% negative predictive values for detecting PROM.

Conclusion: Prolactin and β-hCG have more diagnostic value than urea and creatinine in detecting PROM, and can be used in suspected cases.

Keywords: Premature preterm rupture of membranes, urea, creatinine, prolactin, β-hCG, vaginal fluid

Öz

Amaç: Prematüre preterm membran rüptürü (PMR) tanısında, vajinal sıvıda üre, kreatinin, prolaktin ve insan koryonik gonadotropin hormonu beta alt ünitesinin (β-hCG) etkililiğini değerlendirmek.

Gereç ve Yöntemler: Bu gözlemsel çalışmada, gestasyonel yaşı 28-40 hafta arasında olan 160 gebe kadın, araştırma (dökümante edilmiş PMR) ve kontrol (membranı intakt) grupları olarak iki eşit gruba ayrılmıştır. Tüm katılımcıların vajinalarına 5 cc normal serum fizyolojik verilmiş ve verilen sıvı birkaç dakika sonra bir enjektör ile çekilmiştir. Sıvı, inceleme için bir laboratuvara gönderilmiştir. Veriler t-testi kullanılarak analiz edilmiştir.

Bulgular: Üre, kreatinin, prolaktin ve β-hCG hacimleri iki grup arasında anlamlı olarak farklıdır ($p<0,001$). Alıcı işletim karakteristik eğrisine ve kesim noktasına bağlı olarak, PMR tespitinde β-hCG için, duyarlılık, özgüllük, pozitif ve negatif belirleyicilik değerleri sırası ile %87,5, %86, %86,4 ve %87,3'tür. Ayrıca, PMR tespitinde bu değerler üre için sırası ile %79,7, %82,5, %81,8 ve %80,4'tür. Kreatinin PMR tespitinde, %74,6 duyarlılık, %85 özgüllük, %83 ve %77,2 pozitif ve negatif belirleyicilik değerlerine sahiptir. Son olarak prolaktin, PMR tespitinde %87,5 duyarlılığa, %90 özgüllüğe, %90 pozitif ve %88 negatif belirleyicilik değerlerine sahiptir.

Sonuç: PMR tespitinde prolaktin ve β-hCG, üre ve kreatinine göre daha yüksek tanı değerine sahiptir. Şüpheli olgularda kullanılabilirler.

Anahtar Kelimeler: Prematüre membran rüptürü, üre, kreatinin, prolaktin, β-hCG, vajinal sıvı

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Introduction

Premature rupture of membranes (PROM) refers to fetal membranes' rupture before the onset of labor. If it occurs before 37 weeks of pregnancy, it is called premature preterm rupture of membrane (PPROM)[1]. In term or preterm pregnancies, a long duration between PROM and delivery can result in maternal and neonatal morbidity. This includes intrauterine infections (chorioamnionitis), neonatal and fetal sepsis, fetal prematurity, placental abruption, umbilical cord prolapse, cesarean delivery, and an increased risk of maternal and neonatal mortality[1-6].

Any patient with a history of vaginal leakage during pregnancy or a decreased level of amniotic fluid in ultrasound should be evaluated carefully because of the adverse effects on pregnancy outcomes. Early and accurate diagnosis allows clinicians to design some interventions for optimizing maternal and neonatal outcomes and decrease serious complications[7].

Detecting PROM is sometimes easy in a speculum examination with the observation of amniotic fluid accumulation in the vagina or liquid outflow from cervix[8]. However, when the membrane rupture is small or it is impossible to clearly see amniotic fluid leakage, PROM cannot be detected easily, which might lead to failure in diagnosis and non-performance of necessary interventions[9,10]. There are a few methods for PPROM diagnosis. Fern and nitrazine are two traditional, commonly used tests. Although they are easy and rapid tests, both have high false positive and negative results, for example through blood, semen or cervical mucus contamination or technical errors, which means they are not completely reliabe[8-13].

Ultrasound examination with amniotic fluid determination is not a good test because it cannot differentiate PROM from other causes of oligohydramnios[7]. Although the amnio-dye or tampon test is a standard test for accurate diagnosis, it involves amniocentesis and instillation of dye; therefore, it is an aggressive test and has a risk of placental abruption, miscarriage, bleeding, infection, and iatrogenic uterine perforation[7]. The Amnisure ROM test is another new test that is easy, fast, and minimally invasive, with high sensitivity and specificity. This test identifies trace amounts of placental alpha-microglobulin-1 (PAMG-1), which is abundant in amniotic fluid[14,15]. However, Amnisure it is not available in many centers and it is expensive.

For this reason, a non-invasive, simple, and inexpensive method of detecting PPROM is required. Several markers have been studied such as alpha-fetoprotein, fetal fibronectin, creatinine, insulin growth factor binding protein 1, urea, prolactin, and β-hCG[5,7,13-18].

β-hCG is a glycoprotein that is secreted in the placenta from syncytiotrophoblasts. Prolactin is a single-chain polypeptide that is secreted during pregnancy from the mother's and fetus's pituitary and decidua. Urea and creatinine are both excreted through glomerular filtration. These markers are also available in amniotic fluid and have been examined for finding PPROM in some studies[5,16-18]. The present study evaluated the value of urea, creatinine, prolactin, and β-hCG of vaginal fluid in the diagnosis of PPROM in pregnancy.

Materials and Methods

Between April 2013 and August 2014, 160 pregnant women with gestational age of 28 to 40 weeks were enrolled in the study. All women presented to our center in Zahedan, Sistan and Baluchestan province, Iran. The aim of the study was explained for all participants before their participation and informed consent was received. The study was Approved by the Ethics Committee of Zahedan University of Medical Sciences. All patients were divided into two groups. The PROM group comprised women with ruptured membranes and the control group included women who had just presented to our center for periodic examinations. The mean ages of the investigation and control groups were 25.0±6.5 years and 25.8±5.5 years, respectively (p=0.386).

Gestational age was determined based on the last menstruation period and ultrasound of the first trimester of pregnancy. Membrane rupture was verified in a sterile speculum examination and observation of fluid leakage in the cervix or accumulation of fluid in the posterior fornix of the vagina, or by both nitrazine and Fern tests. Patients with fetal malformations, fetal growth restriction, fetal distress, placenta previa, vaginal bleeding, vaginal infection, maternal disease, hypertension, preeclampsia and other pregnancy complications were excluded.

Five cubic centimeters of normal saline sterile solution was poured by a syringe in all participants posterior vagina fornix. After a few minutes the fluid was aspirated by the same syringe and was sent to a laboratory for examination. The liquid was centrifuged for 10 minutes. The Alcyon automatic biochemical kit was used to measure urea and creatinine (Pars co., Iran) and DiaPlus enzyme-linked immunosorbent assay (ELISA) kit (USA) was used for measuring β-hCG and prolactin.

Statistical Analysis

T-test and chi-square were used to measure the quantitative and qualitative variables. Receiver operating characteristic curve was used to determine a cut-off value. The cut-off point was set at the highest optimal sensitivity and specificity. The statistical package for social sciences (SPSS) software version 16 (Chicago, IL, USA) was used to analyze the data. A p value less than 0.05 was considered significant.

Results

There was no significant difference between the two groups regarding demographic characteristics (Table 1). The means of β-hCG, blood urea nitrogen, creatinine and prolactin were 203.1±180.9 mIU/mL, 8.5±6.3 mg/dL, 0.86±0.68 mg/dL, and 69.8±37.9 mIU/mL in the investigation group and 17.4±9.9 mIU/mL, 2.7±1.4 mg/dL, 0.20±0.16 mg/dL, and 10.9±5.6 mIU/mL in the control group. All of the results were significant (p<0.001) (Table 2).

Based on the receiver operating characteristic curve, the cut-off point for β-hCG was 20.5 mIU/mL. With that cut-off point, the sensitivity, specificity, positive and negative predictive values for detecting PROM were 87.5%, 86%, 86.4%, and 87.3%, respectively. Also, the cut-off point for blood urea nitrogen was 3.5 mg/dL with 79.7% sensitivity, 82.5% specificity, and 81.8% and 80.4% positive and negative predictive values for detecting PROM. The cut-off point for creatinine was 0.25 mg/dL and it had 74.6% sensitivity, 85% specificity, and 83% and 77.2% positive and negative predictive values for detecting PROM. Finally, the cut-off point for prolactin was 16 ng/mL based with 87.5% sensitivity, 90% specificity, and 90% and 88% positive and negative predictive values (Figure 1). A likelyhood ratio was determined for each diagnostic marker (Table 3).

Discussion

If PPROM is diagnosed early in pregnancy, many of its adverse effects can be prevented[1]. Hence, using biochemical tests and its markers in the vagina has been increased for early diagnosis of ruptured membrane. Various factors such as alpha fetoprotein, insulin-like hormone, prolactin, urea, creatinine and β-hCG,[5,16-18] plus alanine transaminase and aspartate transaminase[19]. have been suggested and studied. Researchers are still looking for a simple, fast, and easy way to detect membrane rupture that is accessible and non-invasive. Although PAMG-1 is a good choice for detecting PROM, it is

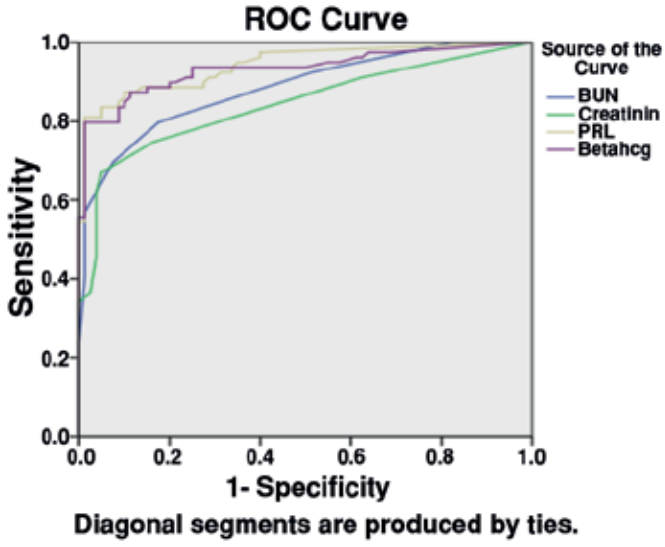

Figure 1. Receiver operating characteristic curve for vaginal beta sub-unit of human chorionic gonadotropin urea, creatinine, prolactin

ROC: Receiver operating characteristic, BUN: Blood urea nitrogen, PRL: Prolaktin

Table 1. Comparison of the demographic characteristics

Variable	Investigation group (mean ± standard deviation)	Control group (mean ± standard deviation)	p
Age (years old)	25.0±5.6	25.8±5.5	0.368
Gestational age	36.4±2.5	35.7±2.4	0.071
No. of pregnancies	2.4±1.7	2.7±1.6	0.245
No. of deliveries	1.2±1.5	1.4±1.4	0.460
No. of abortions	0.21±0.54	0.32±0.63	0.229
The analyses were done using independent t-test			

Table 2. Comparison of means of beta sub-unit of human chorionic gonadotropin urea, creatinine, and prolactin (p<0.001)

Vaginal fluid	β-hCG	Urea	Creatinine	Prolactin markers (mean ± SD)
PPROM group	203.1±180.9	8.5±6.3	0.86±0.68	69.8±37.9
Control group	17.4±9.9	2.7±1.4	0.20±0.16	10.9±5.6

β-hCG: Beta sub-unit of human chorionic gonadotropin, SD: Standard deviation, PPROM: Premature preterm rupture of membrane

Table 3. Evaluation of indicators for diagnostic premature preterm rupture of membrane markers

Variable	Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR
β-hCG	20.5	87.5	86	86.4	87.3	6.25	0.15
Urea	3.5	79.7	82.5	81.8	80.4	4.95	0.25
Creatinine	0.25	74.6	85	83	77.2	4.97	0.30
Prolactin	16	87.5	90	90	88	8.75	0.14

PPV: Positive predictive value, NPV: Negative predictive value, PLR: Positive likelihood ratio, NLR: Negative likelihood ratio, β-hCG: Beta sub-unit of human chorionic gonadotropin

Table 4. Comparison the values of sensitivity, specificity, positive and negative predictive values for vaginal fluid markers other studies

Study first author		Cut-off	Sensitivity %	Specificity %	PPV	NPV
Taheripanah et al.[22]	Prolactin	16	96	79.41	95.2	82.3
	β-hCG	12.5	69.33	69.85	69.4	69.6
Mohamed and Mostafa[21]	Urea	13.2	100	100	100	100
	Creatinine	0.31	100	100	100	100
	β-hCG	20	83	100	100	85.6
Kafali and Oksüzler[23]	Urea	12	100	100	100	100
	Creatinine	0.6				
Buyukbayrak* et al.[2]	Prolactin	30	95	87	84	93
Kariman et al.[24]	Urea	6	90	79	83	87.5
	Creatinine	0.45	100	100	100	100
Bahasadri et al.[20]	β-hCG			79.5	93	84
Shahin and Raslan[5]	Prolactin		76	70	71.4	74.5
	β-hCG		84	72	75	81.5

β-hCG (mIU/mL), Urea, Creatinine (mg/dL); Prolactin (ng/mL), *Buyukbayrak µIU/mL, PPV: Positive predictive value, NPV: Negative predictive value, β-hCG: Beta sub-unit of human chorionic gonadotropin

not available in most centers and is expensive compared with markers such as prolactin or β-hCG. Thus, some researchers have preferred to find a more convenient diagnostic method.

Bahasadri et al.[20] recorded 93% sensitivity and 84% specificity for β-hCG of vaginal fluid, which is in agreement with our findings. The authors reported that there was more β-hCG in vaginal fluid of women with PPROM than in pregnant women with intact membranes; therefore, it could be a reliable and fast way of detecting membrane rupture. In another study in 2011, Mohamed and Mostafa[21] studied the value of urea, creatinine, and β-hCG of vaginal fluid in detecting rupture of membranes of 298 women. They documented 100% sensitivity and specificity for urea and creatinine and 83% sensitivity and 100% specificity for β-hCG, which are consistent with our findings (Table 4).

In 2009, Taheripanah et al.[22] investigated the diagnostic value of prolactin and β-hCG of vaginal liquid in detecting PPROM. They arrived at 96% sensitivity and 79.4% specificity for prolactin, and 69.3% sensitivity and 69.8% specificity for β-hCG. The authors concluded that although β-hCG could help in detecting membranes rupture, it was not as sensitive and specific as prolactin[22]. In our study, when we compared positive likelihood ratios for diagnostic markers, we found prolactin as the marker with the most sensitivity and specificity values. This is in agreement with Taheripanah et al.[22] findings. Kafali and Oksüzler[23] studied urea and creatinine of vaginal liquid with a 12 mg/dL cut-off point for urea and 0.6 mg/dL for creatinine and found that the specificity and sensitivity of both markers was 100%.

In another study, Kariman et al.[24] investigated the diagnostic value of urea and creatinine on vaginal fluid of 179 pregnant women with gestational age of 14 to 42 weeks. For urea, they found 90% sensitivity, 79% specificity, and 83% and 87.5% positive and negative predictive values with a 6.0 mg/dL cut-off point. For creatinine, with a 0.45 mg/dL cut-off point, the authors found 100% sensitivity and specificity. Creatinine had a higher diagnostic value than urea[24]. However, creatinine had less diagnostic value in our study, which might have been because of the difference in laboratory analysis methods and cut-off points (Table 4).

In 2004, Buyukbayrak et al.[2] found that prolactin with a 30 µIU/mL cut-off point had 95% sensitivity, 87% specificity, and 87% accuracy, which is consistent with our study. Also, Shahin and Raslan[5] demonstrated lower predictive values for prolactin than in our study. This may be because of the different cut-off points or smaller sample size.

Prolactin and β-hCG have more diagnostic value than urea and creatinine in detecting PPROM, and can be used in suspected cases. These tests are easy and not expensive, and can be used in any medical center. It is suggested that cut-off value for rupture of membranes in pregnancy be determined in different gestational ages in future studies.

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Ethics

Ethics Committee Approval: Zahedan University of Medical Sciences,
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Authorship Contributions

Surgical and Medical Practices: Marzieh Ghasemi, Reyhaneh Jaami, Ashraf Alleyassin, Concept: Reyhaneh Jaami, Marzieh Ghasemi, Design: Marzieh Ghasemi, Alireza Ansarimoghaddam, Data Collection or Processing: Marzieh Ghasemi, Reyhaneh Jaami, Analysis or Interpretation: Marzieh Ghasemi, Alireza Ansarimoghaddam, Literature Search: Reyhaneh Jaami, Writing: All authors.

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Changes of intraocular pressure in different trimesters of pregnancy among Syrian refugees in Turkey: A cross-sectional study

Türkiye'de yaşayan Suriyeli göçmenlerde gebeliğin farklı trimesterlerinde göz içi basıncındaki değişiklikler: Kesitsel çalışma

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Abstract

Objective: To evaluate the physiologic changes in intraocular pressure associated with pregnancy in healthy Syrian refugee women in Turkey.
Materials and Methods: In this cross-sectional study, intraocular pressures were measured using a Goldmann tonometer in 235 patients in the first, second, and third trimester of pregnancy and puerperium among Syrian refugees in Turkey.
Results: Mean intraocular pressures values of the right eye were 15.5±2.5 mmHg, 14.4±1.4 mmHg, 13.9±1.6 and 14.7±1.9 mmHg in the three trimesters and puerperium, respectively. Mean intraocular pressures values of the left eye were 15.3±1.6 mmHg, 14.3±1.4 mmHg, 13.9±1.6 and 15.3±2.2 mmHg in the three trimesters and puerperium, respectively. The mean intraocular pressures values measured from both eyes were significantly higher in first trimester and puerperal period than in the third trimester ($p<0.001$).
Conclusion: Changes in the intraocular pressure in pregnancy are common and temporary. This study shows the baseline changes in the intraocular pressure during pregnancy in healthy women. Therefore, we cannot extrapolate the results to the whole eye. A decrease in intraocular pressures was shown in healthy pregnant women.
Keywords: Pregnancy, intraocular pressure, Syrian refugees

Öz

Amaç: Türkiye'de yaşayan Suriyeli göçmenlerde gebelik esnasında göz içi basıncındaki fizyolojik değişikliklerin belirlenmesi.
Gereç ve Yöntemler: Bu kesitsel çalışmada Türkiye'de yaşayan 235 Suriyeli göçmenin birinci, ikinci ve üçüncü trimester gebelik ve puerperiyumda göz içi basınçları Goldmann tonometresi ile ölçülmüştür.
Bulgular: Sağ göz için ortalama göz içi basıncı birinci, ikinci ve üçüncü trimester ve puerperiyum için sırasıyla 15,5±2,5 mmHg, 14,4±1,4 mmHg, 13,9±1,6 mmHg ve 14,7±1,9 mmHg olarak ölçülmüştür. Sol göz için sırasıyla göz içi basıncı 15,3±1,6 mmHg, 14,3±1,4 mmHg, 13,9±1,6 ve 15,3±2,2 mmHg olarak ölçülmüştür. Ortalama göz içi basıncı iki göz içinde birinci trimesterde ve puerperiyumda üçüncü trimestere göre daha yüksek olarak bulunmuştur ($p<0,001$).
Sonuç: Gebelikte oküler değişiklikler yaygındır ve geçicidir. Bu çalışma Türkiye'de yaşayan gebe ve puerperiyumdaki Suriyeli göçmenlerden elde edilen verilerle yapılmıştır.
Anahtar Kelimeler: Gebelik, intraoküler basınç, Suriyeli göçmenler

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Introduction

Pregnancy is a complex physiologic process that affects all organic systems. Ocular changes in pregnancy can be physiologic or pathologic. Decrease in intraocular pressure (IOP) in pregnancy has been shown in the literature[1]. Most physiologic changes due to pregnancy are usually marked in last trimester, in which the hormonal status is at its peak[2]. In pregnancy the probable reasons of the reduction of the IOP are the increase in the outflow of aqueous humor (AH), physiologic relaxation of ligaments and reduction of cornea rigidity that occurs in late pregnancy, the reducing effect of diminished venous pressure in upper extremities on episcleral venous pressure, vasodilator effects of pregnancy hormones, and pregnancy acidosis[3].

Due to hormonal influences, physiologic ocular changes have been shown in pregnancy in Caucasian women. Cornea sensitivity, refractive status, IOP, and visual acuity can change in pregnancy[4-6]. Therefore, it is important to be aware of physiologic changes as well as of the potential effects on preexisting disease and complications in order to counsel and advise women who are currently pregnant. The aim of our research was to observe the physiologic varieties of IOP in pregnant Syrian refugees in Turkey.

Materials and Methods

A cross-sectional study was performed after obtaining approval from the Ethics Commission of the Harran University Faculty of Medicine, Şanlıurfa, Turkey. All patients provided written informed consent. Patients were selected in the obstetrics and gynecology outpatients clinic of Suruç State Hospital and Harran University Faculty of Medicine between August 5th, 2015, and September 13th, 2015. Two hundred-eighty pregnant women were evaluated for suitability during the research period. Eighteen patients did not meet the inclusion criteria and 23 patients refused to participate in the study. Four patients were excluded from the study during IOP measurement because of excessive blinking. In total, 235 patients were included in the research. The inclusion criteria were to be a female Syrian refugee aged between 20-35 years with a known last menstrual period. The exclusion criteria were the presence of any systemic disease such as hypertensive disorders, diabetes mellitus, or any ocular disease. Patients with preeclampsia and gestational diabetes were treated and managed in accordance with current guidelines, but these patients were not evaluated in the research. In addition, we did not include any twin pregnancies in the study. The study groups consisted of pregnant women in the first, second, and third trimesters, and women with puerpera. The first third or 14 weeks of pregnancy were defined as the first trimester, 14-28 weeks of pregnancy were defined as the second trimester, and the last third of pregnancy was defined as the third trimester. Puerperium is defined as the period from the end of labor until involution of the uterus is complete, usually lasting between 3 and 6 weeks. Patients were assessed in clinical and ultrasonographic examinations during antenatal screening. Each patient's age, parity, and smoking status was recorded. The smoking rate was low in our patient population. Patients who smoked more than 5 cigarettes a day were not included in the study.

A full ophthalmoscopic examination was performed to exclude any anterior and posterior segment illness. The IOPs were evaluated with the same Goldmann tonometer (Optilasa, S.L., Madrid, Spain). The device was calibrated prior to the study. One drop of 0.5% proparacain was instilled into the each eye of the subjects and both inferior conjunctival sacs were touched with a dry fluorescein strip (Biotech, Gujarat, India) to measure the IOP of the eyes; as soon as a value was established it was recorded. The right eye was always measured first. All measurements were performed in the morning between 08:00 AM and 10:00 AM to avoid the diurnal variation of IOP.

Statistical Analyses

The statistical package for the social sciences (SPSS) version 20.0 for Windows was used for all statistical analyses. The Shapiro-Wilk test was used to test distribution of normality. According to the results, parametric tests were preferred. We used one-way ANOVA test to compare continuous variables. Categorical variables were compared with the chi-square test. A p value <0.05 was considered statistically significant. When we found a statistically significant difference, we performed a post-hoc analysis between all group pairs to determine the source of statistical significance.

Results

There were 61 (25.9%) patients in the 1st trimester, 76 (32.3%) patients in the 2nd trimester, 54 (22.9%) patients in the 3rd trimester, and 44 (18.7%) patients in the puerperal period. The average age of the patients was 27.4±4.7 years. We found no statistically significant difference in age between the groups (p=0.167). The mean parity number of the patients was 3.2±0.2. Similarly, the mean parity number and ratio of smokers within the groups were also comparable (p=0.310, p=0.052, respectively) (Table 1).

Table 1. Demographics of the study groups

	1st trimester n=61	2nd trimester n=76	3rd trimester n=54	Puerperal period n=44	p
Age, years (mean ± SD)	27.1±5.1	28.2±4.3	26.5±4.8	27.9±4.9	0.167
Parity, n (mean ± SD)	2.9±1.7	3.3±1.3	3.1±1.9	3.5±2.4	0.310
Smoking, n (%)	2 (3.3%)	10 (13.2%)	6 (11.1%)	9 (20.5%)	0.052

Table 2 summarizes the mean IOP values of the studied population. The mean IOP values measured from the right eye were significantly higher in the first trimester and puerperal period than in the third trimester (15.5±2.5 mmHg, and 14.7±1.9 mmHg vs. 13.9±1.6 mmHg, respectively; p<0.001). The mean IOP values measured from the left eye were significantly higher in the first trimester and puerperal period than in the second and third trimesters (15.3±1.6, and 15.3±2.2 mmHg, vs. 14.3±1.4 mmHg and 13.9±1.6 mmHg, respectively (p<0.001).

Discussion

This is the first study, to our knowledge, to examine the physiologic changes of IOP in pregnant Syrian refugees in Turkey. The present cross-sectional research was conducted to evaluate the relationship between pregnancy period and IOP. Our results clearly demonstrate that the IOP values decrease as the gestational period progresses and return to normal in the puerperal period. The lowest IOP values were detected in the third trimester of pregnancy. Early studies revealed the effects of pregnancy on the eyes, which in addition to new changes and pre-existing ocular disorders, may change their course owing to the widespread changes during pregnancy, hormonal and otherwise, that may either be exacerbated or ameliorated[7].

The changes in IOP during pregnancy were significant in our study. There was a decline in IOP from the first trimester to the third trimester. These changes are frequently temporary and returned to normal levels after delivery[8]. Our finding of an ocular hypotensive effect in the third trimester of pregnancy is consistent with other studies in the literature[1,3,9,10]. Otherwise, Philips and Gore[11] reported no significant difference in the ocular hypotensive effect of late pregnancy in women who were normotensive and hypertensive.

The main physiological mechanism responsible for the decrease in IOP during pregnancy is not fully known. The decrease in IOP during pregnancy is likely multifactorial[12]. The decreased IOP in pregnancy may be due to elevated hormonal levels, which cause an increase in fluid outflow conductance without altering the rate of fluid entry[13]. It is well documented that progesterone, estrogen, and other placental hormone levels change during pregnancy. Estrogen has a dilatator effect on the vessels. Omoti et al.[10] reported that this vasodilator effect provides a decrease of arterial pressure and thus causes a reduction of AH production.

In pregnancy there is a general decrease in peripheral vascular resistance. Therefore, episcleral venous pressure also decreases in pregnancy[14,15]. AH outflow is facilitated by this decrease. Also estrogen has a protective effect in the vascular pathology by the production of mediators such as nitric oxide, prostacyclins, endothelin-I, and eicosanoids. These vasodilators cause reduction of resistance[8]. In pregnancy with a normal production of AH, the facility of AH drainage is due to the increased levels of the β-hCG and progesterone, and general decreased peripheral vascular resistance. This results in a gradual, statistically significant decrease in IOP during pregnancy[1]. The anti-glucocorticoid features of progesterone may have a role in the reduction of IOP. Endogen corticosteroids have an ocular hypertensive effect and progesterone blocks this effect[16]. During pregnancy, relaxin is released by the high levels of estrogen. Relaxin is a hormone that has softening properties and in late pregnancy these elastic changes decrease cornea-scleral rigidity and this causes a decrease in IOP by the diminished production of AH[11]. The effect of relaxin on outflow facility is thought to be mediated by collagen changes, which in turn affect the rigidity of Schlemm's canal and the trabecular meshwork[17]. Saylık and Saylık[18] reported that the reduction of IOP was more pronounced in twin pregnancies than in singleton pregnancies because of the presence of higher levels of hormones that affect IOP in twin pregnancies. Qureshi et al.[19] showed that the ocular hypotensive effect of late pregnancy was significantly greater in multigravida women than in those who were primigravida. In our study, the average parity number was 3.2±0.2. The multigravida nature of our patients might have increased the relaxin hormone levels, which could explain the diminished IOP in pregnancy in this research.

The small sample size and the cross-sectional study design were the major limitations of the present study, which prevent drawing definitive conclusions about the progression of IOP. A longitudinal design is necessary to establish the change of IOP in different trimesters of pregnancy. However, we were unable to collect data from a cohort during all pregnancy periods due to the inconsistency of Syrian pregnant women in attending ante-natal follow-up programs in this region of the country.

Conclusion

Changes in the IOP in pregnancy are common and temporary. However, these may have an impact on the progression of a preexisting ocular disease. Physicians should know about these

Table 2. Intraocular pressure values of the study groups

	1st trimester n=61	2nd trimester n=76	3rd trimester n=54	Puerperal period n=44	p
IOP-right, mmHg (mean ± SD)	15.5±2.5	14.4±1.4	13.9±1.6	14.7±1.9	<0.001*
IOP-left, mmHg (mean ± SD)	15.3±1.6	14.3±1.4	13.9±1.6	15.3±2.2	<0.001**

*Significance stems from the differences between 1st and 2nd trimesters (p=0.006), 1st and 3rd trimesters (p<0.001), and 3rd trimester and postpartum period (p=0.001). **Significance stems from the differences between 1st and 2nd trimesters (p=0.008), 1st and 3rd trimesters (p<0.001), 2nd trimester and postpartum period (p=0.020) and 3rd trimester and postpartum period (p=0.001). IOP: Intraocular pressure, SD: Standard deviation

physiologic changes in pregnancy so as not to consider these changes pathologic. At the same time, the physiologic decrease in IOP could be advantageous for pre-existing glaucoma. Therefore, physiologic changes should be kept in mind in order to prevent misdiagnoses during routine antenatal investigations.

Ethics

Ethics Committee Approval: The study was approved by the Harran University of Local Ethics Committe, Informed Consent: Consent form was filled out bu all participants.
Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Harun Egemen Tolunay, Sait Coşkun Özcan, Deniz Özarslan Özcan, Fatih Mehmet Adıbelli, Neşe Gül Hilali, Concept: Harun Egemen Tolunay, Sait Coşkun Özcan, Design: Harun Egemen Tolunay, Sait Coşkun Özcan, Data Collection or Processing: Harun Egemen Tolunay, Sait Coşkun Özcan, Analysis or Interpretation: Harun Egemen Tolunay, Sait Coşkun Özcan, Yavuz Emre Şükür, Literature Search: Harun Egemen Tolunay, Sait Coşkun Özcan, Yavuz Emre Şükür, Writing: Harun Egemen Tolunay, Sait Coşkun Özcan, Deniz Özarslan Özcan, Yavuz Emre Şükür.
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Association of first trimester serum uric acid levels gestational diabetes mellitus development

İlk trimester ürik asit yüksekliğinin gestasyonel diabetes mellitus ile ilişkisi

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Abstract

Objective: To investigate the association of first trimester serum uric acid levels with the development of gestational diabetes mellitus (GDM) in low-risk pregnant women.

Materials and Methods: In this retrospective data analysis, the results of pregnant women who completed both first trimester biochemical panel and two-step GDM screening were compared with an age-, body mass index, and gestational age-matched control group. The women were grouped as either GDM or impaired glucose tolerance (IGT) according to 100-g oral glucose challenge results. Uric acid levels were compared between the groups and diagnostic utility was tested with receiver-operating characteristics curves.

Results: Sixty-six women in GDM group and 358 women in the IGT group were compared against 202 healthy pregnant women. The groups did not differ significantly in terms of parity, pre-gestational body mass index and gestational age. Serum samples for uric acid levels were obtained. The mean serum uric acid levels were significantly higher in the GDM and IGT groups (5.95 mg/dL (±0.97 mg/dL) and 4.76 mg/dL (±1.51 mg/dL), respectively) compared with the control group (3.76 mg/dL (±1.07 mg/dL) ($p<0.001$). The area under the curve for uric acid levels was 0.92 (95% confidence interval 0.88-0.95) for diagnosis of GDM. At a diagnostic threshold of 3.95 mg/dL, uric acid levels predicted development of GDM with 60% specificity and 100% sensitivity.

Conclusion: First trimester serum uric acid has a linear association with the development of GDM and IGT.

Keywords: Hyperuricemia, risk assessment, gestational diabetes, screening

Öz

Amaç: Düşük riskli gebelerde ilk trimester ürik asit seviyelerinin gestasyonel diabetes mellitus (GDM) gelişimiyle ilişkisinin saptanması.

Gereç ve Yöntemler: Bu retrospektif veri analizinde birinci trimester biyokimya testi ile iki basamaklı gestasyonel diyabet taramasını tamamlamış gebe kadınların sonuçları; yaş, vücut kitle indeksi ve gestasyonel hafta açısından eşleştirilmiş kontrol grubu karşılaştırıldı. 100-g oral glukoz tolerans testine göre gebeler GDM ve bozulmuş glukoz toleransı (BGT) gruplarına ayrıldı. Gruplar arasında ürik asit seviyeleri karşılaştırıldı ve ürik asit seviyelerinin, GDM ve BGT için tanısal gücü sinyal algılama teorisinde, alıcı işletim karakteristiği eğrisi ile test edildi.

Bulgular: GDM grubundaki 66 kadın ve BGT grubundaki 358 kadın, 202 sağlıklı kadınla karşılaştırıldı. Yaş, parite, gebelik öncesi vücut kitle indeksi ve gestasyonel yaş açısından istatistiksel anlamlı farklılıklar içermeyen grupların birinci trimester serum örnekleri toplandı. Ortalama serum ürik asit seviyesi GDM ve bozulmuş glukoz toleransı gruplarında (sırasıyla, 5,95 mg/dL (±0,97) ve 4,76 mg/dL (±1,51) kontrol grubu ile karşılaştırıldığında (3,76 mg/dL±1,07) ($p<0,001$) daha yüksek olduğu görüldü. GDM tanısında ürik asit seviyeleri için ROC eğrisi altında kalan alan 0,92 idi (%95 güven aralığı 0,88-0,95). 3,95 mg/dL eşik değer olarak alındığında ürik asit seviyeleri GDM gelişme durumunu %60 spesifisite ve %100 sensitivite ile göstermektedir.

Sonuç: İlk trimester ürik asit seviyeleri ile GDM ve BGT gelişimi arasında lineer bir ilişki vardır.

Anahtar Kelimeler: Hiperürisemi, risk hesaplaması, gestasyonel diyabet, tarama

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Introduction

Gestational diabetes mellitus (GDM) is a relatively common disorder of pregnancy. The prevalence of GDM ranges from 1 to 6% depending on the studied population[1,2]. Large population-based studies are lacking in Turkey but some available data from cohort studies suggest that the prevalence of GDM ranges between 4-10% in Turkey[3-5]. Prediction and diagnosis of GDM is important for ongoing pregnancy and has important implications for subsequent health of the mother. GDM is considered a significant risk factor for subsequent development of type II diabetes and is associated with a poorer cardiovascular risk profile compared with women without GDM[6,7].

The method of screening (one-step versus two-step), application of screening (broad versus risk-dependent), and diagnostic criteria of GDM have been the subject to debate. Risk- dependent screening is being abandoned world-wide after the recommendation of the American Diabetes Association for screening all women without prior known diabetes between 24 and 28th gestational week[8]. The recommendation was based upon the inefficiency of the current history-based risk assessment method. However, the benefits of broad screening have not yet been established. A recent study by Koivunen et al.[9] reported no benefit of broad screening on cesarean section rates and birthweight despite increased rates of GDM diagnosis, glucose-challenge test applications, and labor induction. Until the benefits of broad screening are established there is a need of a better risk assessment method.

Uric acid has been investigated as a possible risk factor for the development of GDM. Several researchers reported an association of uric acid levels with development of GDM[10-12]. The aim of the current study was to investigate the association of first trimester serum uric acid levels with development of GDM in a population of low-risk pregnant women.

Materials and Methods

This was a retrospective case-control study including pregnant women who were followed-up entirely at Ankara University Hospital between January 2012 and December 2014. Our antenatal follow-up program includes routine blood tests during the first trimester with biochemical panel and a two-step approach for screening of GDM, in accordance with the American Diabetes Association recommendations[13]. The two-step approach consists of a 50-g oral glucose challenge test (GCT) performed between the 24th and 28th weeks of gestation, followed by a 100-g oral GCT if the initial 50-g oral GCT serial glucose result is over 140 mg/dL. The results of the 100-g oral GCT were interpreted in accordance with the Carpenter and Coustan[14] criteria for diagnosis of GDM. Uric acid levels were analyzed from serum samples obtained in the first trimester. Gestational ages were calculated from crown-rump length measurements in the first trimester[15].

Pregnant women who had completed both first trimester biochemical panel and GDM screening were included for analysis. Women with prior diabetes, hypertension, chronic kidney disease, multiple pregnancy, chronic liver disease, gout arthritis or history of alcohol use were excluded from the analysis. The primary outcome of the study was the association of uric acid levels with occurrence of GDM. For the statistical analysis, the pregnant women were divided into three groups according to their respective GDM screening results. Women who took the 100-g oral GCT were divided into two groups, those whose results indicated GDM (GDM group) and those whose results had at least one abnormal or no abnormal results and did not meet the criteria for diagnosis of GDM (impaired glucose tolerance group). A maternal age-, gestational age-, and body mass index (BMI) matched control group was used to compare uric acid levels between groups.

To determine the size of the case and control groups, first trimester serum uric acid levels of a small group of healthy pregnant women without GDM were used. The mean uric acid level of this group was 3.72 mg/dL ±1.14. To detect a 0.5 mg/dL mean between-group difference in uric acid levels, 41 samples in each group was required for the study to have 80% or more power with a two-sided type I error rate of 0.05.

All statistical analyses were performed using SPSS version 21.0 (IBM Corporation, Armonk NYC, USA). Parameters with normal distribution are described in means with standard deviation. Parameters with non-normal distribution are described in median with minimum maximum values. Student's t-test was used to compare continuous variables between independent groups. For each group, one-way ANOVA was used to test maternal age, pre-gestational BMI, and the gestational week serum samples as covariates to see if adjustment in a multivariable logistic regression model was necessary. Receiver-operating characteristics (ROC) curves were used to test the utility of first trimester serum uric acid levels for diagnosis of GDM. P values below 0.05 were considered statistically significant. This study was considered exempt from ethical approval by the Local Ethics Committee of Ankara University.

Results

A total of 4.812 pregnant women completed their antenatal follow-up in Ankara University Department of Obstetrics and Gynecology outpatient clinic between 2012 and 2014. Five hundred ten pregnant women were scheduled for a 100-g oral GCT because of a positive result of 50-g oral GCT. The results of the 100-g oral GCT revealed that 86 women had GDM and the remaining 410 women were diagnosed as having IGT. The prevalence of GDM was 1.7% in our study group. Twenty-six women in the GDM group and 66 women in the IGT group were excluded from the final analysis because they had co-morbidities (gestational hypertension, chronic liver or kidney diseaes), multiple gestations or missing first trimester serum uric acid levels. Two hundred two healthy age- and BMI-

matched pregnant women were included as a control group. Baseline characteristics of the study groups can be found in Table 1.

In each separate group, one-way ANOVA was used to test for an association of BMI, maternal age, and gestational age serum samples measured for serum uric acid levels, which revealed no association of tested covariates with serum uric acid levels ($p>0.05$).

Student's t test revealed serum uric acid levels were significantly different between the groups with 5.94±0.97 mg/dL in the GDM group, 4.76±1.51 mg/dL in IGT group, and 3.76±1.07 mg/dL in the control group ($p<0.001$). ROC curve was obtained for the first trimester serum uric acid levels to detect GDM (Figure 1). The area under the curve was 0.92 [95% CI: (0.88-0.95)] with a diagnostic threshold of 3.95 mg/dL; first trimester serum uric acid levels had a sensitivity of 100% and specificity of 60% for the prediction of GDM.

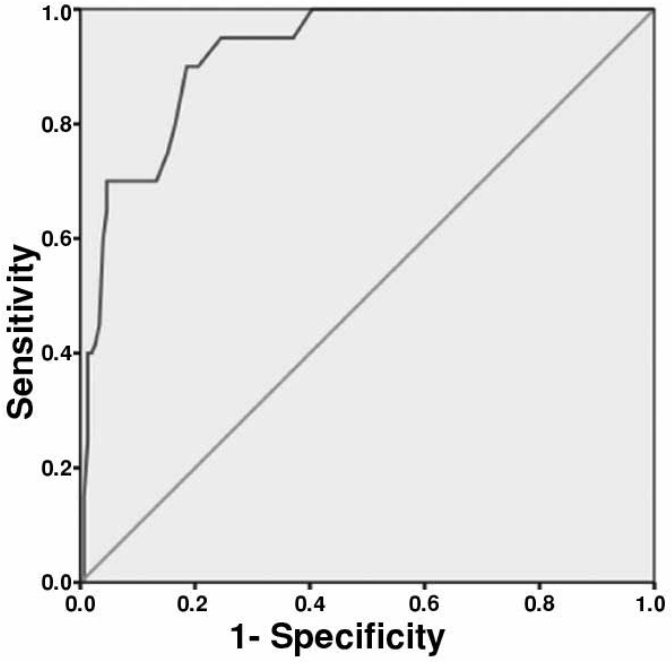

Figure 1. Receiver-operating characteristics curve for prediction of gestational diabetes mellitus with first trimester serum uric acid levels

Discussion

Uric acid is the final product of the oxidation step of purine catabolism and is an important marker for insulin resistance and the future development of metabolic syndrome. The prevalence of GDM is rising across the globe and the benefits of broad screening for GDM has not yet been proven[9,16]. Considering that the prevalence of GDM varies greatly between populations, a better risk assessment model could prevent unnecessary oral GCTs for screening of GDM, especially in populations such as ours where the prevalence of GDM is exceedingly low. In our study, we saw that first trimester uric acid levels had a linear association with the development of GDM and IGT. First trimester serum uric acid levels along with other parameters such as sex-hormone binding globulin, high-sensitive C-reactive protein, and adiponectin could be incorporated into a risk model to assess the need for oral GCT later in pregnancy[17,18].

The strong points of our research are that our test sample was sufficiently powered and also demonstrated the diagnostic power of serum uric acid levels in a population of pregnant women with very low prevalence of GDM. Our study was retrospective in nature and had certain limitations that might have confounded our results, such as missing data in the study group and limited control over the study groups.

Our study adds to the body of literature about the association of serum uric acid levels with the development of GDM[10-12,20]. There is a conflicting study by Maged et al.[21] which suggested no association, but their study was insufficiently powered to demonstrate a lack of difference between groups. Our study is different from the previous study with a GDM prevalence of 1.7%, which is much lower than other studies, and it is the first to report the association and predictive value of the test in a low-prevalence population. Further studies in this field should investigate the predictive value of uric acid levels combined with other biochemical tests in an effort to create a screening model.

Conclusion

In summary, first trimester serum uric acid levels are associated with subsequent development of IGT and GDM. The test has good predictive value for the diagnosis of GDM and it can be used in a risk assessment model.

Table 1. Maternal demographic characteristics

	Group 1 (n=60)	Group 2 (n=403)	Group 3 (n=151)	p value*
Maternal age	32.81±4.93	30.67±5.32	28.53±5.39	<0.001
Gravida (median/min-max)	1 (1-5)	2 (1-6)	2 (1-4)	NS
Parity (median/min-max)	0 (0-3)	0 (0-4)	0 (0-3)	NS
Pre-pregnancy BMI	25.07±4.14	23.78±4.22	23.82±3.89	NS
Gestational age (uric acid level measured) (median/min-max)	7 (6-12)	8 (6-12)	8 (6-12)	NS

*p<0.05 statistically significant, BMI: Body mass index, NS: Non significant

Ethics

Ethics Committee Approval: The study were approved by the Ankara University of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.
Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Seda Şahin Aker, Concept: Seda Şahin Aker, Tuncay Yüce, Design: Erkan Kalafat, Data Collection or Processing: Seda Şahin Aker, Analysis or Interpretation: Tuncay Yüce, Feride Söylemez, Literature Search: Seda Şahin Aker, Murat Seval, Writing: Seda Şahin Aker, Erkan Kalafat.
Conflict of Interest: No conflict of interest was declared by the authors.
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First trimester fetal aortic Doppler for hemoglobinopathies

Hemoglobinopatiler için ilk trimesterde fetal aortik Doppler

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Abstract

Objective: To evaluate fetal aortic Doppler for the prenatal diagnosis of hemoglobinopathies in the first trimester of pregnancy.
Materials and Methods: Between January and November 2014, a total of 108 patients were enrolled in the study. The couples were carriers of either alpha/beta thalassemia, sickle cell disease or combined carriers of these and were admitted to Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology Prenatal Diagnosis Center. One hour before the chorionic villus sampling (CVS), patients were evaluated using fetal aortic Doppler. Pulsatility index, peak systolic velocity, and heart rate were noted.
Results: There were no statistically significant differences in Doppler indices between different groups of CVS results when compared with the healthy controls.
Conclusion: Fetal aortic Doppler investigation was found to be ineffective for the prenatal diagnosis of hemoglobinopathies.
Keywords: Hemoglobinopathies, thalassemia, sickle cell anemia, prenatal diagnosis, Doppler ultrasonography

Öz

Amaç: Gebeliğin ilk üç ayında hemoglobinopatilerin prenatal tanısı için fetal aortik Doppler incelemesinin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Ocak 2014 ve Kasım 2014 tarihleri arasında 108 hasta çalışmaya dahil edildi. Çiftler, Çukurova Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Bölümü Prenatal Tanı Merkezi'ne alfa/beta talasemi, orak hücre anemisi veya bunların kombine taşıyıcısı olup başvuran kişilerden oluşmakta idi. Koryonik villüs örneklemesinden (KVÖ) bir saat önce fetal aortik Doppler incelemesi yapıldı. Pulsatilite indeksi, tepe sistolik hız, kalp hızı not edildi.
Bulgular: Farklı KVÖ sonucu grupları sağlıklı kontrollerle karşılaştırıldıklarında Doppler indeksleri açısından istatistiksel olarak anlamlı fark bulunmamıştır.
Sonuç: Fetal aortik Doppler incelemesi hemoglobinopatilerin prenatal tanısında etkin bulunmamıştır.
Anahtar Kelimeler: Hemoglobinopatiler, talasemi, orak hücreli anemi, prenatal tanı, Doppler ultrasonografi

Introduction

A hemoglobinopathy is a genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule. There are various types of defects known today. They are single gene disorders and generally inherited in an autosomal recessive pattern. The most common are beta/alpha-thalassemia and sickle cell disease.

These diseases have different prevalences around the world. Beta-thalassemia is particularly prevalent among Mediterranean people, whereas alpha-thalassemia is common in sub-Saharan Africa, the Mediterranean Basin, the Middle East, South Asia, and Southeast Asia. Sickle cell disease is found most prevalently in sub-Saharan Africa, tribal regions of India, and the Middle-East[1]. Therefore, these hemoglobinopathies are important health problems for Turkey because of its geographic location.

The heterozygote carriers of these diseases show some degrees of anemia, whereas homozygote mutation carriers exhibit significant health problems. They require intermittent blood transfusions and have many complications such as increased incidence of infections, cholelithiasis (gallstones), cholecystitis, and growth problems during childhood. Carriers also face complications related to treatment such as excess iron load and related complications in thalassemias and other complications of frequent blood transfusions. Bone marrow transplantation is the only known cure for these diseases.

Prenatal diagnosis is very important for carrier couples. Early prenatal diagnosis may provide the chance of early termination for these couples during the first trimester. Current approaches for prenatal diagnosis include invasive [chorionic villus sampling (CVS), amniocentesis or cordocentesis] and non-invasive diagnosis with fetal DNA in maternal plasma[2-4].

Alterations in fetal blood parameters in hemoglobinopathies may cause changes in fetal Doppler parameters. Therefore,

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prenatal diagnosis using Doppler ultrasound may be another noninvasive method for the carriers. In this study we aimed to evaluate the indices of fetal aortic Doppler for the non-invasive diagnosis of hemoglobinopathies.

Materials and Methods

One hundred eight patients were enrolled in this prospective study between January 2014 and November 2014. All patients and their husbands were carriers of either alpha/beta thalassemia, sickle cell disease or combined carriers of these. They were admitted to Çukurova University Faculty of Medicine, Department of Obstetrics and Gynecology Prenatal Diagnosis Center for the prenatal diagnosis of hemoglobinopathies. They all had CVS during 11 and 14 weeks of their pregnancy. Gestational age was based on crown-lump length (CRL). Ethical Committee Approval was obtained from Çukurova University Clinical Research Ethics Committee (Report no: 28/14) and all subjects gave informed consent to participate in the study.

One hour before the CVS, patients were examined using a GE Voluson 730 Pro-ultrasound machine with a convex volumetric transabdominal (RAB 4-8 MHz) probe by a single experienced operator. In order to achieve an insonation angle of <30 degrees, fetuses were examined in a position where the fetal aorta was perpendicular to the probe (Figure 1). The magnification of the image was such that the fetus occupied the whole screen. The range gate was set at 2 mm. Pulsatility index, peak systolic velocity (PSV), and heart rate (HR) were noted.

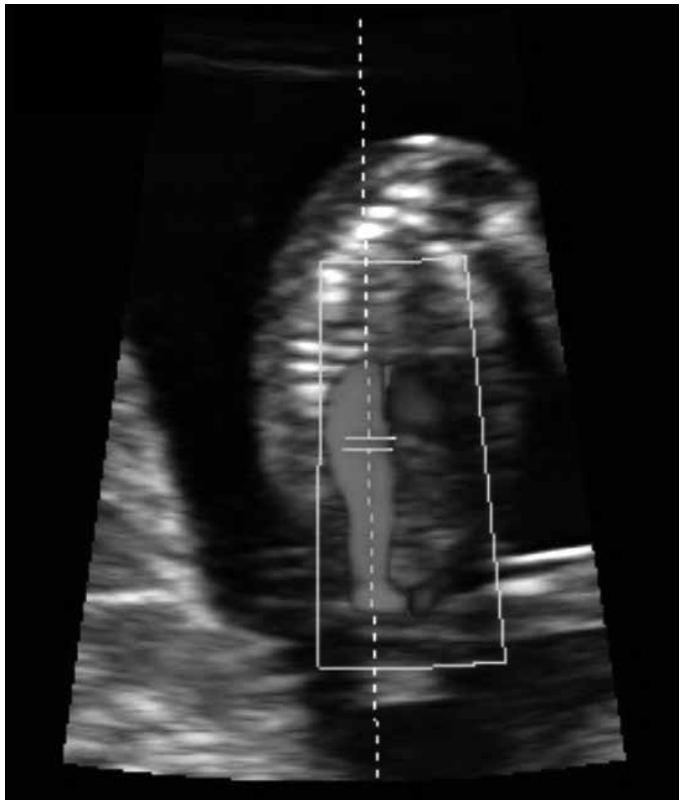

Figure 1. First trimester fetal aortic Doppler

After the ultrasound examination, CVS was performed. Fetal karyotype was also determined for all fetuses. Patients were divided into groups by the results of the hemoglobinopathy status. Therefore, the operator was blinded to the patients' groups.

Student's t-test was used for normally distributed data, and the Mann-Whitney U test was used to compare non-normally distributed data. The p value of <0.05 was considered as statistically significant. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 15 (SPSS, Inc., Chicago, IL).

Results

The mean age of the patients was 28.34±5.2 years (range, 17-40 years). The mean gestational age was 12±0.8 weeks (range, 11-14 weeks) and the mean CRL of the fetuses was 59.90±9.6 mm (range, 41-85 mm). There was no statistically significant differences between the groups regarding the mean age of the women and CRL of fetuses based on CVS results. Eight women were Rh negative but none were isoimmunized. Hemoglobinopathy status of the couples is given in Table 1. All CVS procedures were completed without complication. The karyotypes of all fetuses were normal. Hemoglobinopathy results and the Doppler indices of the fetuses are summarized in Table 2. There were no statistically significant differences in Doppler indices between the different groups of CVS results compared with the healthy (Hemoglobin AA) controls. Comparisons were made in subcategories of gestational weeks (11, 12, 13, and 14 weeks), because Doppler indices normally differ with advancing gestational age. Furthermore, there was no statistically significant difference between subgroups as for fetal HR (mean fetal HR=165.01±3.1 beat/min).

All patients whose fetuses were diagnosed as having sickle cell disease and beta-thalassemia major decided to terminate pregnancy, all were terminated without complication using sublingual misoprostol.

Discussion

Fetal arterial and venous Doppler evaluations are used effectively in different trimesters. For instance, fetal ductus venosus Doppler

Table 1. Hemoglobinopathy status of the couples

Hemoglobinopathy	Number of couples
BT carriers	21
SCD carriers	55
SCD*	1
AT carriers	2
Combined (AT + SCD) carriers	9
Combined (BT + SCD) carriers	20
Total	108

BT: Beta thalassemia, SCD: Sickle cell disease, AT: Alpha thalassemia, *: 1 patient had SCD (Hemoglobin SS) and her husband was SCD carrier.

Table 2. Chorionic villus sampling results and doppler indices of fetuses

CVS Result*	Gestational week	Number of fetuses	PI	PSV
HBAA (Healthy)	Total	22	2.92±0.35	41.87±8.56
	11	3	3.06±0.22	35.80±3.02
	12	12	3.05±0.38	39.73±6.87
	13	6	2.62±0.14	49.29±10.07
	14	1	2.7	41.3
HBAS	Total	34	8.85±15.03	34.98±14.85
	11	7	2.94±0.18	40.91±4.28
	12	21	8.06±13.10	34.38±14.6
	13	4	12.69±20.34	32.09±20.51
	14	2	30.16±39.40	26.27±34.65
HBSS	Total	21	9.16±13.31	33.70±16.64
	11	7	2.98±0.15	37.88±4.80
	12	8	19.34±17.68	22.07±21.05
	13	5	2.65±0.22	44.51±10.22
	14	1	2.40	43.44
BT Minor	Total	13	7.82±18.34	38.06±13.09
	11	3	24.76±38.19	24.95±18.94
	12	8	2.82±0.25	43.01±9.43
	13	1	2.51	36.45
	14	1	2.34	39.41
BT Major	Total	5	2.86±0.26	38.02±5.18
	11	2	3.08±0.11	38.72±3.66
	12	3	2.72±0.22	37.55±6.79
	13	0	-	-
	14	0	-	-
AT silent carrier	Total	4	14.03±22.64	31.83±19.79
	11	0	-	-
	12	2	2.89±0.15	41.69±3.07
	13	2	25.17±32.27	21.97±27.87
	14	0	-	-
S/BT	Total	3	2.76±0.57	43.09±8.57
	11	1	2.86	49.14
	12	1	3.27	33.28
	13	1	2.15	46.85
	14	0	-	-
S/AT	Total	4	10.87±16.01	32.53±20.46
	11	0	-	-
	12	2	2.79±0.16	40.93±4.30
	13	2	18.96±22.53	24.12±30.90
	14	0	-	-

Table 2 continue. Chorionic villus sampling results and doppler indices of fetuses

CVS Result*	Gestational week	Number of fetuses	PI	PSV
BT/AT	Total	2	2.59±0.49	46.47±7.30
	11	1	2.93	41.30
	12	0	-	-
	13	1	2.24	51.63
	14	0	-	-
Total		108		

* HBAA: Healthy fetuses, HBAS: Sickle cell carrier, BT: Beta thalassemia, HBSS: Sickle cell disease, AT: Alpha thalassemia, S/BT: Sickle cell/Beta thalassemia double heterozygote, S/AT: Sickle cell/Alpha thalassemia double heterozygote, BT/AT: Alpha thalassemia/Beta thalassemia double heterozygote, PI: Pulsatility index, PSV: Peak systolic velocity, CVS: Chorionic villus sampling

is found effective in screening for aneuploidies during the first trimester, whereas it is used for fetal well-being from the mid-trimester. The other most commonly used Doppler evaluations include umbilical, uterine, and middle cerebral arteries; each has a different purpose such as fetal well-being, preeclampsia screening, and detection of fetal anemia. Of these, middle cerebral artery PVS is used for the detection of fetal anemia but it is technically used after the 18th week of gestation[5].

Fetal anemia can be expected in hemoglobinopathies, especially in homozygous alpha-thallassemia[6]. During the first trimester, fetal aortic Doppler is found to have significantly different indices in fetuses with homozygous alpha-thalassemia[6]. In a recent study by Karateke et al.[7] ductus venosus Doppler was also found to have significantly different indices during the first trimester in fetuses with beta-thalassemia and sickle cell disease[7].

To the best of our knowledge, this is the first study to investigate fetal aortic Doppler for the prenatal diagnosis of beta-thalassemia and sickle cell disease during the first trimester. We chose fetal aorta for screening because it can be easily visualized during this period. We were able to visualize the fetal aortic blood flow in all patients with transabdominal probe. No transvaginal evaluation was needed.

We found no significant difference in fetuses with beta-thalassemia and sickle cell disease in arterial Doppler parameters during the first trimester compared with the healthy fetuses. Therefore, current fetal aortic Doppler was not found effective for the prenatal diagnosis of these hemoglobinopathies.

Fetal hemoglobin is a tetramer composed of two copies of each of two different peptide chains. The type of chains determine the type of hemoglobin produced. Adult hemoglobin consists of two beta and two alpha chains; however, during embryonic and fetal stages there are various types of hemoglobins. The first fetal hemoglobins are produced in the yolk sac and are called hemoglobin (Hb) Gower 1 (two zeta chains and two epsilon chains), Hb Gower 2 (two alpha chains and two epsilon chains), and Hb Portland (two zeta chains and two gamma chains)[8]. Erythropoiesis then moves to the liver, where Hb F (two alpha and two gamma chains) is produced. Finally, at around 11 weeks, normal Hb A is produced by fetal bone marrow and progressively increases in fetal blood as the fetus matures[9]. The final adult version of the alpha chain is produced exclusively by 6 weeks and there are no functional alternative versions thereafter[10]. If an alpha gene mutation or deletion occurs, there is no alternate alpha type chain that can substitute to form functional hemoglobin. In contrast, at least two versions of the beta chain (gamma and delta) remain in production throughout fetal life and beyond. In the case of a beta gene mutation or deletion, these two other versions of the beta chain often continue to be produced, which results in Hb A2 or Hb F and substitute for the abnormal or missing hemoglobin[10]. The reason that we found no hemodynamic change in fetuses with hemoglobinopathy was probably because of these substitute hemoglobins. In fetuses with hemoglobinopathy, substitute hemoglobins (Hb A2 and Hb F) conceal the disease during fetal life, but the disease manifests after birth. However, fetuses with alpha gene defect may even manifest during fetal life if all three or four genes are affected, because of the lack of substitute chains[11]. We had no fetuses with alpha thalassemia major (Hb Barts or Hb H disease) in our study.

The main limitation of our study is the small number of patients in some subgroups of patients, such as alpha-thalassemia and combined heterozygotes of hemoglobinopathies.

Conclusion

Currently, invasive prenatal diagnostic tests such as CVS, amniocentesis or cordocentesis are the most widely used invasive tests for hemoglobinopathies[12]. Non-invasive diagnostic tests using fetal DNA in maternal plasma shows promising results; however, they are expensive and not yet widely available around the world. Therefore, non-invasive prenatal diagnostic tests like Doppler ultrasound investigations should still be continued.

Ethics

Ethics Committee Approval: The study were approved by Çukurova University Clinical Researchs Ethical Committee, Informed Consent: Consent form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Cihan Çetin, Concept: Cihan Çetin, Selim Büyükkurt, Mete Sucu, Cansun Demir, Design: Cihan Çetin, Selim Büyükkurt, Mehmet Özsürmeli, Cansun Demir, Data

Collection or Processing: Cihan Çetin, Ebru Dündar Yenilmez, Analysis or Interpretation: Cihan Çetin, Mehmet Özsürmeli, Literature Search: Cihan Çetin, Ebru Dündar Yenilmez, Mehmet Özsürmeli, Mete Sucu, Writing: Cihan Çetin, Selim Büyükkurt, Mete Sucu.

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

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Prediction of adverse pregnancy outcomes using uterine artery Doppler imaging at 22-24 weeks of pregnancy: A North Indian experience

Gebeliğin 22.-24. haftasında, uterin arter Doppler görüntüleme ile advers gebelik sonlanımlarının belirlenmesi: Bir Kuzey Hindistan deneyimi

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Abstract

Objective: The aim of this study was to assess the predictive value of uterine artery Doppler imaging at 22-24 weeks of gestation for adverse pregnancy outcomes.

Materials and Methods: This was a prospective study in which uterine artery Doppler was performed at 22-24 weeks of gestation in 165 pregnant women with singleton pregnancies. A pulsatility index (PI) more than 1.45 or bilateral uterine notching was labeled as abnormal Doppler. The pregnancy outcome was assessed in terms of normal outcome, preeclampsia, fetal growth restriction (FGR), low birth weight, spontaneous preterm delivery, oligohydramnios, fetal loss or at least one adverse outcome.

Results: Out of 165 patients, 35 (21.2%) had abnormal second trimester uterine artery Doppler. In pregnancies that resulted in preeclampsia (PE), (n=21), FGR, (n=21), and low birth weight (n=39), the median uterine artery PI was higher (1.52, 1.41, and 1.27 respectively). In the presence of abnormal Doppler, the risk of PE [OR=10.7, 95% confidence interval (CI): (3.91-29.1); p<0.001], FGR [OR=4.34, 95% CI: (1.62-11.6); p=0.002], low birth weight [OR=6.39, 95% CI: (3.16-12.9); p<0.001] and the risk of at least one obstetric complication [OR=8.73, 95% CI: (3.5-21.3); p<0.001] was significantly high. The positive predictive value of abnormal uterine artery Doppler was highest for preeclampsia (36.84%) among all adverse pregnancy outcomes assessed.

Conclusion: Uterine artery Doppler ultrasonography at 22-24 weeks of gestation is a significant predictor of at least one adverse pregnancy outcome, with the highest prediction for preeclampsia.

Keywords: Uterine artery Doppler, preeclampsia, fetal growth restriction, low birth weight, adverse obstetric outcome

Öz

Amaç: Bu çalışmanın amacı, gestasyonun 22.-24. haftasında yapılan uterin arter Doppler görüntülemesinin advers gebelik sonlanımlarını belirleme değerini incelemektir.

Gereç ve Yöntemler: Bu çalışma, gestasyonun 22.-24. haftasında uterin arter Doppler yapılmış, tekil gebeliği olan 165 gebe kadında yürütülmüş bir prospektif çalışmadır. 1,45'in üzerindeki pulsatilite indeksi (Pİ) veya bilateral uterus çentiklenmesi anormal Doppler olarak kabul edilmiştir. Gebelik sonlanımları; normal sonlanım, preeklampsi, fetal büyüme geriliği (FBG), düşük doğum ağırlığı, spontan preterm doğum, oligohidramniyoz, fetal kayıp veya en az bir advers sonlanım bağlamında değerlendirilmiştir.

Bulgular: Yüz altmış beş hastanın 35'inde (%21,2) anormal ikinci trimester uterin arter Doppler mevcuttur. Preeklampsi (PE), (n=21), FBG, (n=21) ve düşük doğum ağırlığı (n=39) ile sonuçlanan gebeliklerde medyan uterin arter Pİ değeri daha yüksektir (sırası ile 1,52; 1,41 ve 1,27). Anormal Doppler varlığında PE [OR=10,7; %95 güven aralığı (GA): (3,91-29,1); p<0,0001], FBG [OR=4,34, %95 GA: (1,62-11,6); p=0,002], düşük doğum ağırlığı [OR=6,39, %95 GA: (3,16-12,9); p<0,001] ve en az bir obstetrikal komplikasyon olma [OR=8,73; %95 GA: (3,5-21,3); p<0,0001] riski anlamlı olarak yüksektir. Değerlendirilen tüm advers gebelik sonlanımları arasında, anormal uterin Doppler görüntüleme pozitif belirleyici değerinin en yüksek olduğu durum preeklampsidir (%36,84).

Sonuç: Gestasyonun 22.-24. haftasında yapılan uterin arter Doppler ultrasonografisi, en fazla preeklampsi belirlenmesi olmak üzere, en az bir advers gebelik sonlanımı için anlamlı bir belirleyicidir.

Anahtar Kelimeler: Uterin arter Doppler, preeklampsi, fetal büyüme geriliği, düşük doğum ağırlığı, advers obstetrik sonlanım

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Introduction

Defective trophoblastic invasion of the spiral arteries is associated with subsequent development of preeclampsia, fetal growth restriction (FGR), and other associated complications. In these pregnancies, the uteroplacental circulation remains in a state of high resistance, which causes generalized endothelial cell injury, compromises vascular integrity, and an atherosis-like process in small arteries that results in vascular occlusion, local ischemia, and necrosis. Under these conditions, the uteroplacental circulation remains in a state of high resistance and low flow. Doppler ultrasonography is a noninvasive tool for evaluating vascular resistance in these otherwise inaccessible maternal vessels. An increase in uterine artery impedance that predates the onset of clinical findings has been shown in preeclampsia and FGR; both disorders are associated with placental insufficiency[1].

Uterine artery Doppler is an attractive screening test in mid pregnancy. In theory, the test is capable of identifying pregnancies that are at premature delivery from a range of clinical complications that are attributable to chronic placental disease, namely intrauterine growth retardation, abruption and preeclampsia. In this study, uterine artery Doppler was done in 20-24 weeks of gestation, contrary to the first trimester and early second trimester in many studies done previously[1-3]. The timing was based on the hypothesis that it is unlikely that the fetal growth and well being are influenced by the transformation of uteroplacental vessels at 11 weeks to 13 weeks 6 days period of gestation because substantial changes in the placental development take place between first and second trimester that have practical importance for the development of the clinical screening program[4].

Uterine artery Doppler velocimetry performed before 16 weeks of gestation is unlikely to be a useful screening test for adverse pregnancy outcomes[5]. Moreover, it is irrational to employ this test for pre-eclampsia in the third trimester because the disease would already be established[6].

This study was performed to assess the role of second trimester uterine artery Doppler in predicting adverse pregnancy outcomes in pregnant women in Northern India.

Materials and Methods

This was a prospective study including 165 pregnant women with singleton pregnancies who attended the antenatal outpatient department of the Maulana Azad Medical College, Department of Obstetrics and Gynecology, New Delhi, India. Ethical committee approval was taken from institutional Ethics Committee, Maulana Azad Medical College, New Delhi (No: F.11/IEC/MAMC/10) and all subjects gave informed consent to participate in the study. Women with insulin-dependent diabetes mellitus, chronic hypertension, hypertension that developed before 24 weeks of pregnancy, cardiac disorders, renal disorders and antiphospholipid syndrome, and multiple gestations were excluded. A detailed informed consent was obtained from all participants after enrolment in the study. Uterine artery Doppler was performed in all these women at 22-24 weeks of gestation using a Philips HD7 ultrasound machine (number-Ci52100333). Uterine artery Doppler was performed by a single observer using a 225-Hz transabdominal probe. The proximal uterine arteries were located at their cross-over point with the external iliac arteries using color flow mapping. The angle of insonation was zero to ten degrees. Pulsed-wave Doppler was obtained for 3 similar consecutive waveforms on both sides. The pulsatility index (PI) was calculated [peak systolic flow minus end diastolic flow divided by mean flow: (A-B)/M]. Women with bilateral uterine artery notches or those with the mean PI of both the arteries ≥1.45 (PI greater than 95th percentile) were classified as abnormal second trimester uterine artery Doppler (Figure 1).

Follow-up continued till delivery and the pregnancy outcome was assessed. Pregnancy outcome was assessed in terms of normal outcome, preeclampsia, FGR, low birth weight (weight <2500 gm), spontaneous preterm delivery, oligohydramnios, fetal loss, or at least one adverse outcome.

The statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) statistical software (SPSS Inc., Chicago, IL, version 17.0 for Windows). Qualitative data was analyzed using chi-square or Fisher's exact test. Quantitative data between groups were analyzed using unpaired t-test and Mann-Whitney U test. A p value less than 0.05 was considered significant.

Results

The results are summarized in Table 1. Out of 165 patients, 35 (21.2%) had abnormal second trimester uterine artery Doppler, based on the criteria mentioned before (PI≥1.45 or

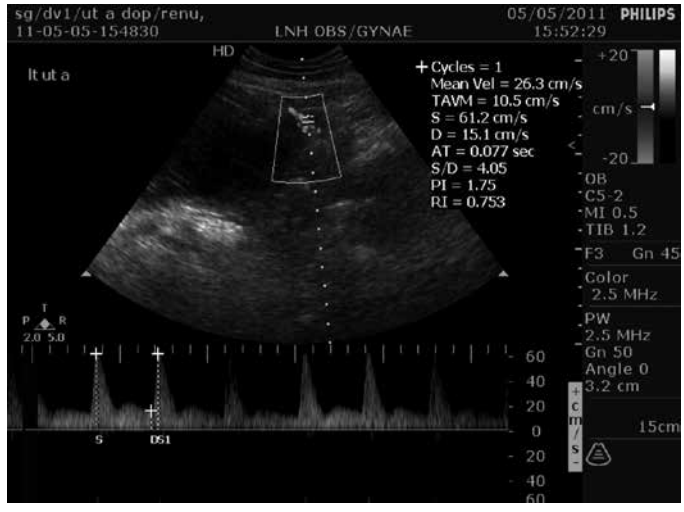

Figure 1. Second trimester uterine artery Doppler showing notching in left uterine artery and pulsatility index >1.45

Table 1. Normal and abnormal second-trimester uterine artery Doppler in adverse pregnancy outcomes and median uterine artery Doppler pulsatility index values

Pregnancy outcome	Normal uterine artery Doppler (n=133)	Abnormal uterine artery Doppler (n=32)	Median uterine artery PI levels	Mean PI ± SD	Odds ratio	95% confidence intervals	p value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Preeclampsia	7 (5.3%)	13 (40.6%)	1.52	1.37±0.39	10.69	3.92-29.18	<0.001	61.9	86.8	36.8	94.0
FGR	9 (6.8%)	10 (31.2%)	1.41	1.15±0.39	4.34	1.62-11.65	0.002	45.0	84.1	28.1	91.7
Low birth weight	12 (9.0%)	4 (12.5%)	1.03	1.13±0.36	6.39	3.16-12.93	<0.001	45.4	84.6	31.3	90.9
Preterm delivery	12 (9.0%)	10 (31.2%)	1.45	1.13±0.48	0.95	0.25-3.57	0.945	57.1	63.2	18.5	91.0
Oligohydramnios	5 (3.7%)	5 (14.3%)	1.27	1.17±0.35	3.92	1.12-13.78	0.024	45.4	82.3	15.6	95.5
At least one adverse outcome	29 (21.8%)	27 (84.3%)	1.39	1.19±0.40	8.74	3.59-21.27	<0.001	48.2	95.4	84.4	78.2

Odds ratio, 95% confidence intervals, p value, sensitivity, specificity, positive and negative predictive value of second trimester uterine artery Doppler in predicting various adverse pregnancy outcomes, FGR: Fetal growth restriction, PI: Pulsatility index, SD: Standard deviation

bilateral uterine artery notches or both). The results indicated that second-trimester uterine artery Doppler significantly determined adverse obstetric outcomes. The incidence of preeclampsia, FGR, small-for-gestational-age neonates, and oligohydramnios was significantly higher in pregnant women with abnormal second-trimester uterine artery Doppler. Second-trimester uterine artery Doppler had high negative predictive value for preeclampsia in this study. No significant association was found between abnormal second-trimester uterine artery Doppler and incidence of preterm delivery in patients in the study group.

One case each of placental abruption and intrauterine fetal death was noted in the study group with abnormal second-trimester uterine artery Doppler.

Abnormal second-trimester uterine artery Doppler was found to have a high predictive value for at least one adverse obstetric outcome. At least one adverse obstetric outcome was found in 84.3% patients with abnormal Doppler indices. Mean uterine artery PI was significantly raised in these patients with a high and statistically significant odds ratio.

The receiver operator characterstics curve for uterine artery Doppler in the detection of composite adverse outcomes in the whole study group revealed that second-trimester uterine artery Doppler was a significant predictor of adverse pregnancy outcomes. The area under the curve was calculated as 0.659 [95% confidence interval (CI): (0.562-0.756); p=0.001] (Figure 2).

Discussion

Although no single screening test in the prediction of adverse pregnancy outcomes, especially preeclampsia, has gained widespread adoption into clinical practice, uterine artery Doppler screening is the best performing of the available

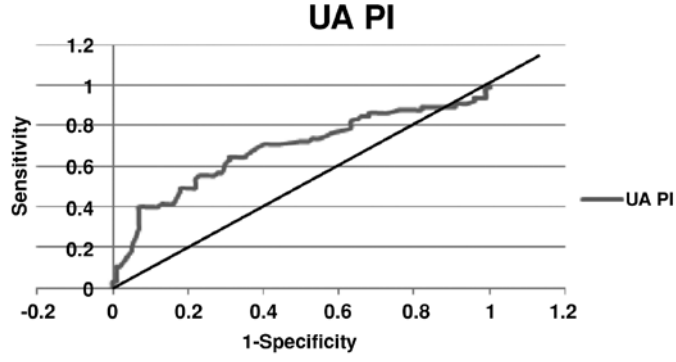

Figure 2. Receiver operator characterstics curve for uterine artery Doppler in depicting composite adverse pregnancy outcomes in the whole study group

The receiver operator characterstics curve for uterine artery Doppler in the whole study group revealed that the second trimester uterine artery Doppler is a significant predictor of adverse pregnancy outcomes. The area under the curve (AUC) is 0.659, 95% CI=0.562-0.756, p=0.001 PI: Pulsatility index, UA: Umbilical arterial

clinical tests to date, and is certainly the most widely studied. The association between increased uterine artery PI and subsequent development of preeclampsia is thought to be the consequence of impaired trophoblastic invasion of maternal spiral arteries[7]. Preeclampsia, which is genetically and immunologically governed, constitutes a disease of circulatory maladaptation to this defective trophoblastic invasion. In normal pregnancy, the luminal diameter of spiral arteries is greatly increased and vascular smooth muscle is replaced by trophoblast cells. In preeclampsia, the process is deficient with a consequent decrease in the vascular capacitance and increased resistance in the uteroplacental circulation, which is reflected as impedance of blood flow in the uterine artery[8]. FGR is also the result of impaired blood flow to the patient.

Abnormal second-trimester uterine artery Doppler indices have a high detection rate of pregnancies at risk of preeclampsia and FGR. In this study, 40.6% of patients with abnormal uterine artery Doppler developed preeclampsia.

FGR was found in 31.2% patients with abnormal uterine artery Doppler. A percentage (31.2%) of the patients also had small-for-gestational-age (SGA) babies.

Albaiges et al.[9] reported similar findings, with a higher detection rate for preeclampsia and SGA. Our study also reported that second-trimester uterine artery Doppler had high negative predictive value for preeclampsia. The sensitivity for preeclampsia was 61.9% and low birth weight was 45.4%, compared with 40% and 20%, respectively, in the study by Albaiges et al.[9] In a separate study by Harrington et al.[10] 81.2% of patients with abnormal uterine artery Doppler developed preeclampsia and 57.6% of these patients had low-birth-weight babies.

In our study, the positive predictive value of abnormal second-trimester uterine artery Doppler in determining at least one adverse outcome was 84.4%, which was significant. The likelihood ratio, sensitivity and specificity for each Doppler index and specific outcome varied among previous studies, but the predictive relationship for adverse outcomes has been consistently reported.

Our study found no association between deranged second–trimester uterine artery Doppler and spontaneous preterm delivery (delivery <37 weeks). This was in contradiction to the study by Fonseca et al.[11] in 2006, which demonstrated a significant association of mean bilateral increased uterine artery PI at 22-24 weeks and spontaneous early delivery. However, the study also concluded that uterine artery Doppler does not provide a significant improvement in the prediction of spontaneous early delivery provided by maternal characteristics and previous obstetric history.

Patients with both elevated PI and bilateral notching in the Doppler were found to be at the maximum risk of adverse pregnancy outcomes, especially preeclampsia and FGR. Albaiges et al.[9] reported the risk of preeclampsia as 40%, and 45% for FGR in these patients. These findings suggest that patients with elevated PI and notching at 23 weeks should be closely monitored for these adverse outcomes.

Our study concludes that second-trimester uterine artery Doppler has a potential role in predicting pregnancies at risk for complications such as preeclampsia, FGR, and small-for-gestational-age babies. This study also noted the high negative predictive value of uterine artery Doppler for adverse perinatal events among unselected women. It signifies that pregnancy outcome is likely to be normal if second-trimester uterine artery Doppler is normal. Although there is no effective intervention at present to alter outcomes in women with an abnormal Doppler study, the level of antenatal surveillance could be modified by the Doppler result[12]. However, uterine artery Doppler alone for predicting pregnancy outcomes has a limiting factor of cost effectiveness. A large number of Doppler studies have to be performed to identify a few high-risk women, which may not be cost effective. It is not logistically feasible in regard to availability and expertise of personnel to perform uterine artery Doppler in all pregnant women.

The strength of this study is its prospective nature, albeit with a small sample size and small number of patients with abnormal second-trimester uterine artery Doppler. Larger randomized controlled trials are required for second-trimester uterine artery Doppler for the extrapolation of the results to the whole population.

Ethics

Ethics Committee Approval: Ethical committee approval was taken from institutional Ethics Committee, Maulana Azad Medical College, New Delhi (No: F.11/IEC/MAMC/10) and all subjects gave informed consent to participate in the study, Informed Consent: It was taken. Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Deepti Verma, Sangeeta Gupta, Concept: Deepti Verma, Sangeeta Gupta, Design: Deepti Verma, Sangeeta Gupta, Data Collection or Processing: Deepti Verma, Analysis or Interpretation: Deepti Verma, Literature Search: Deepti Verma, Sangeeta Gupta, Writing: Deepti Verma, Sangeeta Gupta.

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A comparison of the effects of the most commonly used tocolytic agents on maternal and fetal blood flow

En sık kullanılan tokolitik ilaçların maternal ve fetal kan akımları üzerine olan etkilerinin karşılaştırılması

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Abstract

Objective: To investigate the effects of two tocolytics, nifedipine and magnesium sulfate, on Doppler indices in maternal and fetal vessels.

Materials and Methods: We recruited 100 pregnant women with preterm birth between 24-36 gestational weeks who were admitted to our tertiary center over a two-year period. Patients were allocated to nifedipine (n=49) and magnesium sulfate (n=51) groups and Doppler indices of umbilical, middle cerebral, uterine arteries, and ductus venosus were measured before and after tocolysis.

Results: There were no differences between the groups in terms of maternal age, gestational week, body mass indexes, cervical dilation, effacement at admission, birth weights and latency periods until birth. Nifedipine decreased resistance indexes in uterine arteries but magnesium sulfate increased resistance especially in the right uterine artery. Nifedipine significantly decreased systole to diastole and resistance index in the umbilical artery, magnesium sulfate increased systole to diastole and resistance index but this was not statistically significant. Nifedipine acted variably on resistance index and pulsatility index in the ductus venosus; however, magnesium sulfate increased resistance. Nifedipine decreased pulsatility index in the middle cerebral artery, contrary to magnesium sulfate with which it increased.

Conclusion: Nifedipine had favorable effects on maternal and fetal vessel indexes but magnesium sulfate increased resistance. Despite the proposed neuroprotective benefits of magnesium sulfate, nifedipine seems to be a better and safer tocolytic agent than magnesium sulfate due to its positive beneficial effects on maternal and fetal vessels.

Keywords: Doppler, magnesium sulfate, nifedipine, preterm delivery, adverse effect, tocolytic

Öz

Amaç: Nifedipin ve magnezyum sülfatın maternal ve fetal Doppler kan akımı indekslerine etkilerinin araştırılması.

Gereç ve Yöntemler: İki yıllık süreçte, 24-36. gebelik haftaları arasında preterm eylem tanısıyla perinatoloji kliniğine kabul edilen 100 hasta çalışmaya alındı. Hastalar aldıkları tokolitik tedaviye göre 2 gruba ayrıldı; nifedipin (n=49) ve magnezyum sülfat (n=51). Uterin, umblikal ve orta serebral arterler ve duktus venosus Doppler kan akımı indeksleri tedavi öncesinde ve sonrasında ölçüldü.

Bulgular: Maternal yaş, gebelik haftası, vücut kitle indeksi, servikal açıklık ve silinme, doğum ağırlıkları ve latensi dönemi açısından gruplar arasında fark yoktu. Nifedipin uterin arter rezistans indekslerini azaltırken, magnezyum sülfatın özellikle sağ uterin arterde olmak üzere arttırdığı saptandı. Nifedipin umblikal arterde sistol diastol oranını ve rezistans indeksi anlamlı şekilde azaltırken, magnezyum sülfat istatistiksel olarak anlamlı olmayacak şekilde arttırdı. Duktus venozus Doppler'inde nifedipinin rezistans indeksi ve pulsatilite indeksi üzerinde korele olmayan etkisi görülürken magnezyum sülfatın rezistansı arttırdığı saptandı. Orta serebral arterde nifedipin pulsatil indeksi azaltırken, magnezyum sülfatın arttırdığı saptandı.

Sonuç: Nifedipin genel olarak maternal ve fetal damarlarda olumlu etkilerde bulunurken magnezyumun rezistans indekslerini arttırdığı görüldü. Magnezyum sülfatın beyin koruyucu etkisinden bahsedilmesine rağmen nifedipinin yan etkilerinin daha az ve hafif olması, maternal ve fetal kan akımı üzerindeki etkilerinin olumlu olması nedeniyle nifedipin seçilecek tokolitik ajan olarak bir adım önde görünmektedir.

Anahtar Kelimeler: Doppler, magnezyum sülfat, nifedipin, preterm eylem, yan etki, tokolitik

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PRECIS: Nifedipine seems to be one step ahead as a tocolytic agent to be chosen due to its positive effects on maternal and fetal blood flow, comparing to magnesium sulfate.

Introduction

Despite the developments in medicine and technology, preterm birth (PTB) is still the leading cause of perinatal morbidity and mortality. Early-term complications such as respiratory distress syndrome, necrotizing enterocolitis, and intraventricular hemorrhage are frequent problems in preterm infants[1,2]. Frequent late-term complications include visual impairment, hearing loss, and cerebral palsy[3]. Although the rate of other obstetric complications has declined with the development of contemporary obstetric understanding, the treatment methods developed for preterm labor (PTL) have so far failed to reduce the number of PTBs. However, some benefits are gained through prolongation of pregnancy to enable corticosteroid administration to accelerate fetal lung maturation.

The least harmful drug for the mother and fetus, and the most effective tocolytic medication should be selected and administered without any delay after diagnosing the existence of any preterm pattern. Tocolytics can be used alone and/or in combination. Each tocolytic agent, in addition to their success in stopping the premature uterine contractions, presents maternal and fetal adverse effects. The use of these drugs requires close monitoring of patients during their administration[4]. Although the maternal and adverse effect profiles of these agents are established, the fetal adverse effect profile is relatively unknown. There are limited data on possible effects of tocolytics on fetal and feto-maternal circulation.

The aim of this study was to determine and compare the effects of nifedipine and magnesium sulfate ($MgSO_4$), the most commonly used tocolytic agents, on maternal and fetal blood flows.

Materials and Methods

This was a prospective study conducted on pregnant women with PTL who were administered nifedipine and $MgSO_4$ at a single perinatology clinic throughout a 2-year period (between October 2010 and September 2012). The study was subjected to Etlik Zübeyde Hanım Women's Health Training and Research Hospital of Local Ethics Committee, and written informed consent was obtained from the women who participated in the study. All authors and the study protocol complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects.

Gestational age was determined according to the last menstrual period and/or first trimester ultrasound biometry. PTL was diagnosed using uterine contractions (at least 4 in an hour) associated with cervical dilatation (≥2 cm) and/or effacement as per the American Congress of Obstetricians and Gynecologists recommendations. Our exclusion criteria were 1) cervical dilatation at ≥4 cm and/or cervical effacement ≥80%, 2) multiple gestations, 3) pregnancy complications including preeclampsia, ablatio placentae, intrauterine growth restriction, placenta previa, gestational diabetes 4) presence of preterm premature rupture of membranes, 5) a fetus with any malformation and/or aneuploidy, 6) prior PTL treatment, and 7) women who delivered within 48 hours of administration of tocolytic therapy. Two intramuscular injections of 12 mg betamethasone (Celestone Chronodose, Schering-Eczacıbaşı, Lüleburgaz) were administered 24 hours apart to all pregnant women at 24-34 gestational weeks.

$MgSO_4$ treatment protocol: 100 mL 5% dextrose 4-6 gr $MgSO_4$ ($MgSO_4$ 15% amp. Biosel, İstanbul) as intravenous (IV) loading dose administered over 20 minutes, followed by 2 gr/hour IV as a maintenance dosage, while continuing $MgSO_4$ infusion[4]. During the treatment, patients were monitored at hourly intervals for urination pattern, deep tendon reflexes, and respiratory rate per minute. The treatment was terminated after 6 hours following the cessation of contractions.

Nifedipine treatment protocol: Initial dose administered orally at 20 minutes intervals at 4 doses/10 mg, followed by 10 mg at 6-hour intervals as a maintenance dosage; the total daily dosage was 80 mg. The treatment was terminated in all patients at the end of 48 hours.

All sonographic evaluations were performed at a single perinatology department, using Voluson 730 ultrasound equipment (General Electric, Tiefenbach, Austria) fitted with a 2-7 MHz convex abdominal probe. All measurements were performed by the same operator to avoid any examiner-dependent bias. Doppler index measurements were performed prior to nifedipine and $MgSO_4$ administration and repeated after 48 hours of initiation.

The Doppler measurements of vessels were performed as described in the literature[5]. The absence of uterine contractions and fetal inactivity was a required condition to obtain a precise evaluation. A low-frequency (100 Hz) was used for visualizing of ductus venosus (DV) with color and pulsatile Doppler. The sample volume size was adjusted due to the diameter of the vessel. The insonation angle was established as close to zero degrees as possible and never exceeded more than 30 degrees. Color flow imaging was used to visualize the flow through the main uterine artery medial to the external iliac artery. Furthermore, the ascending branch was selected for pulsatility index (PI) calculation. The waveforms were assessed for the possible presence of notch and the uterine artery score was calculated. This technique was the same for both sides. For umbilical artery Doppler, the sampling site was located halfway between the fetal and placental end of the cord. The circle of Willis and the middle cerebral artery (MCA) were identified when a transverse view of the fetal brain was obtained. The measurements were taken in the middle part of the MCA.

Peak systolic velocity, and resistance index (RI) and PI were calculated for both vessels.

Data were analyzed using IBM SPSS 17.0 software (SPSS Inc., IBM, Chicago, IL, USA), and descriptive data are expressed as mean ± standard deviations and range. Continuous variable data obtained from the groups were analyzed using the Kolmogorov-Smirnov test against compliance with typical distribution. The average statistical analysis of the dependent and independent groups that distributed atypically was subjected to Wilcoxon and Mann-Whitney U tests, respectively. Chi-square and Fisher's exact tests were used for the comparison of groups. Student's t-test was to compare the averages of normally-distributed independent groups. P<0.05 was considered significant in all analyses.

Results

There was no significant difference in demographic characteristics as shown in Table 1. Before the treatment, the correlation between the RI and PI of the right and the left uterine arteries was strong in the $MgSO_4$ and nifedipine groups (r=0.961, p<0.001, r=0.974, p<0.001; and r=0.968, p<0.001, r=0.979, p<0.001, respectively). When uterine artery indexes were examined after the treatment, the correlation between the right and the left uterine RI and PI was strong in both groups (r=0.916, p<0.001, r=0.670, p<0.001; and r=0.876, p<0.001, r=0.796, p<0.001, respectively).

In the magnesium group, the PI of the right uterine and the RIs were significantly higher following the treatment (p=0.001 and p=0.018). However, no changes were observed in the left artery PI and RI (p=0.072 and p=0.901). No statistically significant difference was observed in umbilical artery PI, RI and systole to diastole (S/D) rates (p=0.358, p=0.556, and p=0.534, respectively). The PI in DV remained unchanged, but the RI revealed an increase (p=0.710 and p<0.001). In addition, an increase in the PI of the MCA was observed, whereas a decrease in the RI was seen (p=0.024 and p<0.001).

In the nifedipine group, a decrease in PI in right uterine artery was observed, and no change was determined in the RI (p=0.026 and p=0.054). However, a significant decrease was determined in the left uterine artery PI (p=0.001 and p=0.012). Although a significant decrease was detected in the umbilical artery RI and S/D rates, an increase was observed in the PI (p=0.021, p<0.001, and p=0.028, respectively). An increase in RI was detected while the PI was decreasing in the DV (p=0.006 and p=0.018). However, while the PI was decreasing, no change was detected in RI of MCA (p=0.001 and p=0.414).

When the pre- and post-treatment values were compared between the groups, all maternal and fetal Doppler measurements were significant except the pre- and post-treatment values of PI and pre-treatment RI of DV, as shown in Table 2.

Discussion

Nifedipine and $MgSO_4$ have direct effects on vascular structures due to their receptors, which are found throughout the body. Therefore, unexpected adverse effects might be seen in both uterine and fetal veins.

The use of nifedipine has been discredited owing its potential adverse effects in utero-placental perfusion and fetal oxygenation[6]. Nifedipine blocks calcium channels and reduces uterine vascular resistance by inhibiting the contraction of smooth muscles[7]. Although nifedipine does not seem to be a teratogenic agent, it is cause for concern because of its potential effects on the fetal circulation system[8]. Conflicting results were obtained from animal studies for nifedipine Ducsay et al.[9] and Harake et al.[10] determined that nifedipine led to decreased uterine blood flow on Rhesus monkey and sheep. In contrast, some studies conducted on other animals reported that nifedipine caused no changes in fetal and uterine blood flow[11,12]. In our study, it was determined that nifedipine had a positive effect on uterine blood flow.

Early studies reported no significant change prior and after nifedipine treatment in umbilical and fetal MCA Doppler indexes[13,14]. These findings suggest that nifedipine does not cause any adverse effects on the utero-placental and fetal vascular system. In our study, nifedipine reduced the resistance of uterine arteries, and $MgSO_4$ increased resistance, especially in the right uterine artery. This finding shows that nifedipine increases uterine blood flow rather than preventing it. The increased resistance of blood flow to the right uterine artery caused by magnesium stands out as a negative result. It was not possible to determine the effects of placentation on the RIs because the placenta lateralization

Table 1. Demographic characteristics of the groups

	$MgSO_4$ treatment (n=49)	Nifedipine treatment (n=51)	p
Age (y) (min-max)	24.0 (18.0-37.0)	22 (19.0-39.0)	0.320
Gestational age (min-max)	33.0 (28.3-35.4)	34.0 (30.5-35.2)	0.840
Gravida	2 (1-2)	1 (1-3)	0.810
Parity	0 (0-1)	0 (0-2)	0.710
Body mass index (kg/m^2)	26.0 (24.3-28.3)	28.0 (24.3-29.0)	0.078

Data are given as median (interquartile range within brackets), $MgSO_4$: Magnesium sulfate

(right-left) was not specified at the time we recorded the data.

In another study, nifedipine treatment showed no changes in umbilical artery PI values on Doppler measurements tested 24-48 hours following the treatment; however, uterine artery PI and MCA, PI values showed a significant decrease[15]. These changes were interpreted as a visible redistribution in the fetal vascular system and change in cerebral blood flow caused by nifedipine[15]. In our study, nifedipine reduced the S/D and RI in the umbilical artery. On the other hand, although magnesium increased these indexes, this change was not statistically significant. These findings showed the positive effects of nifedipine on fetal circulation and potential negative effects of magnesium. Furthermore, nifedipine reduced the MCA PI, but magnesium had the opposite effect. The effect of nifedipine was in favor of redistribution. Nifedipine increased the RI in the DV and reduced the PI.

A previous study showed an increase in MCA PI, a decrease on both sides of the uterine artery PI, and no significant changes in umbilical artery PI at 24-35 weeks before and after $MgSO_4$ treatment[16]. These findings are similar to the results of our study. The increase of PI in the MCA was attributed to the cerebral blood flow increase during PTL and the normalization of MCA PI following $MgSO_4$ treatment, thus the cessation of PTL. The decrease in uterine arteries PI has been explained as vasodilation in $MgSO_4$-treated uterine arteries and thus the increase in blood flow. The changes that occur in these vessels were described as physiological changes that occur with the removal of PTL stress on the fetus.

Wright et al.[17] administered $MgSO_4$ tocolysis to 16 pregnant women with PTL, and performed Doppler measurements before tocolysis, and 1 and 24 hours after treatment. The authors stated that there was no effect of $MgSO_4$ on umbilical artery Doppler flow, similar with our data. In another study, 15 pregnant women were administered $MgSO_4$ tocolysis and a significant decrease in MCA PI was identified[18]. Conversely, $MgSO_4$ led to an increase in MCA PI in our study.

A recent study showed increased resistance in the DV against flow in the 48th hour of $MgSO_4$ treatment administered to pregnant women, and reported that this effect became apparent after the 32nd week. Therefore, the authors suggested being cautious when planning to administer a $MgSO_4$ tocolytic on pregnant

Table 2. Doppler indexes in groups before treatment and at 48 hours post-treatment

	$MgSO_4$			Nifedipine			p values	
	B.T1	P.T1	p	B.T1	P.T1	p	B.T	P.T
Right UtA PI	0.65 (0.52-0.83)	0.70 (0.59-0.89)	0.001	0.86 (0.66-0.94)	0.85 (0.73-0.99)	0.026	<0.001	0.018
Right UtA RI	0.45 (0.38-0.53)	0.49 (0.41-0.53)	0.018	0.52 (0.44-0.60)	0.56 (0.50-0.61)	0.054	<0.001	0.006
Left UtA PI	0.77 (0.59-0.88)	0.74 (0.54-0.91)	0.072	1.06 (0.84-1.10)	0.91 (0.78-1.02)	0.001	<0.001	<0.001
Left UtA RI	0.51 (0.40-0.55)	0.48 (0.41-0.57)	0.901	0.59 (0.57-0.63)	0.55 (0.53-0.64)	0.012	<0.001	<0.001
UmbA PI	0.88 (0.80-1.03)	0.90 (0.79-1.12)	0.358	0.78 (0.75-0.95)	0.82 (0.69-0.97)	0.028	<0.001	<0.001
UmbA RI	0.59 (0.56-0.67)	0.62 (0.55-0.68)	0.566	0.55 (0.52-0.61)	0.52 (0.65-0.68)	0.021	<0.001	0.016
UmbA S/D	2.45 (2.32-2.93)	2.56 (2.28-3.1)	0.534	2.29 (2.07-2.53)	2.01 (1.72-2.29)	<0.001	<0.001	<0.001
DV PI	0.71 (0.53-0.97)	0.73 (0.55-1.0)	0.710	0.80 (0.71-0.92)	0.76 (0.52-1.1)	0.006	0.810	0.568
DV RI	0.32 (0.12-0.36)	0.40 (0.20-0.62)	<0.001	0.13 (0.05-0.23)	0.15 (0.12-0.23)	0.018	0.240	<0.001
MCA PI	1.68 (1.44-2.24)	1.74 (1.58-2.0)	0.024	2.48 (1.89-2.75)	2.42 (1.77-2.66)	0.001	<0.001	0.001
MCA RI	0.78 (0.71-0.86)	0.76 (0.60-0.80)	<0.001	1.00 (0.83-1.00)	1.00 (0.77-1.10)	0.414	<0.001	<0.001

Data are given as median (interquartile range within brackets), B.T: Before treatment, P.T: Post-treatment, UtA: Uterine arteries, PI: Pulsatility index, RI: Resistance index, MCA: Middle cerebral artery, DV: Ductus venosus, UmbA: Umbilical artery, S/D: Systole to diastole

women in 32nd weeks' gestation[18]. Parallel to the study above, we identified that $MgSO_4$ significantly increased DV RI values.

Conclusion

In pregnant women with PTL, $MgSO_4$ and nifedipine tocolysis may have some implications after 48 hours of the onset of treatment on maternal and fetal vascular blood flow patterns. Therefore, while choosing tocolytics, the adverse effect profile and the applicability of the drug should be considered. To interpret the effects of nifedipine and magnesium on fetal and maternal circulation based on the results obtained in this study, nifedipine reduces vascular resistance and thus increases uterine and fetal blood flows; the effect of magnesium on veins is less pronounced. Moreover (even though is the better definition than moreover in this sentence), $MgSO_4$ is said to have neuroprotective effects. Nifedipine seems to be ahead as a tocolytic agent and should be chosen owing to its positive effects on maternal and fetal blood flow, and its milder and lower adverse effect characteristics.

Ethics

Ethics Committee Approval: The study were approved by the Etlik Zübeyde Hanım Women's Health Training and Research Hospital of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Mahmut Güden, Bora Coşkun, Concept: Mahmut Güden, Bülent Yirci, Design: Mehmet Özgür Akkurt, Mahmut Güden, Data Collection or Processing: Mahmut Güden, Iltac Akkurt, And Yavuz, Analysis or Interpretation: Bülent Yirci, Necmi Ömer Kandemir, Literature Search: And Yavuz, Writing: Mahmut Güden, Mehmet Özgür Akkurt, Serenat Yalçın.
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Luteal phase support in intrauterine insemination cycles

İntrauterin inseminasyon sikluslarında luteal faz desteği

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Abstract

Intrauterine insemination (IUI) treatment aims to increase the rate of conception by increasing the chances that the maximum number of healthy sperm reach the site of fertilization. IUI with controlled ovarian stimulation is frequently used in assisted reproduction practice. Although widely used, the efficacy of luteal support in IUI remains controversial. In this article, we aimed to review what we know regarding luteal support in IUI cycles and to adjudicate about the clinical use and benefits of this treatment. Based on the study results available in the literature, it appears to be beneficial to supplement the luteal phase in gonadotropin-stimulated IUI cycles that yield more than one follicle.

Keywords: Intrauterine insemination, controlled ovarian stimulation, luteal phase support

Öz

İntrauterin inseminasyon (İUİ) tedavisi, maksimum sayıda sağlıklı spermin fertilizasyon bölgesine ulaşma şansını arttırarak gebelik oranını arttırmayı hedefler. Kontrollü ovaryan stimülasyon ile İUİ, yardımıyla üreme pratiği sıklıkla kullanılmaktadır. Yaygın kullanımına rağmen, İUİ'de luteal desteğin etkinliği halen tartışmalıdır. Bu makalede, İUİ sikluslarında luteal destek hakkında bildiklerimizi gözden geçirmeyi ve bu tedavinin klinik kullanımı ve faydaları hakkında hükme varmayı hedefledik. Literatürdeki çalışma sonuçlarına göre, birden fazla follikül elde edilen gonadotropinle indüklenmiş İUİ sikluslarında luteal fazing desteklenmesi faydalı gibi görünmektedir.

Anahtar Kelimeler: İntrauterin inseminasyon, kontrollü ovaryan stimülasyon, luteal faz desteği

PRECIS: Based on the study results in the literature, it appears to be beneficial to supplement the luteal phase in gonadotropin-stimulated intrauterine insemination cycles that yield more than one follicle.

Introduction

The aim, frequency, and success rate of intrauterine insemination

Intrauterine insemination (IUI) is an artificial insemination technique in which sperm is introduced directly into the uterine cavity irrespective of whether ovulation has been triggered. The purpose of this process is to increase the rate of conception by improving the sperm quality and concentration and by transporting the maximum number of healthy sperm to the site of fertilization. Controlled ovarian stimulation (COS), which involves a variety of ovulation induction (OI) agents, is used before the procedure in an effort to increase the number of oocytes, eliminate ovulation disorders that are not detected during regular examinations, and provide the optimal conditions for insemination.

Although the cost-effectiveness of IUI has been questioned, it is a widely-used fertility treatment that gives patients a good chance of pregnancy with relatively lower cost as compared with in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI); however, the difference between the two is significant. Within the context of the European IVF Monitoring Programme in 2004, data from 19 countries were analyzed. It was reported that IUI was performed in 98.388 of a total of 245.099 cycles, or 40.1% of infertile patients, (52.866 IVF cycles and 93.845 ICSI cycles), and 12.081 births (12% per cycle) were reported in these IUI cycles, with a multiple pregnancy rate of 13%. The same data indicated that the number of donor inseminations was relatively high (with a 2004 donor insemination cycle number of 17.592, a pregnancy rate per cycle of 17.7%, and a multiple pregnancy rate of 11.8%)[(1)]. The European Society of Human

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Reproduction and Embryology (ESHRE) also reported that IUI practices in Europe have increased over the years, and 162.843 IUI cycles with 29.235 donor cycles for IUI were carried out in 2009[2]. This outcome is not surprising given that IUI is employed in a broad range of indications including mild male infertility, unexplained infertility, and minimal or mild endometriosis. The effectiveness of IUI depends on a set of variables including the extent of IUI use, the indications for IUI, the optimal procedures for sperm preparation, insemination methods and timing, and the need to prevent premature luteinizing hormone (LH) surges and luteal deficiency in stimulated IUI cycles[3].

The main problem with IUI cycles with respect to the data in question is that pregnancy rates per cycle are lower as compared with IVF/ICSI, for which there are many possible reason. The leading one may be that the amount of research focused on IUI is not sufficient. A literature search on PubMed using the phrase IUI revealed that the first publication on this issue was published by in 1962 and also yielded 2.233 publications since October 2015. In contrast, when PubMed is searched using the phrase IVF, the resulting number of hits is 38.340. There are nearly 17 times as many IVF studies as there are IUI studies. Consequently, these data indicate that although frequently employed, IUI is not adequately studied. For this reason, we suppose that there are many unknowns to be analyzed. One of which is whether luteal phase support (LPS) is necessary for IUI cycles. When the current literature was investigated using the phrase LPS in IUI, PubMed yielded 42 publications since October 2015 on this subject. These publications include two meta-analyses in 2013 and very few controlled prospective studies to date. Moreover, the Cochrane database does not have any articles on the topic.

Known facts and observational study results

An LH surge is needed for follicle rupture and oocyte maturation. Similarly, progesterone (P) is needed to support early pregnancy and implantation. P is a product of the corpus luteum (CL) and provides secretory transformation of the endometrium during the luteal phase. In order for P receptors to diffuse sufficiently, a sufficient amount of estrogen (E) is needed[4]. In all COS cycles, multiple follicular development and the resultant supraphysiologic estradiol block the hypothalamic-pituitary axis with negative feedback, and hence the necessary LH oscillation for CL function does not occur. Consequently, this situation causes defective P and premature luteolysis[5].

Analysis of the available observational studies revealed that the luteal phase in gonadotropin-stimulated cycles is 20% shorter (an average luteal phase lasts 11 days), and that this shortness can be normalized by administering mid-luteal human chorionic gonadotropin (hCG), and that groups receiving LPS had significantly higher levels of mid-luteal P than those receiving no support[6-9]. However, mid-luteal P levels were <10 ng/mL in only one-third of the cycles that had a shortened luteal phase. This demonstrates that mid-luteal P levels are not directly related with luteal function[6]. Additionally, P plays a crucial role in LH secretion modulation, and long-term exposure to P or E+P can reduce LH secretion[10,11].

Exogen hCG administration can reduce LH concentrations through short and long feedback mechanisms[12]. However, more comprehensive studies demonstrated that hCG injection does not induce down-regulation of LH secretion during the luteal phase in normoovulatory women in spite of multifollicular development in unstimulated cycles and therefore supraphysiologic steroid concentration[13]. On the other hand, ovarian stimulation and the related multifollicular development are associated with abnormal endometrium progression during the early luteal period in almost 50% of cases[14].

Premature luteinizing hormone surge in intrauterine insemination cycles

An LH surge is triggered by the increasing levels of E secreted by the dominant follicle and is a requirement for follicular rupture and oocyte maturation. A premature LH surge is defined as a premature rise of LH (>10 IU/L) accompanied by a concomitant rise in P (>1 mg/L-3.2 nM/L)[15]. With the exception of natural cycles in older women, premature LH surge is a rarely-encountered phenomenon, although in stimulated IUI cycles its rate approaches 25-30%[15,16]. Premature LH surges may result in cycle cancellation or treatment failures. Gonadotropin-releasing hormone antagonists correct the premature LH surge but do not affect pregnancy rates, which indicates that it is not completely a case of early luteinization or premature LH surge[15].

Meta-analysis and prospective randomized controlled studies for luteal phase support in intrauterine insemination cycles

Despite some controversies in the published literature, luteal deficit and its causes are clarified thanks to progress in assisted reproduction technique. From this perspective, the usefulness of P supplementation during the luteal phase in IVF/ICSI cycles for reproductive outcomes is notably accepted in the Cochrane study. However, the timing of initiation, duration, route and amount of administration are still debated[17]. On the other hand, the effects of LPS in IUI cycles are unclear. To date, a very limited number of randomized controlled prospective studies has been conducted regarding the necessity of LPS treatment in stimulated IUI cycles. IUI cycles resemble IVF cycles in terms of multifollicular production and represent supraphysiologic steroid production. In this regard, it could be plausible to consider that the number of follicles in stimulated IUI cycles does matter and that LPS may be needed in multifollicular cases. The relevant literature investigation with regard to stimulated IUI cycles + LPS produced only two studies that addressed the

issue of follicle number. The first is the report of ESHRE Capri Workshop Group in 2009, which assessed six randomized controlled studies including 456 patients[3]. In this report, it was stressed that LPS treatment was not a major requisite for mild gonadotropin-stimulation IUI cycles (1-2 follicles). The second was the study by Seckin et al.[18] in 2014, which compared women who received a vaginal P gel with controls in an unexplained infertility population undergoing gonadotropin-stimulated IUI cycles. According to the results of this study, it was suggested that there were no difference between groups that received and did not receive LPS in terms of clinical pregnancy rates (CPR) and live birth rates (LBR) per cycle and per patient; however, in the IUI cycles that yielded a multifollicular response (>1 dominant follicle), LPS treatment statistically increased the CPR per patient compared with those with monofollicular response (28.2% vs. 11.4%, respectively, p=0.04). This result was the first evidence that LPS affected the success of the multifollicular resulst in gonadotropin-stimulated IUI cycles.

A literature search with regards to prospective randomized controlled trials (RCT) revealed the first study was conducted by Erdem et al.[19] in 2009. In this study, groups receiving and not receiving vaginal P gel for LPS two days after IUI in rFSH-stimulated IUI cycles in patients with unexplained infertility were compared. The results revealed that both CPR per cycle (21.1% and 12.7%, p=0.028, respectively) and per patient (39.4% and 23.8%, p=0.01, respectively) and LBR per cycle (17.4% and 9.3%, p=0.016, respectively), and per patient (35.8% and 18.1%, p=0.003, respectively) were significantly higher in the LPS-receiving group. Multiple pregnancy rates (MPR) did not significantly differ between the groups. This was the first prospective randomized controlled study to be conducted in this field that provided live birth rates. To date, there have been two meta-analyses,[20,21] including five RCTs[19,22-25] regarding this issue. Although all five RCTs included in the two meta-analyses provided biochemical pregnancy rate (BPR), CPR, MPR, and miscarriage rates (MR), the studies conducted by Erdem et al.[19], Ebrahimi et al.[23], and Maher[24], also assessed LBR. None of the studies indicated a difference between MPR and MR. Vaginal P was used for LPS in all these studies. Meta-analyses by Hill et al.[20] and Miralpeix et al.[21] revealed significantly higher CPRs [OR: 1.47; 95% CI: (1.15-1.98) and RR: 1.41; 95% CI: (1.14-1.76), respectively] and LBRs [OR: 2.11; 95% CI: (1.21-3.67) and RR: 1.94; 95% CI: (1.36-2.77), respectively] in LPS-administered groups as compared with LPS-free groups. In the subgroup analyses, LPS was reported to significantly increase BPR, CPR, and LBR only in the gonadotropin-stimulated IUI cycles compared with the LPS-free group. However, none of the

Table 1. Characteristics of the randomized controlled trials that assessed luteal phase support in women undergoing intrauterine insemination cycles

	Total no of patients	No of treatment / control cycles	Stimulation of ovulation	Luteal support, dosage, duration	Outcomes
Erdem et al.[19], 2009	214	223/204	rFSH	Vaginal progesterone gel, 90 mg/24 h, once daily, until 12th wk.	CPR, BPR, LBR, MPR, MR
Kyrou et al.[22], 2010	468	196/204	Clomiphene citrate	Vaginal progesterone suppositories, 200 mg/8 h, until 7th wk.	CPR, BPR, MPR, MR
Ebrahimi et al.[23], 2010	200	252/259	Clomiphene citrate + hMG	Vaginal progesterone suppositories, 400 mg/24 h, once daily, until 10th wk.	CPR, BPR, LBR, MPR, MR
Maher[24], 2011	71	132/126	rFSH	Vaginal progesterone gel, 90 mg/24 h, once daily, for 14 days.	CPR, BPR, LBR, MPR, MR
Agha-Hosseini et al.[25], 2012	300	148/142	Clomiphene citrate, Clomiphene citrate + hMG, Letrozole, Letrozole + hMG	Vaginal progesterone suppositories, 400 mg/24 h, once daily, until 12th wk.	CPR, BPR, MPR, MR
Hossein Rashidi et al.[26], 2014	253	NA	Clomiphene citrate + hMG	Vaginal progesterone suppositories, 400 mg/12 h, twice Daily, until 8th wk.	CPR, BPR, MR
Khosravi et al.[27], 2015*	150	NA	Clomiphene citrate + rFSH	Vaginal progesterone suppositories, 400 mg/24 h, once daily.	CPR, MR

rFSH: Recombinant follicular stimulating hormone, hMG: Human menopausal gonadotropin, CPR: Clinical pregnancy rate, BPR: Biochemical, pregnancy rate, LBR: Live birth rate, MPR: Multiple pregnancy rate, MR: Miscarriage rate, Wk: Week, NA: Not available, *Groups received oral dydrogesterone versus vaginal progesterone suppositories for luteal phase support.

Table 2. Reproductive outcomes of randomized controlled trials that assessed luteal phase support in women undergoing intrauterine insemination cycles

	BPR (%) (p)	CPR (%) (p)	LBR (%) (p)
Erdem et al.[19], 2009	25.1%-13.7% (0.002)	21.2%-12.7% (0.028)	17.4%-9.3% (0.016)
Kyroue et al.[22], 2010	-	7.3%-8.7% (NS)	-
Ebrahimi et al.[23], 2010	13.5%-11.2% (NS)	11.5%-10% (NS)	7.5%-5.7% (NS)
Maher[24], 2011	37.1%-20.6% (0.004)	29.5%-19.8% (0.07)	18.9%-5.5% (<0.001)
Agha-Hosseini et al.[25], 2012	29%-21.8% (NS)	24.3%-14.1% (0.02)	-
Hossein Rashidi et al.[26], 2014	30.8%-22.2% (NS)	15.8%-12.7%, (NS)	-
Khosravi et al.[27], 2015*	-	25.7%-29.7% (NS)	-

Data are presented as percentage rates in groups receiving luteal phase support versus no support. BPR: Biochemical pregnancy rate, CPR: Clinical pregnancy rate, LBR: Live birth rate, NS: Not significant, *Groups received oral dydrogesterone versus

studies present data regarding the number of follicles yielded. In addition, Miralpeix et al.[21] categorized the five studies assessed in their meta-analyses as either low risk of bias (if all the questions were answered yes) or high risk of bias (if at least one question was answered no) with respect to the responses to six parameters, which were "sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and other issues." Consequently, studies conducted by Kyrou et al.[22] and Ebrahimi et al.[23] were defined as having a high risk of bias, whereas the others had a low risk of bias.

After the above-mentioned meta-analyses, two more double-blinded prospective randomized controlled studies were published, both of which were conducted in Iran. The first was a placebo-controlled study in 2014 by Hossein Rashidi et al.[26] which involved vaginal P for LPS administered until the 8th gestational week. COS was achieved with clomiphene citrate (100 mg/d) and human menopausal gonadotropin (75 IU/d) in the study groups. No statistically significant difference was detected between the groups receiving LPS (n=127) and placebo (n=126) in terms of BPR (30.8-22.2%, p=0.15, respectively), CPR (15.8-12.7%, p=0.30, respectively), MR (10-18.8%, p=0.45, respectively) and the ongoing pregnancy rate (OPR) (46.2-46.4%, p=0.98, respectively). The second study was conducted in 2015 by Khosravi et al.[27] in which vaginal P (400 mg) and oral dydrogesterone (20 mg) groups were compared for LPS with regards to CPR, MR, and mid-luteal P values (seven days after IUI). Although there was no statistical difference, the mid-luteal P level in the oral dydrogesterone arm was higher and, accordingly, MR was lower. Consequently, this study demonstrated that oral dydrogesterone could also be effective for LPS as vaginal P in COS+IUI cycles. The characteristics and reproductive outcomes of the RCTs available in the literature are demonstrated in detail in Tables 1 and 2.

Conclusion

In conclusion, COS+IUI cycles are similar to IVF/ICSI cycles in terms of multifollicular development. Only a limited number of randomized controlled trials are available about LPS in IUI cycles. Based on the results of these studies, it appears to be beneficial to support the luteal phase in gonadotropin-stimulated IUI cycles that yield more than one follicle. There is still a need for further randomized controlled trials to evaluate the effectiveness of LPS treatment in stimulated IUI cycles.

Ethics

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: İsmet Gün, Özkan Özdamar, Design: İsmet Gün, Özkan Özdamar, Data Collection or Processing: İsmet Gün, Özkan Özdamar, Ali Yılmaz, Analysis or Interpretation: İsmet Gün, Özkan Özdamar, Ali Yılmaz, Literature Search: İsmet Gün, Özkan Özdamar, Ali Yılmaz, Writing: İsmet Gün, Özkan Özdamar.

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Live birth after transfer of a tripronuclear embryo: An intracytoplasmic sperm injection as a combination of microarray and time-lapse technology

Tripronükleer bir embriyonun transferiyle elde edilen canlı doğum: Mikroarray ve time lapse teknolojisinin kombinasyonu olan bir intra-sitoplazmik sperm enjeksiyonu olgusu

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Abstract

Although around 1-4% of human zygotes have been found to be tripronuclear, there is little information about the subsequent development and chromosomal composition of embryos that derive from these zygotes. Herein, we report a pregnancy and subsequent delivery of a healthy newborn after the transfer of a blastocyst that developed from a tripronuclear zygote that had a euploid microarray result.
Keywords: Tripronuclear, microarray, euploid

Öz

İnsan zigotlarının %1-4'ünün tripronükleer olduğu gözlenmiş olsada, bu zigotlardan elde edilen embriyoların gelişimleri ve kromozomal içeriğine dair oldukça az bilgi vardır. Bu olgu sunumunda, tripronükleer bir zigottan gelişen fakat mikroarray sonucuna göre öploid olduğu görülen bir embriyonun transferiyle elde edilen bir gebelik ve canlı doğumu sunuyoruz.
Anahtar Kelimeler: Tripronükleer, mikroarray, öploid

Introduction

During assisted reproduction treatments, successful fertilization is determined through the presence of two visible pronuclei in the cytoplasm of the oocyte on the first day. Also, two common abnormal fertilization patterns have been reported in clinical practice. These are 1 pronucleus (PN) and 3PN zygotes. It is known that triploid human embryos are associated with spontaneous abortions after implantation[1]. For this reason, it is believed that it is crucial to recognize 3PN formation in the early period and not to transfer the embryos that develop from 3PN oocytes during in vitro fertilization (IVF) treatment.

Aneuploidies are commonly observed in early human embryos[2,3]. Preimplantation genetic diagnosis (PGD) has been applied as a method in assisted reproductive technology to select genetically normal embryos for transfer that have the highest implantation potential. It is generally recommended for the people who are referred to IVF clinics with an etiology such as repeated implantation failure, recurrent pregnancy loss, and advanced age. After the introduction of microarrays in assisted reproduction practice, physicians and embryologists began to obtain more detailed information about the whole genomic constitution of the embryos.

In this case report, we aimed to present a healthy delivery by a woman whose transferred embryo was developed from a 3PN zygote until the blastocyst stage and yielded a euploid result with microarray analysis.

Case Report

A woman aged 36 years was admitted to our IVF clinic with the etiology of diminished ovarian reserve. She had experienced two previous pregnancies; however, both pregnancies were terminated with missed abortions at around 13 weeks due to trisomy XXY. All sperm parameters were within normal range based on World Health Organization (WHO) 2010 criteria. The

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patient was offered genetic testing for a possible aneuploidy along with intrcytoplasmic sperm injection (ICSI) treatment due to the previous abortions, which she accepted. She underwent an antagonist protocol with the administration of 450 IU recombinant follicle stimulating hormone (Gonal F, Merck Serono, Italy) for 7 days. The antagonist (Cetrotide, 0.25 mg) was administered after the observation of a leading follicle of 15 mm. Human choriogonadotrophin (hCG) was administered when more than three ≥17 mm oocytes were seen. Three oocytes were collected 36 hours after hCG (Ovitrelle, Merck Serono, Italy) administration. All oocytes were at metaphase II (MII) stage and they all underwent ICSI. ICSI is the preferred method of fertilization in our clinic irrespective of semen parameters due to its higher fertilization rates. A fertilization check was performed 17 hours after microinjection. Two oocytes were normally fertilized (2PN) and one was abnormally fertilized (3PN). All zygotes were cultured in *ISM1* medium (Medicult, Origio, Denmark) until the end of day 2 in Blast Assist medium (Medicult, Origio, Denmark) until the blastocyst stage in a closed incubation system including time-lapse photography (Embryoscope, Unisense Fertilitech, Denmark) under the culture conditions of 37 °C, 6.0% CO_2 and 7.0% O_2. We use lower oxygen concentrations for embryo culture in our laboratory becayse there are higher blastocyst development and clinical success rates reported in the literatüre[4]. All three embryos were good quality at day 3 (including ≥6 even blastomeres and no fragmentation). One single blastomere was biopsied from all day 3 embryos and sent for genetic analysis. All 24 chromosomes were screened using a comparative genomic hybridization array following genome amplification in single cell. The genetic results arrived in the morning of day 5, and that only embryo that developed was from 3PN zygote and it was found to be normal (euploid) based on the report. The patient was informed about all possible consequences of transferring an embryo developed from a 3PN zygote and she gave consent for its transfer. The embryo cleaved properly based on the expected timing intervals of Meseguer's hierarchical model, and reached hatching blastocyst stage on day 5[5]. An image of the 3PN embryo that was taken from the embryoscope screen is shown in Figure 1. One of the other embryos (2PNs) also developed to blastocyst stage and the other arrested at the compaction stage (Figure 2). The microarray results of the embryos are given in Table 1. A hatching blastocyst was transferred to the patient by the common decision of the geneticist, embryologist, physician, and the patient. The patient was considered as pregnant after obtaining a βhCG result of 652 mIU/mL after 10 days following embryo transfer and the quantitative hCG result doubled after two days. The patient delivered a healthy baby girl after 39 weeks by cesarian section. The weight of the baby was 3410 gr and her height was 51 cm at birth.

Discussion

Although around 1-4% of human zygotes were found to be tripronuclear, there is little information about subsequent development and chromosomal composition of embryos that derive from these zygotes[6]. The reason is that these tripronuclear zygotes are generally discarded in routine IVF practice because they are believed to be genetically abnormal. Despite this general belief, it was shown in some studies that some of these tripronuclear human oocytes do not always develop into triploid embryos. Kola et al.[6] showed that a large majority of tripronuclear human oocytes do not develop into triploid embryos, and that the first cleavage is the critical stage that affects the subsequent chromosomal constitution of tripronuclear human oocytes. In some other studies that evaluated the chromosomal composition of 3PN zygotes and embryos developed from these zygotes, various amounts of them were found to be diploid[7]. These data are one of the reasons why we performed blastomere biopsy also to the 3PN embryo in this case. The second reason was the few embryos with which we had to work.

3PN formation has been acounted for by two main features: Dispermic fertilization and nonextrusion of the second polar body[3]. After the introduction of ICSI to the assisted reproduction armoury, it has become the preferred method of many IVF clinics because of its high fertilization and comparable pregnancy rates for male factor infertility problems and other etiologies. We could rule out the possibility of dispermic fertilization in this case because it is the only technique routinely used in our IVF clinic. Therefore, nonextrusion of the second polar body was the only possible explanation for this 3PN formation.

In the review by Munne and Cohen[3] on chromosome abnormalities in human embryos, the different percentages

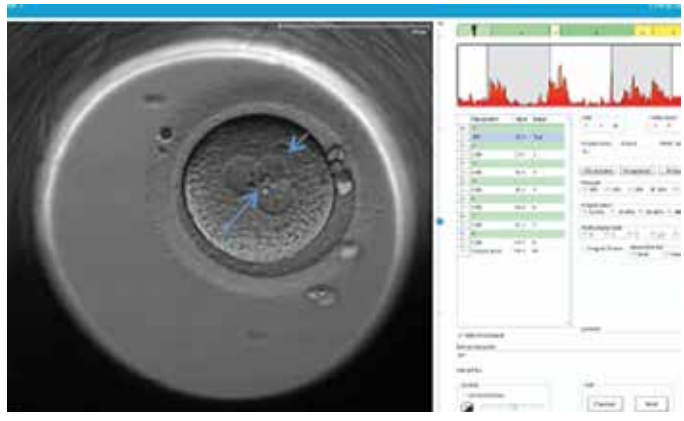

Figure 1. The image of 3PN embryo on embryoscope screen at day 1. Blue arrows indicate the exact location of third pronucleus

Table 1. Microarray outcome of the biopsied blastomeres

Embryo#	Microarray result	Interpretation	Final disposition
1	45,XX, -16	Aneuploid	Discarded
2	46,XX	Euploid	Transfer
3	47,XY, +6	Aneuploid	Discarded

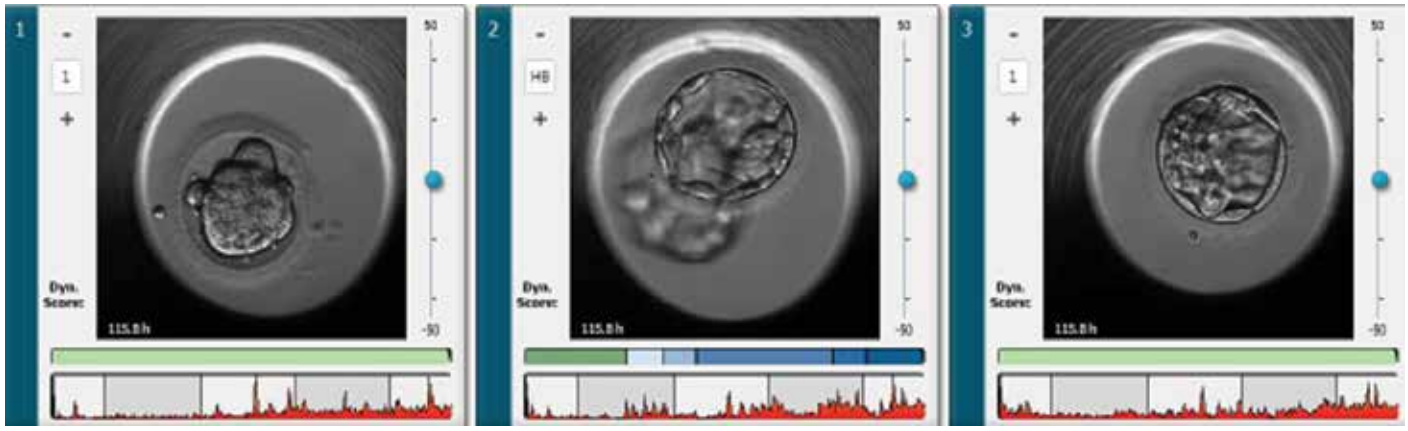

Figure 2. All three embryos at day 5, 1) 2PN (45,XX, -16) 2) 3PN (46,XX) (euploid) 3) 47,XY, +6

of diploid 3PN embryos observed in different centers were attributed to differences in recording vacuoles and pronuclei. In our study, our embryo was cultured in a time-lapse incubator (Embryoscope, Unisense Fertilitech, Aarhus, Denmark) and we had the opportunity to check the pronuclear status of the embryo repeatedly by four experienced embryologists; it was determined that the third formation included pronucleolar bodies inside. We can also suggest that it would be better to put oocytes into a time-lapse system directly after ICSI such that the details of 3PN formations can be seen. The reason for this suggestion is based on our experiences with time lapse, which shows that 2PN zygotes may sometimes later appear as 3PN following fragmentation of one of its pronuclei.

In previous studies, 25-62% of human day 3 preimplantation embryos were found chromosomally mosaic and the average percentage of aneuploid blastomeres in the mosaic embryo ranges between 40-52%[8]. The genetic results obtained from a single blastomere may not be an exact representation of the whole embryo because of the high ratio of mosaicism in preimplantation day 3 embryos. Thus, two approaches may improve PGD accuracy. The first is the biopsy of two blastomeres on day 3, and the second is biopsy at a more advanced development stage such as blastocyst. In an unselected population of human blastocysts, it could be expected that nearly 40% could be chromosomally abnormal[9]. Thus, it can be concluded that the development of an embryo up to the blastocyst stage does not mean that the embryo is genetically normal. Accordingly, the main limitation in our study seems to be the timing of biopsy. As such, we could not determine the final chromosomal constitution of the two blastocysts we obtained during the treatment. The reason why we performed day 3 biopsy was that the patient was living abroad and did not want to come back for a thaw cycle. In addition, the reason for performing only a single blastomere biopsy instead of two was the double financial burden of the microarray, which was not approved by the patient.

Conclusion

As far we know, this is the first study to show time-lapse imaging of a 3PN embryo that was found to be euploid using microarray-based genetic testing, which reulted with a healthy newborn. We conclude that it would be useful to perform aneuploidy testing also for abnormally-fertilized oocytes during IVF treatment, especially for patients with a low number of embryos. However, further studies on this subject should focus on a day-5 biopsy to minimize the confounding effect of mosaicism.

Ethics

Informed Consent: Written informed consent was obtained from patients who participated in this study.
Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: Alev Özay, Elif Ergin, Design: Ender Yalçınkaya, Data Collection or Processing: Alev Özay, Zeynep Öztel, Ender Yalçınkaya, Analysis or Interpretation: Elif Ergin, Hakan Özörnek, Literature Search: Ender Yalçınkaya, Writing: Ender Yalçınkaya.
Conflict of Interest: No conflict of interest was declared by the authors.
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Case Report / Olgu Sunumu

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Amphricrine carcinoma of the cervix-adeno neuroendocrine tumor: A case report

Servikal ampikrin adeno-nöroendokrin karsinom: Olgu sunumu

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Abstract

Adenoneuroendocrine carcinoma is a very rare form of cervical carcinoma that includes both endocrine and exocrine components. In general terms, these carcinomas progress aggressively and show early metastases due to the neuroendocrine component. The most important criteria related to prognosis is the stage of the disease. Without clearly determined therapeutic protocols this carcinoma is generally seen at earlier ages and causes high mortality. Many radiotherapy and multidrug chemotherapy protocols are used after surgical intervention. Detection of the neuroendocrine component of cervical tumors is achieved through immunohistochemical staining. Herein, we present a woman aged 50 years who was admitted to the hospital with abdominal pain and postmenopausal vaginal bleeding whose examination revealed a cervical tumor. A pathologic examination after surgery resulted as "adenocarcinoma and large cell neuroendocrine carcinoma." Afterwards, a combined chemotherapy regimen (cisplatin + etoposid) was administered to the patient and 6 months of progress is evaluated in this report.

Keywords: Cervical carcinoma, amphicrine tumor, neuroendochrine tumor

Öz

Adenonöroendokrin karsinomalar servikal karsinomların çok nadir rastlanan bir türü olup hem endokrin hem ekzokrin komponent içermektedirler. Genel anlamda bu tümörler içerdikleri nöroendokrin komponent nedeniyle erken metastazlarla agresif bir seyir gösterirler. Prognoza ilişkin en önemli kriter olarak hastalığın evresi gösterilmektedir. Tam olarak belirlenmiş bir tedavi protokolü olmayan bu karsinomalar, erken yaşlarda izlenmekte ve yüksek mortaliteyle seyretmektedirler. Cerrahi müdahale takiben birçok radyoterapi ve multi-ilaç kemoterapi protokolü kullanılabilmektedir. Hastalığın nöroendokrin komponentinin belirlenmesi ise tanı için önemli olup immünohistokimya aracılığıyla yapılmaktadır. Bu olgu sunumunda 50 yaşında karın ağrısı ve postmenopozal kanama şikayetleriyle başvuran hastanın servikal tümörü saptanmış olup, cerrahi takiben patolojik tanısı "adenokarsinoma ve büyük hücreli nöroendokrin karsinoma"olarak belirlenmiştir. Takiben sisplatin + etoposid şeklinde kombine kemoterapi alan hastanın 6 aylık takibi sunulmuştur.

Anahtar Kelimeler: Servikal karsinom, ampikrin tümör, nöroendokrin tümör

Introduction

Adenoneuroendocrine carcinoma includes both endocrine and exocrine components. The histopathologic classification of these tumors was defined by Lewin[1] as listed below:

1. Mixed tumors (both tissues exist together),
2. Both tissues exist completely separately,
3. Tumors that exist of amphricrine cells.

These tumors may occur in the sinonasal cavity, larynx, lungs, gastrointestinal system, and uterine cervix. Neuroendocrine tumors account for one percent of all cervical carcinomas and are classified into three groups histopathologically; (i) carcinoid tumor, (ii) atypical carcinoid tumor, and (iii) high grade neuroendocrine carcinoma; small cell or large cell types[2].

Early nodal and distant metastases are typical for these tumors. Between all pathologic groups, small-cell carcinomas show the worst prognosis and have similar survival to small cell carcinomas of the lungs. Compared with other cervical carcinoma histopathologies, distant metastases occur frequently and there are shorter survival periods in neuroendocrine carcinoma[3]. Due to the lack of data, there is no consensus on the definitive treatment of the disease. Surgery alone is not adequate because of the frequent metastases and aggressive behavior. Following surgery, adjuvant chemotherapy and

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radiotherapy present better survivals in patients with non-small cell disease[4].

The definitive diagnosis of neuroendocrine tumors can be achieved through immunohistochemical staining. Epithelial membrane antigen (EMA), cytokeratin 7 (CK7), cytokeratin 19 (CK19), chromogranin, and synaptophysin staining are diagnostic. Polymerase chain reaction (PCR) study of these viruses is also crucial because of the strong relationship with human papillomavirus (HPV) type 16 and 18.

Cervical adenoneuroendocrine carcinoma can be very similar to adenocarcinoma of the cervix. The differential diagnosis is very important because of the aggressive behavior of these tumors and the need for a more aggressive treatment than with other cervix carcinomas.

Case Report

A woman aged 50 years who had been post-menopausal for 8 years was admitted to the hospital with abdominal pain and ongoing postmenopausal vaginal bleeding, which had lasted for ten days. A gynecologic examination revealed a 2x1.5-cm polypoid lesion that originated from the endocervical canal. Also, the whole vagina seemed fragile and the cervix seemed to fuse with the vagina, and the vaginal fornix appeared to be erased. A 3x4-cm cervical tumor was diagnosed. The left parametrium was shortened and there was suspected involvement seen in the right parametrium. Abdominal ultrasonography showed normal uterine fundus and corpus with an endometrial thickness of 15 mm. The right adnexal area included a solid lesion that was 89x50 mm in diameter with cystic areas, and a 72x74 mm complicated cystic mass was seen in the left adnexal area, which was adjacent to the other mass. Abdominopelvic tomography revealed increased dimensions of uterus and cervix with heterogeneous structure. There was minimal fluid in the endometrial cavity. The right adnexal area included a mass of 80 mm that could not be distinguished from the uterus and showed retention of contrast matter. This mass was regular-edged and hypo-dense. The left adnexal area included a 90 mm complicated cyst. The peritoneal surfaces had implants, the largest of which was 53 mm. Massive ascites was present. The right lobe of the liver had 15 and 20 mm lesions that retained contrast matter.

The tumor marker results were as follows; CA-125: 1870 IU/mL, CA 19-9: 234 IU/mL, CA 15-3: 36.7 IU/mL, and carcinoembryonic antigen: 1.98 IU/mL. A cervical and endometrial biopsy were performed and both results were reported as adenocarcinoma. Accordingly, a laparotomy with a midline incision was performed. Nearly 7000 mL of serous ascites was explored. Adnexal masses were explored as they had been imaged on ultrasonography and tomography. Tumoral implants were seen on the peritoneum of the douglas pouch, hepatorenal area and paracolic area, serosa of rectosigmoid colon, and mesothelium of the bowel. The pancreas and spleen were normal and omental cake was observed. Right unilateral salpingo-oophorectomy was performed and frozen sections were reported as "poorly differentiated adenocarcinoma of the cervix." As a result, surgery was expanded to type 2 radical hysterectomy, left unilateral salpingo-oophorectomy, resection of the sigmoid colon, right hepatorenal peritoneum, and tumoral implants both on the liver and mesothelium of bowel and appendectomy. Right diaphragm stripping was performed due to the tumoral implants of the right diaphragm. Bilateral pelvic and para-aortic complete lymphadenectomy was performed until the level of left renal vein. The operation was completed with optimal cytoreduction, which resulted with the existence only of residual tumor with a greatest diameter of 5 mm.

Pathology

Macroscopic: The first examined tissue was frozen sections of right salpingo-oophorectomy. The tumoral lesion was a solid structure with minimal cystic structures within, with white-yellowish color and necrosis. Ovarian and tubal tissues were had tumoral nodules on their surface.

Microscopic: After fixation with 10% formalin and staining with Hematoxylin and Eosin on paraffin sections, two different malignant tissue was remarkable on cervix. The first tumor was characterized with pseudo-stratified prismatic epithelium with hyperchromatic nuclei and different diameters of atypical glands with mitosis. These atypical glands were at a random distribution both at mucinous stroma and desmoplastic stroma.

The second tumor comprised large cells with atypical nuclei and significant nucleoli. There were a high number of mitotic cells forming trabecular, cordon-like structures with a palisade sequence. Necrosis and lymphovascular invasion were also noticed (Figure 1).

More than 50% percent of cervical muscle tissue was invaded with tumoral tissue. The vagina, both parametrium, uterine

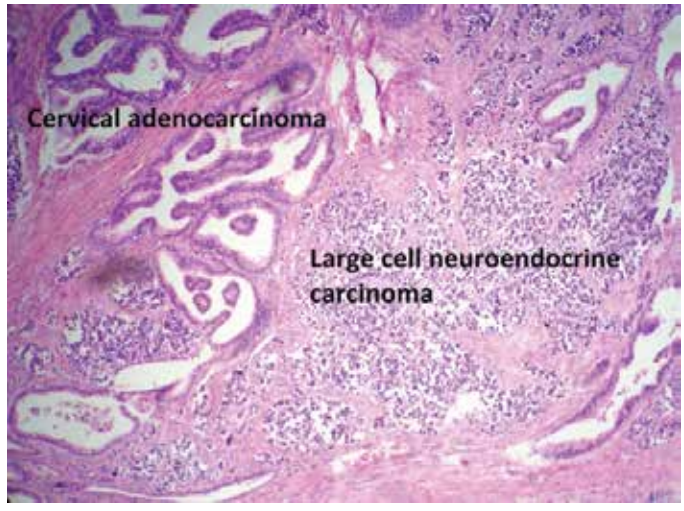

Figure 1. Mix cervical adenocarcinoma and large cell neuroendocrine carcinoma

and cervical peritoneum were also showing invasion. The endometrium was inactive and only adenomyosis was noticeable in the myometrium.

The tumoral tissue on the right ovary was also showing characteristics of the second tumor described above. The cystic structure on left ovary and tumoral tissues on both tuba uterine, serosa of the recto-sigmoid colon and adjacent adipose tissue were showing the same structural features (Figure 2).

Immunohistochemical Study: For the definitive diagnosis, proper immunohistochemical tests were performed. The tumoral tissue on right ovary was stained diffusely positive with EMA, CK7, chromogranin, synaptophysin, focally positive for progesterone receptor, and negative for estrogen receptor, thyroid transcription factor-1 (TTF-1), CK, gross cystic disease fluid protein 15 (GCDFP15), CK K-20, CD-56. These results were compatible; both the ovarian and cervical tumors were large cell neuroendocrine carcinoma. HPV PCR studies also revealed HPV type 16 positivity for both tumors. The Ki 67 proliferation index was 80%. According to all of these data, the ovarian tumor was a metastasis of cervical neuroendocrine carcinoma. The definitive pathologic diagnosis was reported as mixed adenoneuroendocrine carcinoma of the cervix (mucinous adenocarcinoma, endocervical type + large cell neuroendocrine carcinoma). The stage of disease was 4B according to 2009 International Federation of Gynecology and Obstetrics criteria.

The other tumoral implants were metastasis of the cervical primary tumor. Also 1 right hypogastric and 1 presacral lymph node were infiltrated with tumor. Other lymph nodes and abdominal cytology were clear.

After the operation, 6 cycles of etoposide + cisplatin combined chemotherapy were given with 2-week intervals. During that period, abdominal and thoracic tomography showed no new tumor lesions. Tumor markers 6 months after the surgery were: Carcinoembryonic antigen: 0.30 ng/mL, CA 15-3: 10.6 IU/mL, and CA 125: 14.3 IU/mL.

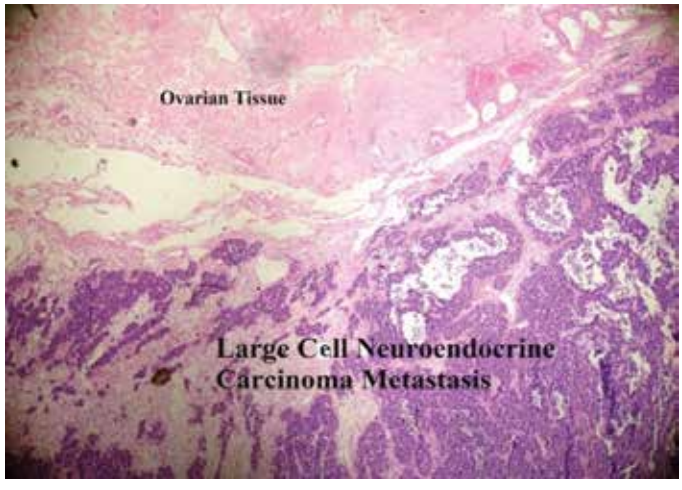

Figure 2. Large cell neuroendocrine carcinoma (ovarian metastasis)

Discussion

Cervical adenoneuroendocrine carcinomas are extremely rare. These malignancies progress aggressively with distant metastasis and high mortality rates. There is no consensus on suitable therapeutic protocols because of the lack of data[5]. According to the available data, the most important prognostic factor is the stage of the disease[6]. Studies reflecting data about small-cell neuroendocrine carcinoma suggest multidrug aggressive chemotherapeutic protocols including cisplatin and etoposide[7]. Despite the higher stage of the presented case, 6 months of follow-up showed a very good prognosis with surgery and cisplatin + etoposide chemotherapy regimen.

One of the most important prognostic features of adenoneuroendocrine carcinomas is that the malignant potential is mostly related to the neuroendocrine component. The presented case was consistent with this and the metastases originated from neuroendocrine component. Despite these data, ovarian metastasis may rarely originate from lower grade adenocarcinoma components and the differential diagnosis of these malignancies with primary ovarian carcinomas can be made through HPV DNA positivity[8].

An association between cervical neuroendocrine carcinomas and high-risk HPV (type 16 and 18) has been described[9]. HPV produces E6 oncoprotein, which causes the demolition of p53 rather than the effects on retinoblastoma[10]. Another molecular mechanism is the loss of heterozygosity on 3p loci as seen in the pathophysiology of small-cell lung cancers[11]. Considering all of these pathways, it may be speculated that the malignant transformation of a cervical cell is a multifactorial process.

Another point is that neuroendocrine carcinomas may cause paraneoplastic syndromes. No paraneoplastic syndromes were observed in our follow-up, but different syndromes have been experienced[12].

Conclusion

Cervical adenoneuroendocrine carcinoma is a very rare form of cervical malignancies and there is no consensus for proper treatment. The prognosis is mostly related to neuroendocrine component. The most important prognostic factor is indicated as the stage of the disease. The association between neuroendocrine malignancies and HPV subtypes are clinically important for diagnosis. It must be highlighted that differential diagnosis between adenocarcinoma and adenoneuroendocrine carcinomas may be challenging, but is essential because of the different clinical approach and prognosis. Immunohistochemistry is widely used in the differential diagnosis. More extended data should be presented to provide for definitive treatment protocols and clinical approaches.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of Etlik Zübeyde Hanım Women's Health Training

and Research Hospital, Informed Consent: Consent form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Taner Turan, Heyecan Ökten, Reyhan Öçalan, Concept: Taner Turan, Gökhan Tulunay, Design: Taner Turan, Gökhan Tulunay, Data Collection or Processing: Erdem Fadıloğlu, Şeyma Asiltürk, Analysis or Interpretation: Nurettin Boran, Literature Search: Erdem Fadıloğlu, Şeyma Asiltürk, Writing: Erdem Fadıloğlu, Alper Karalök, Osman Türkmen.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study has received no financial support.

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Do preeclampsia symptoms resolve after intrauterine death of a fetus?

Preeklampsi semptomları fetal ölüm sonrası kaybolur mu?

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Abstract

We present two cases of twin pregnancies without resolution of preeclamptic symptoms after intrauterine death of one twin.

Case 1: A nulliparous woman aged 37 years was referred at 26 weeks of gestation because of arterial hypertension, edema, and growth restriction in one twin. Three weeks later the restricted twin died. During the following three weeks, ultrasound examinations showed a reduced growth velocity of the surviving fetus and reversed umbilical flow. At the end of the 34th week of gestation, cesarean section was performed and a healthy female infant was delivered.

Case 2: A nulliparous woman aged 33 years with a 27-week twin pregnancy was referred because of arterial hypertension and discordant growth. The restricted twin died at 31 weeks of gestation. Following the death, within two weeks the growth of the co-twin started to slow down and reversed end diastolic flow presented. At the end of the 33rd week of gestation, cesarean section was performed and a healthy female infant was delivered.

The interesting point of these cases was the secondary effects on the co-twins. During the time after intrauterine deaths of one twin, the surviving fetuses started to show a reduced growth velocity and reversed umbilical flow and mothers had increased blood pressure and proteinuria again. We think that both cases are evidence of late on-set systemic maternal effects (such as systemic maternal endothelial activation and/or systemic maternal inflammatory response) depends on preeclampsia.

Keywords: Preeclampsia, twin pregnancy, growth restriction

Öz

İkiz eşinin ölümünden sonra preeklamptik semptomların devam ettiği iki olguyu sunuyoruz.

Olgu 1: Otuz yedi yaşında, nullipar, 26 hafta ikiz gebelik kan basıncı yüksekliği, ödem ve bir fetüste gelişme geriliği nedeniyle kliniğimize refere edildi. Üç hafta sonra geri kalan fetüs öldü. Takip eden üç haftada, ultrason ölçümlerinde yaşayan fetüsün gelişiminde yavaşlama ve umblikal arterde ters akım saptandı. Otuz dördüncü haftanın sonunda sezaryen gerçekleştirildi ve sağlıklı kız bebek doğurtuldu.

Olgu 2: Otuz üç yaşında, nullipar, 27 hafta ikiz gebelik kan basıncı yüksekliği ve bir fetüste gelişme geriliği nedeniyle kliniğimize refere edildi. Otuz birinci gebelik haftasında geri kalan fetüs öldü. Takip eden 2 haftada, ultrason ölçümlerinde yaşayan fetüsün gelişiminde yavaşlama ve umblikal arterde ters akım saptandı. Otuz üçüncü haftanın sonunda sezaryen gerçekleştirildi ve sağlıklı kız bebek doğurtuldu.

Bu olgulardaki ilginç nokta yaşayan ikiz eşlerindeki ikincil etkilerdi. İkiz eşinin ölümünden sonraki periyotta yaşayan fetüste gelişmede yavaşlama, ters umblikal akım ve annede kan basıncında artış ve proteinüri görüldü. Biz bu belirtilerin preeklampsinin geç başlangıçlı sistemik etkilerinin (sistemik maternal endotelyal aktivasyon ve/veya sistemik maternak enflamatuvar yanıt gibi) bir kanıtı olduğunu düşünmekteyiz.

Anahtar Kelimeler: Preeklampsi, ikiz gebelik, gelişme geriliği

Introduction

Preeclampsia originates from the placenta, and its progressive clinical course is only treated by delivery of the placenta[1]. The incidence of disease is 3-5% and it is known as a major cause of maternal and perinatal mortality[2]. Although the etiology and pathogenesis remain to be clarified, termination of pregnancy or delivery of the placenta eradicates the disease; therefore, the placenta is undoubtedly related to preeclampsia[3]. Twin gestations occur in 3.2% of pregnancies and are associated with increased risks of gestational diabetes mellitus, preterm delivery, intrauterine growth restriction, hypertension and hemorrahage[4]. Additionally, the risk of preeclampsia in twin pregnancies increases more than twice compared with singleton

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pregnancies[4]. We present two cases of twin pregnancies without resolution of preeclamptic symptoms after the intrauterine death of one twin.

Case Reports

Case 1

A nulliparous woman aged 37 years was referred at 26 weeks of gestation because of arterial hypertension, edema, and growth restriction in one twin. On admission her blood pressure was 160/100 mmHg. In our centre, ultrasound examination confirmed growth restriction (weight estimation: 389 g) and reversed umbilical flow with cerebral redistribution in one twin, with a normal co-twin (estimated weight: 855 g). This was a dichorionic, diamniotic twin pregnancy. The quantitative analysis of proteinuria in a 24-hour urine sample taken after admission showed 2.708 g/day. To avoid prematurity, expectant management was planned. The patient received alpha-methyldopa in a dosage of 1000 mg daily for blood pressure control and magnesium sulfate at a daily dosage of 1500 mg to prevent convulsions. Two days later, because of intolerable nausea and vomiting, we had to stop the magnesium sulfate but we kept going on alpha-methyldopa. During the following three weeks, the blood pressure was under partial control, ultrasound examinations showed no evidence of growth of the restricted twin and confirmed severe doppler abnormalities; the co-twin's assessment was reassuring. Some days later restricted twin died but doppler flows of the surviving twin were normal. Accordingly, we decided to prolong the pregnancy under close observation. Significant proteinuria occured again at 31 weeks of gestation, accompanied by a rise in blood pressure. During the following three weeks, ultrasound examinations showed a reduced growth velocity of the surviving fetus and reversed umbilical flow. At the end of the 34th week of gestation, cesarean section was performed and a healthy female infant weighing 1670 g was delivered, followed by a macerated female fetus of nearly 200 g. The mother recoverd quickly and her blood pressure was normal on the third day postpartum.

Case 2

A nulliparous woman aged 33 years with a 27-week twin pregnancy was referred because of arterial hypertension and discordant growth. On admission her blood pressure was 170/105 mmHg. Our ultrasound check showed growth restriction (weight estimation: 698 g) and reversed umbilical flow in one twin, with a normal co-twin (estimated weight: 1.060 g). The examination also revealed a dichorionic, diamniotic twin pregnancy. In a 24-hour urine sample showed proteinuria (4.850 g/day). Expectant management was planned to prevent prematurity. The patient received alpha-methyldopa in a daily dosage of 1000 mg for blood pressure control and magnesium sulfate at a dosage of 1500 mg daily to prevent convulsions. During the following two weeks, beside the partial blood pressure control, ultrasound examinations revealed no growth in the restricted twin and abnormal umblical artery flow; the co-twin's assessment was good. At 31 weeks of gestation, the restricted twin died and a significant proteinuria (6.650 g/day) reappeared but the co-twins doppler flows were normal. Following the death, growth of co-twin started to draw back within two weeks and reversed end diastolic flow presented. At the end of the 33rd week of gestation, cesarean section was performed and a healthy female infant weighing 1.370 g was delivered, followed by a macerated female fetus of almost 800 g. We discharged the mother from hospital after her blood pressure normalized on the fourth day postpartum.

Discussion

It is certain that the placenta takes a central role in the pathogenesis of preeclampsia. The importance of poor placentation as a feature of the disorder is well documented[5]. However, we still do not know the reasons of poor placentation. In these cases there are two separate placentas. One of them is preeclamptic, the pathologic report documented low weights with fibrin and thrombosis inside the vessels, and the others were normal. We still do not know why one of the twin placentas has abnormal development when both have the same maternal factors such as immunity or blood pressure. One explanation is local factors such as fetus or placental localization, which could possibly affect placental growth.

Another interesting point of these cases was the secondary effects on the co-twins. After intrauterine deaths of one twin, the surviving fetuses started to show a reduced growth velocity and reversed umblical flow and the mothers had increased blood pressure and proteinuria again. We think that this is evidence of late on-set systemic maternal effects such as systemic maternal endothelial activation and/or systemic maternal inflammatory response) depends on preeclampsia.

Usually preeclampsia progresses without any recovery until birth. Few cases of resolution of preeclampsia after spontaneous intrauterine death of one twin or selective termination of a dichorionic pregnancy have been reported[1,6,7]. Narasimhulu et al.[8] reported resolution of superimposed preeclampsia in a surviving fetus after the intrauterine demise of its co-twin and suggested that placental involution after fetal demise was the key to resolution of preeclampsia (resolution period take 1 to 3 weeks). However, according to our cases, even if the fetus dies, preeclampsia could not be curable unless placental seperation occurred. The greatest disparity of our cases compared with those in the literature was the amount of proteinuria, nearly 5 g/day in 24-hour urine sample. Consequently, severe preeclampsia may differ from mild preeclampsia, without any resolution or resolution in longer time (more than 3 weeks). In addition, a retrospective analysis of outcomes in pregnant women who had expectant managemant with severe preeclampsia at less than 27 weeks' gestation revealed that overall perinatal survival was 57% (9% of them were

multifetal gestations)[9]. As a result, resolution of preeclampsia in dichorionic twin pregnancies following intrauterine death of restricted fetus is still uncertain, especially in severe preeclampsia.

To our knowledge, these are the first cases to describe the return of preeclamptic symptoms in twins after the intrauterine death of one twin in the literature. Further studies are needed for the comprehension of preeclampsia and recognition of placental factors responsible for the persistence of the disease.

Ethics

Ethics Committee Approval: The study were approved by the Dokuz Eylül University of Local Ethics Committee, Informed Consent: Consnt form was filled out by all participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Bahadır Saatli, Sabahattin Altınyurt, Concept: Taylan Bodur, Design: Serdar Balcı, Data Collection or Processing: Serdar Balcı, Analysis or Interpretation: Recep Emre Okyay, Literature Search: Yusuf Aytaç Tohma, Writing: Serdar Balcı.
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Case Report / Olgu Sunumu

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Early prenatal diagnosis of thoraco-omphalopagus twins at ten weeks of gestation by ultrasound

On hafta dört günlük torako-omfalofagus ikiz olgusunun ultrason ile erken prenatal tanısı

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Abstract

Early prenatal diagnosis of conjoined twins, an extreme form of monozygotic twinning, is very important for the further management and counselling of parents because they are associated with high perinatal mortality. We present a case of thoraco-omphalopagus twins diagnosed at ten weeks and four days of gestation by two-dimensional Doppler ultrasound, which was then terminated.
Keywords: Monozygotic twins, conjoined twins, ultrasonography, doppler ultrasonography

Öz

Monozigotik ikiz gebeliğin en nadir formu olan yapışık ikiz olgularında erken prenatal tanı, yönetim ve ebeveyn bilgilendirilmesi açısından çok önemlidir. On hafta dört günlük gebelik iken iki-boyutlu Doppler ultrasonografi ile tanısı konularak sonlandırılan torako-omfalofaguslu yapışık ikiz olgusu sunulmuştur.
Anahtar Kelimeler: Monozigotik ikiz, bitişik ikiz, ultrasonografi, doppler ultrasonografi

PRECIS: We present a case of thoraco-omphalopagus twins diagnosed at ten weeks and four days of gestation using two-dimensional doppler ultrasound.

Introduction

Conjoined twins are a rare, complex complication seen in 1% of monochorionic twins that occurs with an estimated incidence of 1 in 50.000 to 1 in 200.000 live births[1]. Even though the degree and location of conjunction and the shared internal organs determine the prognosis of conjoined twins, they are associated with a high perinatal mortality rate; the overall survival rate is 25%. Although a smaller fraction born alive have anomalies incompatible with life, survivors need to have various correction operations because of many coexisting diseases[1,2]. Therefore, early prenatal diagnosis of conjoined twins plays a crucial role in management and allows appropriate and timely counselling of the parents to decide among various options, which are (a) continuation of pregnancy with planned postnatal surgery, (b) termination of pregnancy, and (c) in cases of high-order multifetal pregnancies with a component of conjoined fetuses, multifetal pregnancy reduction or selective fetocide[1].

In this report, we present a case of thoraco-omphalopagus twins diagnosed prenatally using two-dimensional (2-D) Doppler ultrasound (US) at ten weeks and four days of gestation.

Case Report

A gravida five, parity two, abortus two woman aged 32 years woman was referred to our perinatology clinic for first trimester screening with a combined test. Her obstetric history revealed two spontaneous vaginal deliveries and was unremarkable with respect to medication use or births with structural or chromosomal anomalies. She had no family history of multiple gestations.

Transabdominal US scan using a 5 MHz probe with 2-D Doppler US (Voluson 730 PRO, GE Medical system) revealed a monochorionic monoamniotic twin pregnancy of 10 weeks and four days of gestation, according to her last menstrual date. One of the fetuses was anencephalic. Both fetuses had

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cystic hygroma. The fetuses were fused to each other at the chest (thoracopagus) and the umblicus (omphalopagus). Two upper and two lower extremities were seen for each fetus. There was only one heart beat. This was confirmed using a 5 MHz transvaginal probe. A diagnosis of conjoined twins was made using sonography (Figure 1).

The couple was informed about the US findings and counseled about management options. They decided to terminate the pregnancy. The induced abortion material was sent for pathogic examination. The result was conjoined twins with two bodies fused from the upper thorax to lower belly (Figure 2). Both fetuses were female. Two upper and two lower extremities were seen for each fetus. The pathology report revealed one heart, one liver, two stomachs, and two kidneys shared by the twins. Therefore, the ultrasonographic diagnosis of conjoined twins of thoraco-omphalopagus type was confirmed.

Discussion

Conjoined twins is an extreme form of monozygotic twinning. Incomplete fusion of a single zygote at the primitive streak stage (15-17 days) during blastogenesis is considered to be responsible for this condition[3]. Therefore, when there is a case of monochorionic-monoamniotic pregnancy, the possibility of conjoined twins should always be kept in mind.

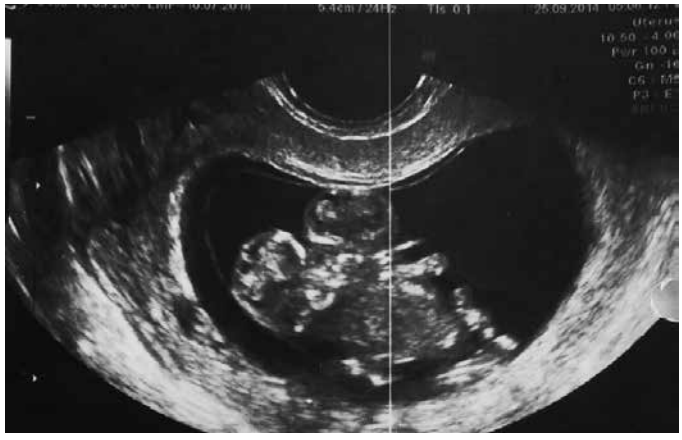

Figure 1. Ultrasound image

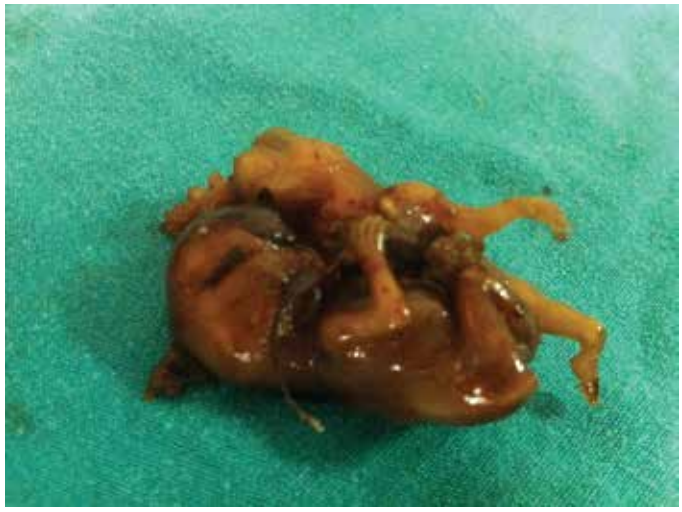

Figure 2. Macroscopic image of the induced abortion material

Sonographic characteristics that raise the suspicion of conjoined fetuses are polyhydramnios seen in 50-76% of cases, bi-breech and face-to-face presentation of twins[4]. Moreover, for early diagnosis of conjoined twins, specific ultrasound features have been proposed and reports of diagnosis as early as seven weeks and six days of gestation have been published. However, it is still possible to miss or misdiagnose conjoined twins beacuse of various misleading sonographic signs[5,6]. Increased nuchal translucency (NT) was observed in six fetuses of four conjoined twins. In the present case, both fetuses had cyctic hygromas. This observation highlights the importance of increased NT in multifetal pregnancies during the 11-14 weeks scan by raising the possibility of conjoined twins so that a more careful examination should be considered[7]. Therefore, the 11-14 weeks 2-D Doppler US scan remains the mainstay of prenatal diagnosis of conjoined twins[1]. This was also the diagnostic method used in the prenatal diagnosis in this case report. Moreover, very early diagnosis seems not to add any practical information compared with detection in the 11-14 weeks scan because either repeat US, magnetic resonance imaging (MRI) or 3-D US had to be used to confirm the diagnosis of conjoined twins in such reports[8]. It must be stressed that even though ultrasonographic features such as an absent separating membrane in monochorionic twins, bifid appearance of the fetal pole, and inseparable bodies or heads despite fetal movements suggest the diagnosis, the 11-14 week scan with 2-D Doppler US is still the diagnostic imaging modality in these cases[9]. However, ultrasound does have pitfalls; therefore, modern imaging modalities like 3-D US, 4-D US or fetal rapid MRI can be useful to overcome these disadvantages in the diagnosis of conjoined twins. In addition, if the parents decide to continue the pregnancy, MRI is used to determine the site and percentage of conjunction to further predict the success of surgical seperation and postnatal prognosis.

Conjoined twins are very rarely seen in human pregnancies. However, they should be kept in mind when monochorionic twins are being examined during the 11-14 week scan, especially when increased NT is observed because early and accurate prenatal recognition is essential for parental counseling to decide for or against continuation of pregnancy. If parents decide to continue the pregnancy, prenatal surveillance and postnatal management should be planned. The obstetricians role in prenatal diagnosis, counseling, and organization of interdiciplinary medical care is indispensible in cases of conjoined twin.

Ethics

Informed Consent: Not applicable.

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Authorship Contributions

Surgical and Medical Practices: Başak Baksu, Oya Pekin, Concept: Başak Baksu, Dilşat Herkiloğlu, Oya Pekin, Design: Dilşat Herkiloğlu, Başak Baksu, Oya Pekin, Data Collection or Processing: Başak Baksu, Dilşat Herkiloğlu, Oya Pekin, Analysis or Interpretation: Başak Baksu, Dilşat Herkiloğlu, Literature Search: Dilşat Herkiloğlu, Başak Baksu, Writing: Dilşat Herkiloğlu, Başak Baksu, Oya Pekin.

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