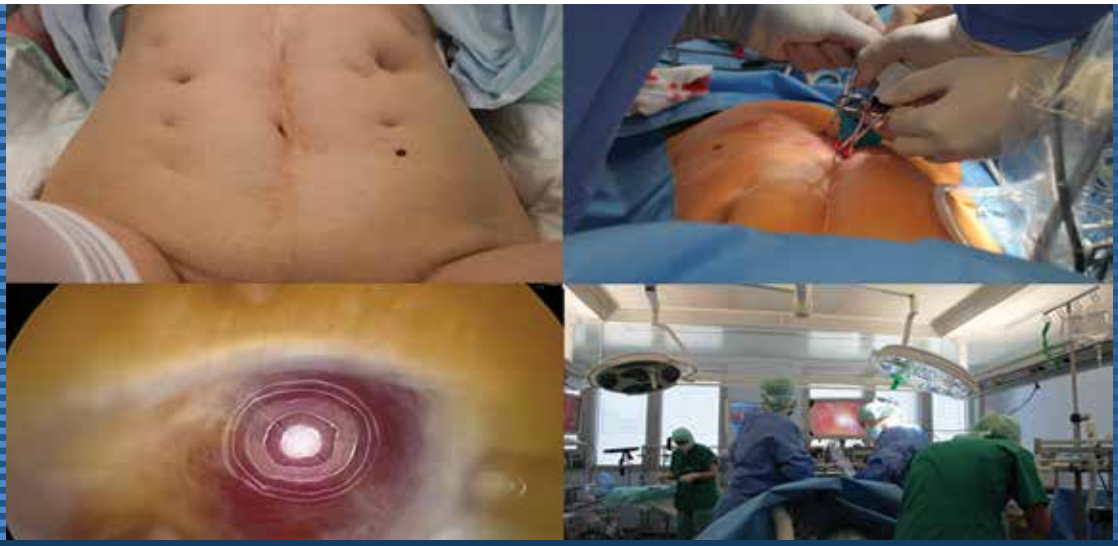
ISSN 1309-0399 • EISSN 1309-0380

Indexed in PubMed and Web of Science

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association



Volume 16
Issue 4
December
2015

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Original Investigations

Official Journal of the Turkish-German Gynecological Education and Research Foundation
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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December.

The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the Turkish-German Gynecological Association 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.

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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/),

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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

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Contents

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Editorial

Dear Colleagues,

I am delighted to introduce the fourth issue of the "Journal of the Turkish German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2015.

We are proud to say that J Turk Ger Gynecol Assoc is now more popular than it's used to be. J Turk Ger Gynecol Assoc, which publishes original studies on all aspects of gynecology, has been in PubMed Central. Besides it is indexed in PubMed Central, EMBASE, Scopus, CINAHL, Gale/ Cengage Learning, EBSCO, DOAJ, ProQuest and Index Copernicus, I am very glad and proud to say that it has been accepted for Emerging Sources Citation Index (ESCI).

This year, Thomson Reuters has launched the Emerging Sources Citation Index (ESCI), which will extend the universe of publications in Web of Science to include high-quality, peer-reviewed publications of regional importance and in emerging scientific fields. ESCI will also make content important to funders, key opinion leaders, and evaluators visible in Web of Science Core Collection even if it has not yet demonstrated citation impact on an international audience. Journals in ESCI have passed an initial editorial evaluation and can continue to be considered for inclusion in products such as SCIE, SSCI, and AHCI, which have rigorous evaluation processes and selection criteria. All ESCI journals will be indexed according to the same data standards, including cover-to-cover indexing, cited reference indexing, subject category assignment, and indexing all authors and addresses.

We would like to invite you to join us for our 11th Turkish - German Gynecology Congress to be held in Antalya on May 11-15th of 2016. We are confident that this global meeting in Antalya will attract many participants. This time our congress venue will be the newly constructed Sueno Hotels Deluxe's convention center in Belek. The high standard of the scientific program will be attractive for the international gynecology and obstetrics community world and we look forward to welcoming you to Antalya.

In this issue, we are dealing with very interesting research articles and case reports. We worked hard to deliver you the journal with the best manuscripts in time. In this issue, you will read several good papers from all over the world. A manuscript from United States will explain how can we detect the uterine magnetomyographic signals for diagnosis of preterm labor. You will read a very informative review from Germany, that shows different port placement and trocar systems as well as their correct and professional usage in correlation with the abdominal functional anatomy. I hope you will see and read all other papers either from local or global like Egypt, India, United Kingdom. Please also enjoy solving a challenging quiz.

I would like to wish you a happy new year in 2016 and we are looking forward to receiving your valuable submissions.

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

Hilbert–Huang transform in detecting and analyzing the uterine contraction activities

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Abstract

Objective: The diagnosis of labor is currently one of the most difficult problems encountered by obstetrical healthcare providers. A major health problem is the increase in the rate of preterm delivery, which is responsible for 75% of all deaths in newborns. In addition, preterm delivery is associated with several cognitive and health problems in later life and enormous costs for the health system. A better understanding of myometrial activities could help to reduce preterm deliveries and the costs associated with prematurity in the following years. Therefore, the objective of this study was to determine whether using the Hilbert–Huang transform (HHT) to analyze the uterine contraction data would help us gain a better insight of the myometrial activities of the human uterus during pregnancy.

Material and Methods: Uterine magnetomyographic (MMG) signals were recorded from pregnant patients at gestational ages of 32–38 weeks. The study was approved by the Human Research Advisory Board of the University of Arkansas for Medical Sciences (UAMS) and performed after obtaining written consent from each patient. The recording of transabdominal MMG signals was conducted with the SQUID Array for Reproductive Assessment (SARA, VSM MedTech Inc; Coquitlam, BC, Canada) system, which has 151 primary magnetic sensors allocated approximately 3 cm apart over an area of 850 cm^2. The arrangement of sensors is concave in nature and, in a similar lateral distance, spans the maternal abdomen longitudinally from the symphysis pubis to the uterine fundus. The recording times ranged from 12 to 28 min, and the sampling rate was 250 Hz. The data were down-sampled to 25 Hz to reduce the computational complexity and post-processed with a bandpass filter (0.05–1 Hz) because the uterine contraction activity is a band-limited process (0.05–1 Hz). The recordings of one intrauterine pressure catheter (IUPC) dataset and two mother-perceived contraction datasets were compared with the HHT results, and HHT's potential was explored through the development of a module and a series of experiments. The local energy and the instantaneous frequency derived from the intrinsic mode functions (IMFs) through HHT provide a full energy-frequency-time distribution of the data. Our objective was to determine whether HHT for each channel can help identify and localize contractions in the uterus. Human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000.

Results: After comparing the IUPC and other mother-perceived contraction (STIM) datasets with HHT results, we were able to visually detect contraction locations in the HHT-processed uterine signals. For verification and validation purposes, when we further analyzed the delay time between two signals, the mechanical activity (i.e., IUPC) following the electrical activity (i.e., magnetic signal) was observed. In conclusion, our experimentations using the method introduced here revealed that there is a 75% correlation between the results obtained by HHT and IUPC data.

Conclusion: This study compared uterine contractions and changes in the intrauterine pressure with results obtained by HHT. In addition, using IUPC data as a validation guide, we showed that the HHT approach can be used for noise removal. There is a need for time-saving and non-subjective automatic contraction detection in the field of prenatal examination. (J Turk Ger Gynecol Assoc 2015; 16: 195-202)

Keywords: Uterine contraction, myometrium, magnetomyographic activity, Hilbert–Huang transform, empirical mode decomposition, contraction analysis

Received: 12 March, 2015 **Accepted:** 23 August, 2015 **Available Online Date:** 02 November, 2015

Introduction

The purpose of this work was to identify uterine contractions using the Hilbert–Huang transform (HHT) to gain a better insight of the myometrial activities of the human uterus during pregnancy. The myometrium is the muscle layer in the human uterus and consists of small smooth muscle cells. The coordinated activity of these muscles is responsible for the generation of uterine mechanical contractions, which leads to the delivery of the fetus. The diagnosis of labor is currently one of the most difficult problems encountered by obstetrical healthcare providers. Prediction of labor is important in both normal and complicated pregnancies. A major health problem is the increase in the number of preterm deliveries, which are responsible for 75% of all deaths in newborns (1, 2). In addition, preterm delivery is associated with several cognitive and health problems in later life and enormous costs for the health system (3). A better understanding of myometrial

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DOI:10.5152/jtgga.2015.15021

activities could help reduce preterm deliveries and the costs of prematurity in the following years. Apart from recording the changes in the cervical state, the progress of labor is monitored by measuring the rate, duration, and amplitude of uterine contraction using a tocodynamometer (TOCO) or the measurement of intrauterine pressure using an intrauterine pressure catheter (IUPC). Because of its simplicity and almost risk-free nature for the mother and fetus, the TOCO technique is widely used by physicians. However, its susceptibility to maternal motion artifacts is known to be the major drawback. Compared with IUPC, which is an invasive procedure that requires the rupture of the amniotic membranes, TOCO provides less reliable results. Because of the poor predictive power of TOCO and the invasive nature and limited use of IUPC (4), neither technique has been beneficial in the prediction of preterm labor or the diagnosis of true labor at term. If physicians were able to more objectively differentiate between true and false labor, unnecessary visitations to the hospital and the associated treatment could be avoided.

The SQUID Array for Reproductive Assessment (SARA) (4) system is a non-invasive device that is designed to specifically study different aspects of maternal and fetal physiology, particularly in detecting weak biomagnetic fields associated with the electrophysiological activity in the human body (5). The role of SARA (6) can be summarized as the process of capturing the fluctuating magnetic field activities "generated during the polarization of the biological tissue, using a set of sensors (an array of 151 SQUID sensors) covering the complete area of the maternal abdomen starting from the perineum to the top of the uterine fundus." The advantage of using SARA is that it records magnetomyographic (MMG) activity related to the uterine electrophysiological activity, and this information could provide an opportunity to track the parameters that would aid in the prediction of the onset of active labor. This ability would be of great clinical benefit for the management of term patients and particularly for the management of patients at high risk for premature delivery. Myometrial activity patterns may reveal whether a contraction will lead to a delivery. As a novel approach in gaining new insights into uterine data, HHT, which has already been proven to be successful in various non-stationary and non-linear data analysis (6-10), was examined. HHT can be used to better explain its application in the identification of contraction patterns and data analysis properties. Some of these properties include decomposition and expansion of the data into components that are called intrinsic mode functions (IMF) (11), which reveal interesting information about the original data, localizing events in the time-frequency domain using temporal frequency energy distribution, and noise removal.

Material and Methods

Data recording

Uterine MMG signals were recorded from pregnant patients at gestational ages of 32–38 weeks. The study was approved by the University of Arkansas for Medical Sciences (UAMS) Human Research Advisory Board and performed after obtaining written consent from each patient. Transabdominal MMG signals were recorded with the SARA (VSM MedTech Inc., Cocuitlam, BC, Canada) system with an array of magnetic sensors [i.e., 151 primary magnetic sensors spaced approximately 3 cm apart over an area of 850 cm^2 (4)]. The sensors are arranged in a concave array that spans the maternal abdomen longitudinally from the symphysis pubis to the uterine fundus and a similar lateral distance.

Because of the invasive nature of IUPC recording, simultaneous recordings with SARA were performed only on a single patient. Although for all the subjects, maternal perception of the contraction was also recorded simultaneously with SARA measurements. Therefore, during the recording process, the subjects were asked to use their finger in the up-position as a duration indicator of each perceived contraction to inform the operator to mark the beginning and end time points of the contraction in the record.

The recording times ranged from 12 to 28 min, and the sampling rate was 250 Hz. The data were down-sampled to 25 Hz to reduce the computational complexity and post-processed with a bandpass filter (0.05–1 Hz) because the uterine contraction activity is a band-limited process (0.05–1 Hz) (8).

Data analyses

Empirical mode decomposition (EMD) (12) is the decomposition of the signal into IMFs and is based on the direct extraction of the energy associated with various intrinsic time scales. These time scales are the most important features of complex biological systems such as the uterus. Among the decomposed IMFs, we selected the one that has the highest standard deviation (SD). The choice of IMF with the highest SD is based on the fact that the manifestation of a contraction will make a large deviation from the signal's average, thereby making the SD higher. The use of Hilbert transform to drive the local energy and the instantaneous frequency from the IMFs provides a full energy-frequency-time distribution of the data. Such representation is designated as the Hilbert spectrum. They have well-behaved Hilbert transforms, as expressed in IMFs, from which the instantaneous frequencies can be calculated. Thus, any contraction-related event can be localized based upon time.

Decomposition can also be viewed as an expansion of the data in terms of the IMFs. Subsequently, these IMFs based on and derived from the data can serve as the basis of the non-linear expansion of data. The most interesting property of the method is its adaptive nature to data.

Hilbert–Huang transform (HHT) in uterine contractions

Our aim was to determine whether HHT of each channel can reveal and localize contractions in the uterus. To explore the potentiality of HHT, we developed a system to conduct a series of experiments. We want to note that HHT is more of an empirical approach rather than a theoretical method. It has been successfully applied in many areas such as non-linear ocean wave evolution data; earthquake signals and structure responses; bridge and structural health monitoring; biomedical signals such as blood pressure fluctuations; long-term environmental data , including global temperature variations,

Antarctic ice extents records, solar irradiance variance; hydromachinery design; and machine vibration. The key feature of HHT is EMD, which provides a unique basis called IMFs for the underlying hidden parts of the data that are derived from data and adaptive to the data.

Empirical mode decomposition (EMD) method

Two conditions need to be satisfied for any IMF. The first condition refers to the fact where the number of extrema and zero crossings are either equal or differ at the most by one. The second condition refers to the mean value of the envelope defined by the local minima and local maxima being zero at any point. According to these two conditions, the sifting process is applied. In the sifting process, the cubic splines from the local maxima and local minima points of uterine contraction data are calculated. These cubic splines are called envelopes. Then, the average is calculated from the envelopes using cubic spline approximations, and this average is subtracted from the signal. The process is repeated until the resulting mean satisfies the two aforementioned properties of IMF. More details on the use of EMD can be found in (9).

The HHT methodology is described as follows: Let X(t) be the MMG signal, $IMF_i(t)$ be the i^{th} IMF function, and R(t) be the residue of the signal. Then, the original signal X(t) can be written as follows:

$$X(t) = \sum_{i=1}^{n} IMF_i(t) + R(t) \qquad (1)$$

The sifting process is terminated if the difference between the successive IMFs is less than a prefixed tolerance defined as follows:

$$SD = \sum_{t=1}^{T} \left[\frac{\left(IMF_{(k)}(t) - IMF_{(k-1)}(t)\right)^2}{IMF_{(k-1)}(t)} \right] \qquad (2)$$

SD can have a value between 0.2 and 0.3 according to (9). In all of our calculations, we set SD to 0.2 for a more sensitive difference between siftings.

The Hilbert transform is applied to IMFs to obtain the local energy of the contraction. However, applying the Hilbert transform to a non-stationary signal may result in two cases: 1) frequencies that are outside the Fourier spectrum or 2) frequencies that go outside the band limit for a band-limited signal. To prevent these two situations, the Hilbert transform should be applied to narrow band data (10). Fortunately, IMFs are narrow band signals, and they behave well with the Hilbert transform.

The Hilbert transform, Y(t), of a given signal, S(t), is as follows:

$$Y(t) = \frac{1}{\pi} PV \int \frac{S(t')}{t - t'} dt', \qquad (3)$$

where PV denotes the Cauchy principal value. With this definition, S(t) and Y(t) can be used to form the analytical signal Z(t), defined as follows:

$$Z(t) = S(t) + iY(t) = a(t)e^{i\theta(t)} \qquad (4)$$

where time-dependent amplitude a(t), phase θ(t), and the instantaneous frequency ω(t) are defined as follows:

$$a(t) = \sqrt{S^2(t) + Y^2(t)} \qquad (5)$$

$$\theta(t) = \arctan\left(\frac{Y(t)}{S(t)}\right) \qquad (6)$$

$$\omega(t) = \frac{d\theta(t)}{dt} \qquad (7)$$

S(t) can be calculated from IMF components using the Hilbert transform, as a generalized expansion of the Fourier transform with the time variable amplitudes and frequencies:

$$S(t) = \mathrm{Re} \sum_{k}^{n} a_k(t) e^{i \int \omega_k(t) dt} \qquad (8)$$

Results

We applied HHT to the uterine contraction data obtained from 12 patients. In 11 datasets, there was no quantitative estimate of the performance of HHT because the maternal perception of contraction is a subjective measure. However, in all of the 11 cases, there was a good (qualitative) agreement between the two entities (maternal perceived contractions and the ones identified by HHT). We have recorded the maternal perception of the contractions for all the datasets and compared the instances at which the mothers perceived the contractions with the instances of the ones identified by the HHT approach. In most of the recordings, HHT also identified the low-amplitude contractions, which mothers could not perceive and report as contractions. Because there was a good degree of correlation between the events perceived by mothers (the high-amplitude contractions) and those identified by HHT, any quantification of these two entities would make the approach over-appealing; hence, we have not quantified the same.

We were able to visually detect contraction locations in the HHT-processed uterine signals. For verification and validation purposes, we compared our findings with the IUPC data of one patient who had both MMG and IUPC recordings. For this patient, recordings in each sensor lasted for 720 s (at a sampling frequency of 25 Hz, resulting in 18000 data points). To facilitate the comparison of the result with IUPC data (Figure 1), we added the results of HHT for all sensors together (Figure 2). As mentioned in the earlier section, IUPC was recorded simultaneously with MMG for this subject. We observed that the mechanical

(IUPC) activity followed the electrical activity. Apart from this delay, there was a significant correlation between them.

To quantify the correlations between the Hilbert energy and IUPC data, a cross-correlation analysis was performed. The cross-correlation function (CCF) between the Hilbert energy and IUPC data is shown in Figure 3. There was a maximum correlation (coefficient) of 0.774 (square of this gives the % of correlation) between the two quantities at a time shift of −9.72 s. For a negative shift, the IUPC data was shifted backwards in time. Thus, there was a delay of 9.72 s between the two signals, indicating that mechanical activity (i.e., IUPC) followed the electrical activity (i.e., magnetic signal).

Because MMG obtained from different sensors are heterogeneous in nature, the resulting Hilbert energy obtained (by adding the HHT results across all the sensors) is noisy. To improve the correlation between the two signals, the results obtained from HHT were smoothed by performing moving average (non-causal/zero-phase filter) with a window width of 10 s. The correlation between the smoothed data and IUPC data is shown in Figure 4. The correlation between the signals increased from 0.774 to 0.8684, with the delay remaining fairly the same value of approximately 9.72 s (Figure 4).

Because the correlation analysis of a smoothed data is subjective, we performed the analysis as a function of the width of the smoothing window to see the influence of the window width on the correlation. Naively, one would expect the correlation to increase as a function of window width and reach a maximum value when the optimal window width is reached. Figure 4 shows the correlation analysis as a function of the window width. As anticipated, the correlation increased with the increase in window width and reached a maximum value for an optimal width of 27 s. A further increase in the window width resulted in a decrease of correlation because the smoothing process destroyed relevant signal content in addition to minimizing the noise.

The dependence of delay on the window width is shown in Figure 5. The delay remained fairly constant (stable) at around −9.72 s to a window width of 56 s. Although the smoothing process reduced the correlation beyond the width of 27 s (Figure 5), the delay was quite robust to window width.

Noise separation with HHT

The reliability measurement was conducted in two different forms: a) the addition of a Gaussian noise (white noise) and b) the addition of a filtered noise. With Gaussian Noise, we show the reliability rate of HHT in the process of removing noise from the signal. Therefore, we added white noise to the signal and then used HHT to retrieve the original signal (Figure 6). Here, the first plot shows the original signal contaminated with a white noise. The second plot shows that the noise was removed fairly well. The third plot is the corresponding IUPC data. To test the strength of HHT, we increased the noise level and conducted additional experiments to show the noise removal from the mixture for obtaining the original signal. The small deviation encountered in the resulting HHT signal is insignificant because the noise is quite large and it still corresponds to the IUPC (80%). We further increased the noise level and conducted the test again and, as expected, some small contractions were lost, but were still capable of decomposing the dominant contractions from a signal which resembled nothing but a noise. Aiming to take the reliability testing to the next level, we made

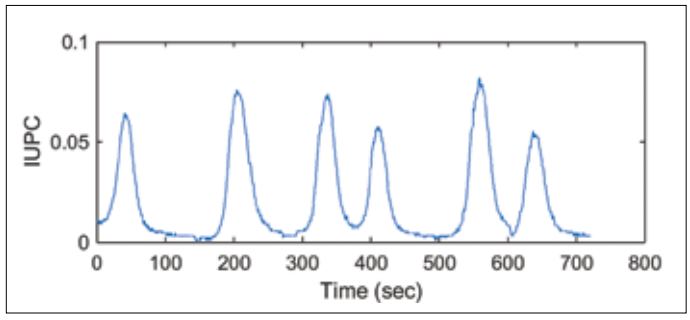

Figure 1. IUPC data
IUPC: intrauterine pressure catheter

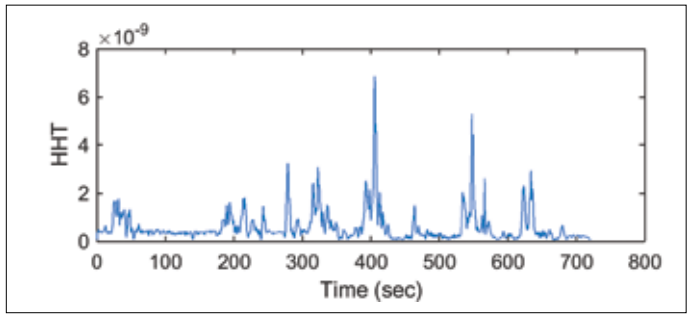

Figure 2. HHT (sum of all channels)
HHT: Hilbert–Huang transform

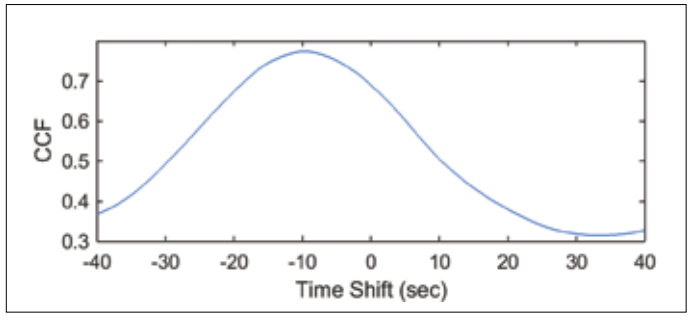

Figure 3. Cross-correlation between the HHT and IUPC data
HHT: Hilbert–Huang transform; IUPC: intrauterine pressure catheter; CCF: cross correlation function

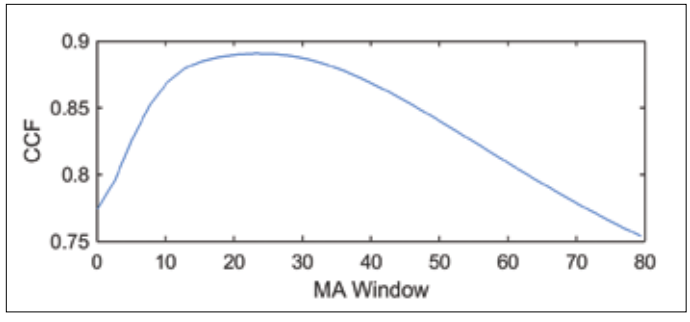

Figure 4. Cross-correlation Function at time shift corresponding to the delay vs. moving average window width
CCF: cross correlation function; MA Window: moving average window

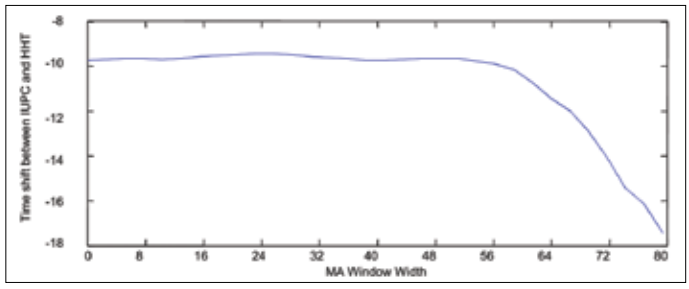

Figure 5. Time shift between IUPC and HHT vs. moving average window length

IUPC: intrauterine pressure catheter; HHT: Hilbert–Huang transform

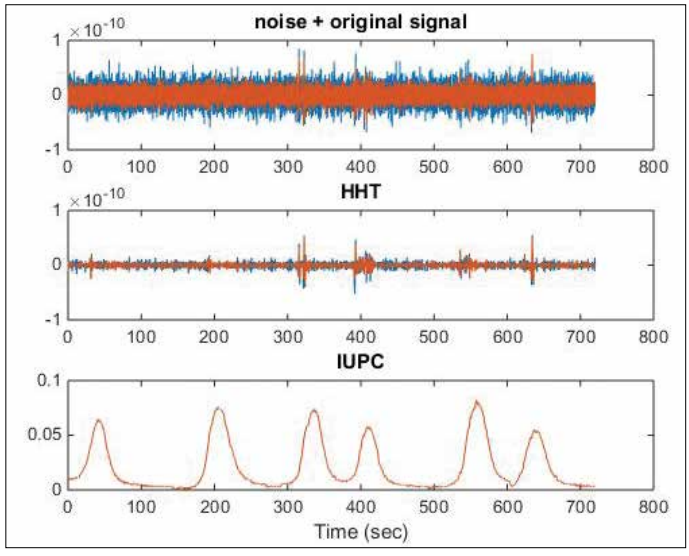

Figure 6. Demonstration of the reliability of HHT using white noise

HHT: Hilbert–Huang transform; IUPC: intrauterine pressure catheter

Top panel represents the magnetomyographic (MMG) signal from one of the The SQUID Array for Reproductive Assessment (SARA) sensors with white noises (signal-to-noise ratio (SNR)=0.4501 dB, SNR=−5.5705 dB, SNR=−9.0923 dB) added to it. Middle panel represents the Hilbert amplitude of the signal shown in the top panel. Bottom panel represents the intrauterine pressure recorded by intrauterine pressure catheter (IUPC).

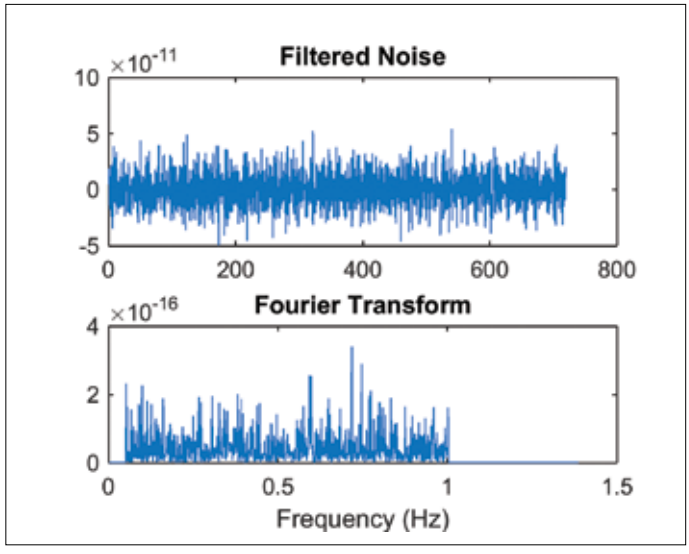

Figure 7. The filtered noise and its Fourier amplitude

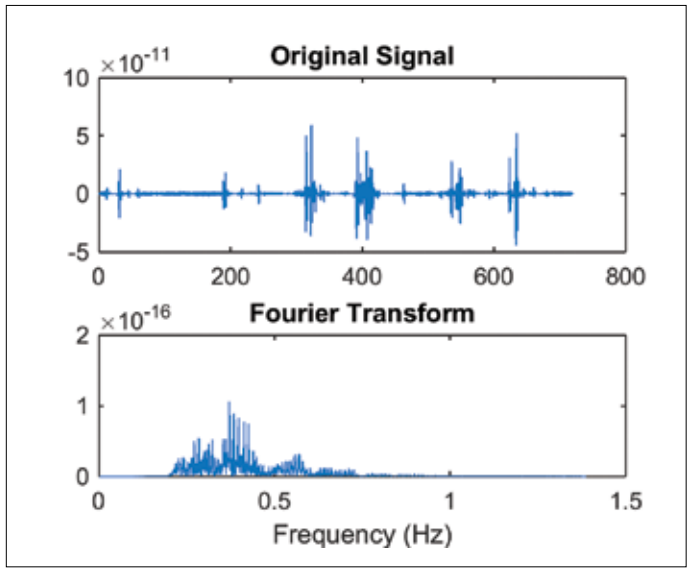

Figure 8. Original signal and its frequency content

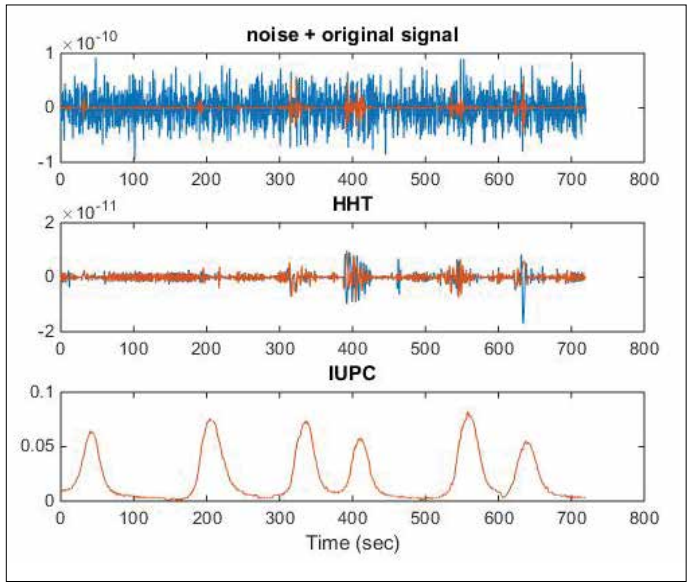

Figure 9. Demonstration of the reliability of HHT using filtered white noise

HHT: Hilbert–Huang transform; IUPC: intrauterine pressure catheter

The quantities plotted are the same as in Figure 6, but with different signal-to-noise ratios (SNRs), i.e., SNR=0.4501 dB, SNR=−5.5705 dB, SNR=−9.0923 dB, SNR=−11.5911 dB.

the frequency content of the noise, similar to the original signal in the following section.

In the previous section, the use of HHT revealed the success of white noise removal from the signal. However, the noise used has a different frequency content compared with the original signal which makes separation easier. More realistic results can only be obtained by employing filtered noise. Therefore, the white noise was filtered between 0.05 and 1 Hz to complement the original signal's frequency range (also 0.05–1 Hz). Figure 7 depicts the graph of the filtered noise (0.05– 1 Hz) and its frequency content, while Figure 8 depicts the graph of the original signal and its frequency content.

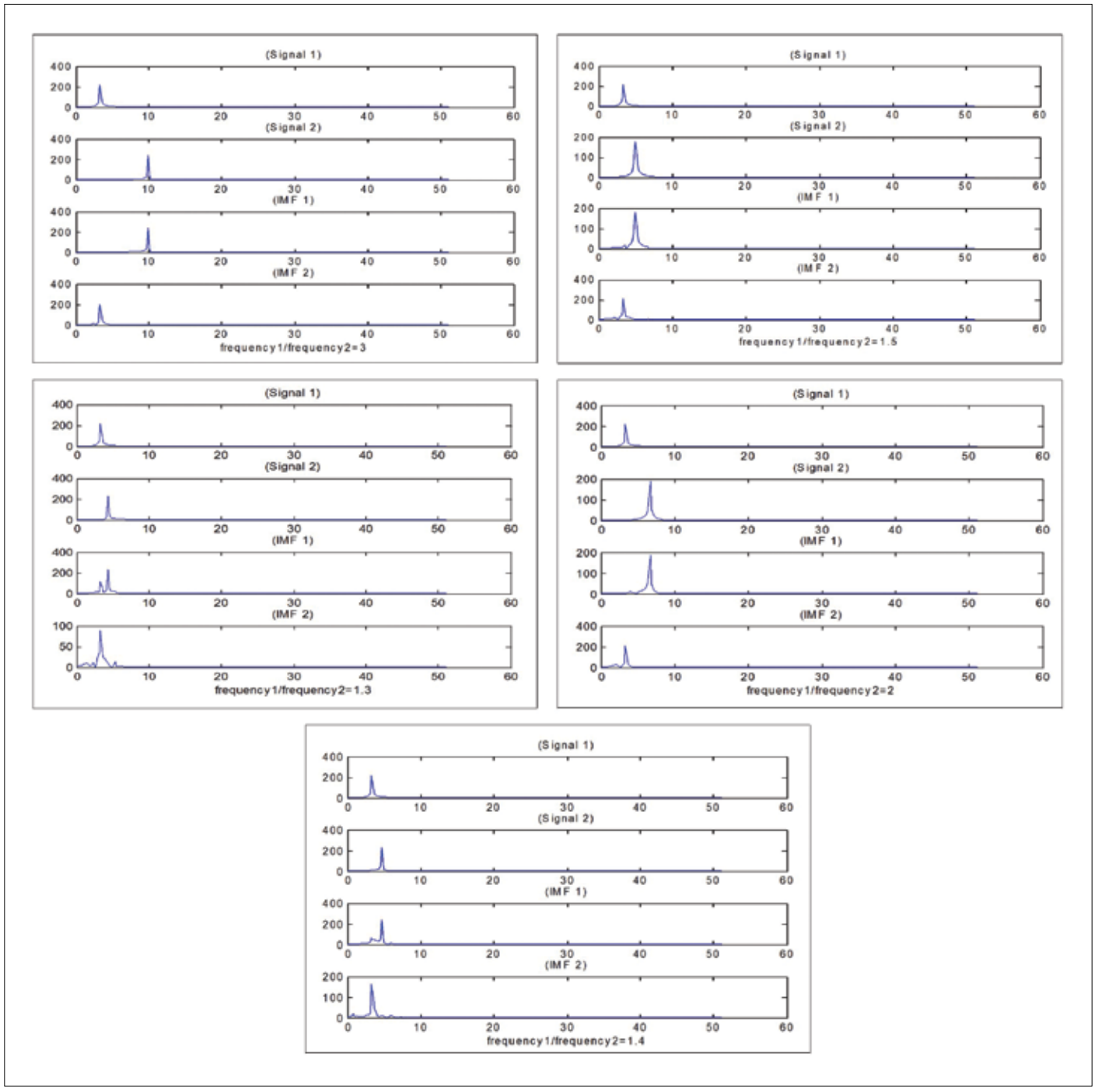

Figure 10. Frequency content of the signals
IMF: intrinsic mode function
The first two plots show the frequency content of each signal, and the second two plots show the frequency of the decomposed IMFs.

Figure 9 show the decomposition of the original signal from a noise-contaminated signal. Here, both the signal and the noise have the same frequency content. Therefore, the separation is an extremely difficult task. The success in separating noise decreased when the noise had the same frequency range as the original signal. Therefore, what should the minimum frequency ratio between two signals be to reliably separate those signals? To provide an answer, we used synthetic data to investigate this issue. Our experiments showed that if the ratio decreases below 1.5, then separating two signals becomes problematic.

Verification and validation of HHT in the separation of signals

For each figure in Figure 10, the first and second plots show the frequency contents of each signal. Those signals were mixed together, and HHT was then used to decompose them back from this mixture. The third and fourth plots of each figure show

the frequency content of the decomposed IMFs. Notably, there are no problems in decomposing two signals if they have a frequency ratio of 1.5 or higher. In other words, if the ratio is greater than or equal to 1.5, decomposed IMFs give the exact frequency contents of the original signals.
As seen in Figure 10, the EMD algorithm is able to separate signals if their frequency ratio is higher than 1.5. When the ratio decreases below this point, the resulting IMFs decomposed from the original signal, start to include data from both signals.

Comparison of HHT with mother perception

Mother-perceived contractions were recorded in the STIM channel. We compared our findings with STIM data for the additional reliability test of HHT approach. If we encountered a contraction which corresponds to STIM data, we counted it as a true positive, but if we encountered a contraction which did not correspond to STIM data, we did not count it as false positive because we do not expect the mother to feel every contraction. There may be a different threshold for different mothers where they can feel the contraction. Data used in this work were gathered from the records of two patients.
Figure 11 depicts HHT with mother's perception. When a mother felt a contraction, she pushed the STIM button when she thinks it has ended, and she pushed the button again with the assumption a new contraction started. In the figure, the mother perceived two contractions between 90–210 s and 610–695 s. Our HHT plot overlaps with those results; in addition, it shows another contraction at 310 s and 410 s, which is not recorded by the mother. Figure 12 shows another contraction perceived by the mother between 410 and 550 s. However, the mother did not record the contraction between 600 and 660 s. This may be due to the pressure not reaching the threshold level because HHT shows an increase followed by a large decrease in the electrical activity around 610 s and then the same activity is repeated around 640 s.

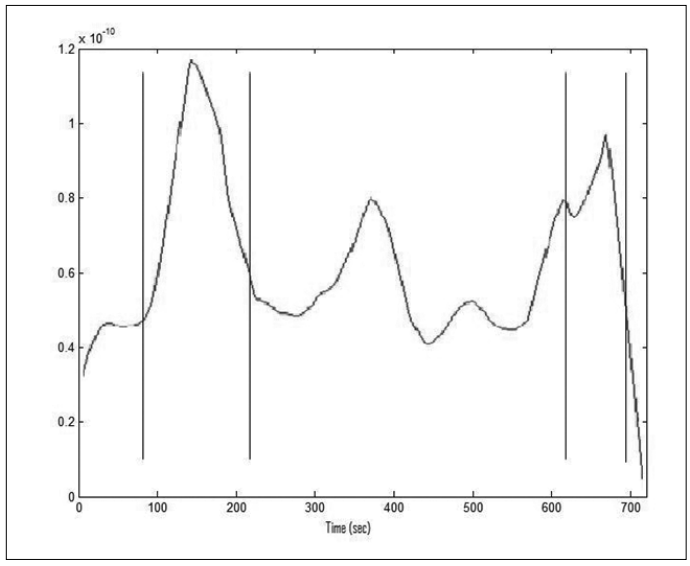

Figure 11. Mother's perception of two contractions

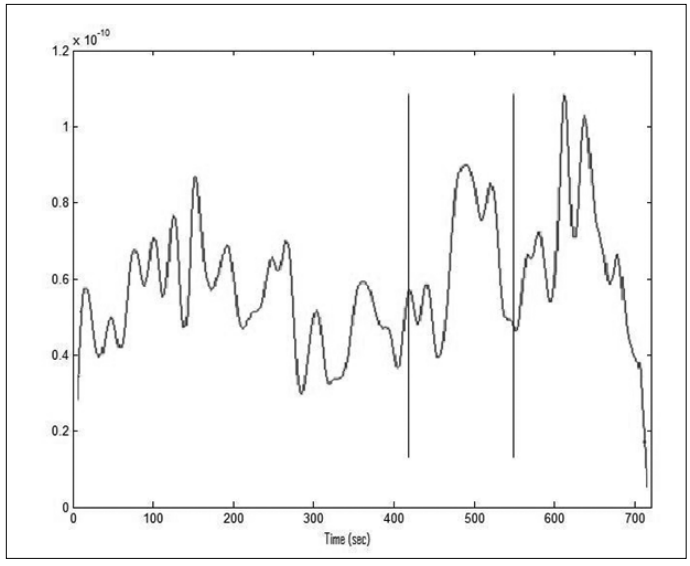

Figure 12. Mother's perception of one contraction

Discussion

The objective of this study was to determine whether using HHT to analyze the uterine contraction data would help us gain a better insight of the myometrial activities of the human uterus during pregnancy. Based on the results obtained from the experiments, HHT appears to be a promising approach in detecting uterine contractions and shows pressure changes in the womb without any invasive measurement such as IUPC. With appropriate filtering, a 75% correlation (square of the correlation coefficient, 0.8684) was revealed between the HHT and IUPC data, and there exists a delay of 9.72 s between electrical activity and mechanical activity. In addition to correlation, mother-perceived contractions also support HHT results.
Through the use of the EMD algorithm, noise was removed from the signal, thus discovering if the noise has the same frequency content as the original signal, it becomes harder to remove the noise from the signal. Our experiments showed that for a reliable separation of signal from noise, the ratio of the frequency contents should be at least 1.5. There is a need for timely, efficient, optimized, and non-subjective automatic contraction detection approaches in the field of prenatal examination. It is the intension of this study group to revise and refine the exiting contraction detection algorithm to be useful in the near future. With the current system, it is possible to identify the antipodal activities in the cellular community.

Limitations

Although the SARA system covers the entire pregnant abdomen with 151 sensors recording the magnetic field, at the rate of 250 samples/s and re-sampled with 25 samples/s, generated by the uterine myometrium, the primary frequency band of the uterine contractions are restricted between 0.1 Hz and 0.2 Hz, which represents underlying contraction activities (13). At this low frequency band rate, we are forced to use a window size of 20 s for computing the synchronization indices. To avoid the unneces-

sary distribution of the information collected from the uterine contractions, it is necessary to use small steps, more realistic windows size, and magnetic field data between 0.3 and 1 Hz for accurate analyses.

Also, the data used in this experimentation is pre-processed, i.e., that the original noise has been removed, and the effectiveness test was conducted using white noise. To test the reliability of the system, a new study is in progress to validate the complimentary findings of the method introduced here.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of the Institutional Review Board (IRB) (Protocol No #15-057).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - K.A., R.M.D., C.B.; Design - K.A., C.B.; Supervision - C.B.; Resource - K.A., C.B.; Materials - K.A., C.B.; Data Collection and/or Processing - K.A.; Analysis and/or Interpretation - R.M.D., C.B.; Literature Search - K.A., R.M.D., C.B.; Writing - K.A., C.B.; Critical Reviews - R.M.D.*

Acknowledgements: *This research is partly supported by National Institute of Biomedical Imaging and Bioengineering (NIBIB) of National Institute of Health under the grant number 1R33-EB00978-02. We also extend our thanks for Rathinaswamy B. Govindan for his valuable comments and feedbacks.*

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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Method comparison between Munich II and III nomenclature for Pap smear samples

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Abstract

Objective: Munich Nomenclature III for cervical smear evaluation also known as Papanicolaou (Pap) smear was launched in Germany in July 2014, and it is the only used system in Germany. The study aims at a method comparison between the previously used nomenclature Munich II and the currently used Munich III.

Material and Methods: A method comparison was performed by analyzing 117 Pap smear samples (pss) in the cytological laboratory of the department of Obstetrics and Gynecology of Luebeck University between January and March 2014. The samples were evaluated twice using both nomenclatures (Munich II and Munich III).

Results: One out of the 117 pss showed a loss of cellular material. According to Munich III, this Pap smear should be linked to group 0. Concerning Pap I, Munich II showed 0/117 pss (0%) and Munich III showed 55/117 pss (47%) cases ($p<0.001$). Pap II results were seen less frequently in Munich III than in Munich II (47% vs 93%, $p<0.001$). Pap IVa, IVb, and V stay similar in both nomenclatures [IVa: 1/117 pss (0.85%), IVb: 0/117 pss (0%) and V: 1/117 pss (0.85%)].

Conclusion: Patients at risk are clearly separated by Munich III from those with no evidence of pathology. The former clusters have been extended by distinctly defined subgroups, resulting in a more precise way to differentiate cytological findings. Differentiating between Pap IIID 1 and IIID 2 clearly separates mild and moderate dysplasia [cervical intraepithelial neoplasia (CIN) 1 (CIN 1) and CIN 2)].

(J Turk Ger Gynecol Assoc 2015; 16: 203-7)

Keywords: Munich nomenclature II, Munich nomenclature III, Pap smear

Received: 30 July, 2015 **Accepted:** 14 October, 2015

Introduction

Papanicolaou (Pap) smear samples have been shown to detect cervical cancer even without a minor surgical procedure. This was first demonstrated by Traut et al. (1) 1943. Cytological findings were originally classified into 5 groups by Papanicolaou et al. (2) in 1963 (Table 1).

Different classification systems for Pap smear samples have been internationally used (3). Germany applies the Munich nomenclature for Pap smear evaluation. The Munich nomenclature was established in 1975 as a modification of Papanicolaou's classification. This modification was necessary to meet the international requirements of a descriptive classification (4). In 1990, the Munich nomenclature was updated by the creation of Munich II. The revision of Munich II in 2013 led to the Munich III nomenclature in July 2014. Since January 1 2015, Munich III has been established as the only system officially used in Germany.

With Munich III, new subgroups were created to categorize different grads of dysplasia. Unclear findings that are neither clearly reactive nor meet certain criteria of dysplasia are now marked. Munich III further differentiates between squamous, epithelial, and glandular cells (5).

Furthermore, the Munich III system is attempting to make cytological findings transferrable to the internationally more commonly used The Bethesda System. This offers the opportunity to compare them with international studies (Table 2).

The aim of the study was a method comparison between Munich II and the newly defined nomenclature Munich III.

Material and Methods

Method comparison was done by analyzing 117 Pap smear samples in the cytological laboratory at the department of Obstetrics and Gynecology of Luebeck University between January and March 2014. All Pap smear samples were

Table 1. Papanicolaou's classification of cervical smear samples (2)

Class I	Absence of atypical or abnormal cells
Class II	Atypical cytology, but no evidence of malignancy
Class III	Cytology suggestive of but not conclusive of malignancy
Class IV	Cytology strongly suggestive of malignancy
Class V	Cytology conclusive for malignancy

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DOI: 10.5152/jtgga.2015.0147

Table 2. Comparison of classifications Munich II, Munich III, and The Bethesda System (modified after Griesser et al. (5), 2013)

Munich II Nomenclature	Munich III Nomenclature		The Bethesda System
	0	Unsatisfactory specimen → *repeat Pap smear*	Unsatisfactory for evaluation
I Normal cell pattern	I	Normal or unsuspicious cell pattern → *Pap smear next routine checkup*	NILM
	IIa	Normal cell pattern with suspicious patient history → *consider control Pap smear due to suspicious patient history (cytologic/ histologic/colposcopic/clinical findings)*	NILM
II Mild inflammatory, regenerative, metaplastic, or degenerative changes	II	Findings with low protective value	
	II-p	Squamous epithelium with low-grade changes of the nucleus; less than CIN 1 , also with coilocystic cytoplasm/paraceratotic changes → *if applicable, control Pap smear considering patient history and clinical findings (possibly after inflammation treatment and/or hormonal treatment; in special cases additional diagnostic methods and/or colposcopy)*	ASC-US
	II-g	Abnormal cervical glandular cells; more than reactive changes → *consider control Pap smear depending on patient history and clinical findings (possibly after inflammation treatment, in special cases additional methods and/or colposcopy)*	AGC endocervical NOS
	II-e	Endometrial cells; women >40 y.o. and second half of the cycle → *clinical checkup considering patient history and clinical findings*	Endometrial cells
III Unclear findings: severely inflammatory or degenerative and/or poorly preserved cell material; abnormal glandular or stromal cells; dysplasia, carcinoma in situ, or invasive carcinoma not excluded	III	Unclear findings	
	III-p	CIN 2/CIN 3/squamous cell carcinoma cannot be excluded → *colposcopy, if applicable additional diagnostic methods, possibly short-term re-pap smear after inflammatory treatment and/or hormonal treatment*	ASC-H
	III-g	Distinctive atypia of glandular cells, adenocarcinoma in situ/invasive adenocarcinoma cannot be excluded → *colposcopy, if applicable additional diagnostic methods*	AGC endocervical favor neoplastic
	III-e	Abnormal endometrial cells → *further clinical diagnostics, if applicable* with histological support	AGC entometrial
	III-x	Unclear glandular cells of unknown origin → *further diagnostics (e.g. diagnostic curettage; if applicable additional diagnostic methods/colposcopy)*	AGC favor neoplastic
IIID Cells of mild or moderate dysplasia	IIID	Dysplastic findings with greater tendency of regression	
	IIID 1	Cells of mild dysplasia (CIN 1) → *control Pap smear in 6 months, if persisting for >12 months; colposcopy, if applicable additional diagnostic methods*	LSIL

Table 2. Continue

	IIID 2	Cells of moderate dysplasia (CIN 2) → *control Pap smear in 3 months, if* persisting for >6 months; colposcopy, if applicable additional diagnostic methods	HSIL
	IV	direct pre-stages of cervical carcinoma → *colposcopy and therapy*	
IVA Cells of severe dysplasia or carcinoma in situ	IVa-p	Cells of severe dysplasia or carcinoma in situ (CIN 3)	HLIS
	IVa-g	Cells of adenocarcinoma in situ	AIS
IVB Cells of severe dysplasia or carcinoma in situ; cells of invasive carcinoma not safely excluded	IVb-p	CIN 3, invasion cannot be excluded	HSIL with features suspicious for invasion
	IVb-g	Cells of adenocarcinoma in situ, invasion cannot be excluded	AIS with features suspicious for invasion
V Cells of invasive cervical carcinoma or of other malignant tumors	V	Cells of invasive cervical carcinoma or of other malignant tumors → *further diagnostics including histology and therapy*	
	V-p	Squamous cell carcinoma	Squamous cell carcinoma
	V-g	Endocervical adenocarcinoma	Endocervical adenocarci noma
	V-e	Endometrial adenocarcinoma	Endometrial adenocarci noma
	V-x	Other malignant tumors, also of unclear origin	Other malignant neoplasms

NILM: negative for intraepithelial lesions or malignancy; ASC-US: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; AGC: atypical glandular endocervical cells; NOS: not otherwise specified; y.o.:years old; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells of undetermined significance cannot exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; AIS: adenocarcinoma in situ
Printed in italics: treatment recommendations in accordance with the involved medical societies (5)

analyzed at our cytological laboratory during this time period. No exclusion criteria were in use. All patient samples were evaluated twice by certified cytologists using the Munich II and Munich III nomenclatures. Informed patient consent and ethical approval (#12-234 Luebeck University) was obtained. Statistical analysis was performed with SPSS Statistics Version 22 (IBM Corporation; Armonk, USA).

Results

The classification of Pap I significantly differs in Munich II and III ($p<0.001$). Results are presented in table 3. While 0 of the 117 analyzed samples were classified as Pap I ("normal cell pattern") in Munich II, 55 were categorized as Pap I ("normal or unsuspicious cell pattern") in Munich III.

New subcategories in categories II and III of Munich III allow a more specific classification of Pap smear samples. Pap II findings were less frequently seen in Munich III (47% vs 94%, $p< 0.001$). Pap smear samples categorized in group III as "unclear findings" of the Munich nomenclatures stayed almost the same in both systems. One Pap smear sample showed the loss of cellular material. In Munich III, this Pap smear sample is now linked to the category 0 "unsatisfactory for evaluation." In Munich II, it has been classified in category III as an "unclear finding."

IIID Pap smear samples are subcategorized in Munich III in mild dysplasia (IIID 1) and moderate dysplasia (IIID 2).

The Pap smear samples classified as Pap IVa and Pap V in Munich II were classified as Pap IVa-p and Pap V-p due to the squamous cell origin.

Discussion

Many different cytology classification systems exist worldwide. European guidelines highly recommend that different systems should be transferrable into the internationally accepted and used The Bethesda System (3, 6). The German system, the Munich nomenclature, was created on the basis of the numerical Papanicolaou classification system for Pap smear samples (2). Pap smear evaluation and categorization are important for cervical cancer checkup. The incidences of cervical cancer have been reduced due to Pap smear examinations (5). Non-participation in cervical cancer screening is the most significant cause for persistent cervical cancer (7).

A detailed and exact classification system is essential to take necessary actions needed for treating cytological findings.

The new group 0 in Munich III clearly marks Pap smear samples unsatisfactory for evaluation and clears the former group III in Munich II. Pap smear findings with a benign background and findings that do not imply an increased risk of neoplasia

Table 3. Comparison of Pap smear samples classified in Munich II and Munich III

Munich II	Pss	Munich III	Pss	Significant difference between groups
I	0	I	55	p<0.001
II	110	II-a	32	p<0.001
		II-p	21	
		II-g	2	
		II-e	0	
III	4	III-p	3	ns
		III-e	0	
		III-x	0	
III D	1	IIID 1	1	ns
		IIID 2	0	
IVa	1	IVa-p	1	ns
		IVa-g	0	
IVb	0	IVb-p	0	ns
		IVb-g	0	
V	1	V-p	1	ns
		V-g	0	
		V-e	0	
		V-x	0	
		0	1	
n	117	n	117	

Pss: Pap smear samples; ns: not significant

(the Bethesda category "negative for intraepithelial lesions or malignancy" NILM) are now classified as Pap I. These findings include hormonal patterns, repair changes, microglandular hyperplasia, tubo-endometrioid metaplasia, tubal metaplasia, irradiation changes, alterations resulting from inflammation, or the presence of an intrauterine contraceptive device (8). These normal or unsuspicious cell patterns are classified as Pap I in Munich III. These findings were formerly classified as Pap II. Pap II is now reserved for findings of low protective value.

Pap III still marks unclear findings, but categorizes in the same way as the new Pap IV and V on histological characteristics now. This means that cells of squamous (-p), glandular (-g), or endometrial (-e) origin are clearly made visible with their suffixes. Cells of unknown origin get suffixed with "-x".

The Munich II system was criticized to link moderate with mild dysplasia (8, 9). In Munich III, Pap IIID is now subcategorized in IIID 1 [cervical intraepithelial neoplasia grade 1 (CIN 1)] and IIID 2 (CIN 2). Also, compared to The Bethesda System, Munich III differentiates between moderate- and high-grade dysplasia. CIN 2 is possibly remissible, which means that depending on colposcopic findings, surgery can be avoided (10). This fact made the new group IIID 2 necessary.

A limitation of our study is that we only investigated 117 Pap smear samples. The single-center design is another limitation. However, we still demonstrated with our study results the differences of the nomenclatures Munich II and III. The former clusters of Munich II have been extended by distinctly defined subgroups, resulting in a more precise way to differentiate cytological findings. Munich III clearly separates patients at risk from those with no evidence of pathology by the new definition of Pap I and II. In our case, 55 patients (47% of all Pap smear samples) now categorized as Pap I (normal or unsuspicious) in Munich III will receive the next Pap smear in the regular routine checkup interval. The same patients in Munich II (Pap II) had no statement concerning the unsuspicious presentation of the cervical smear. Due to the restrictive use of Pap II and the more precisely defined Pap III, the new nomenclature Munich III improves the positive predictive value (11).

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Luebeck University (#12-234).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - C.C., C.B., D.B.; Design - C.C., C.B., D.B.; Supervision - D.B.; Materials - D.B.; Data Collection and/or Processing - D.B.; Analysis and/or Interpretation - C.C., C.B., D.B.; Literature Search - C.C.; Writing - C.C., C.B.; Critical Reviews - C.C.*

Acknowledgements: *We thank Prof. Husstedt for his collaboration in the laboratory.*

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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Changing perspectives of infectious causes of maternal mortality

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Abstract

Objective: Infections significantly contribute to maternal mortality. There is a perceived change in the spectrum of such infections. This study aims to estimate the contribution of various types of infections to maternal mortality.

Material and Methods: We retrospectively reviewed records of maternal death cases that took place between 2003 and 2012 in the Christian Medical College, Vellore, India. The International Classification of Diseases-Maternal Mortality was used to classify the causes of deaths and World Health Organization near-miss criteria were used to identify organ dysfunction that occurred before death. Infections during pregnancy were divided into three groups, i.e., pregnancy-related infections, pregnancy-unrelated infections, and nosocomial infections.

Results: In this study, 32.53% of maternal deaths were because of some type of infection as the primary cause. The contribution of pregnancy-related infections was comparable with that of pregnancy-unrelated infections (16.03% vs. 16.50%). Metritis with pelvic cellulitis, septic abortions, tuberculosis, malaria, scrub typhus, and H_1N_1 influenza (influenza A virus subtype) were among the most commonly encountered causes of maternal death due to infections. Another 7.07% of cases developed severe systemic infection during the course of illness as nosocomial infection. A significant majority of mothers were below 30 years of age, were primiparae, had advanced gestational age, and had operative delivery. Cardiovascular and respiratory system dysfunctions were the most common organ dysfunctions encountered.

Conclusion: The contribution of pregnancy-unrelated infections to maternal deaths is significant. Control of these diverse community-acquired infections holds the key to a reduction in maternal mortality along with the promotion of clean birthing practices. Nosocomial infections should not be underestimated as a contributor to maternal mortality. (J Turk Ger Gynecol Assoc 2015; 16: 208-13)

Keywords: Infections, maternal mortality, organ dysfunction

Received: 03 July, 2015 **Accepted:** 14 October, 2015

Introduction

Puerperal sepsis was the most common cause of maternal death in the 19th century, accounting for up to 50% of all maternal deaths (1). The introduction of hand hygiene and sterilization practices, followed by the use of antibiotics, resulted in a sharp fall in the incidence of sepsis. However, puerperal infections are still reported as one of the major causes of maternal death, accounting for up to 15% of deaths (2-4). Puerperal sepsis is a polymicrobial infection of the genital tract caused by bacteria that normally inhabit the birth canal (5). In contrast, the term puerperal infection is used for genital tract infection along with other generalized infections that are incidental to pregnancy, such as tuberculosis, malaria, H1N1 influenza, and other endemic infections, including scrub typhus and dengue hemorrhagic fever (5). With the growing awareness and better availability of antiseptics and antibiotics, followed by control of bacterial genital sepsis, incidental systemic infections contributing to maternal death have increased. For example, in the sub-Saharan Africa, tuberculosis, pneumonia and meningitis, which are infections related to human immunodeficiency virus-acquired immunodeficiency syndrome (HIV-AIDS), have become major contributors to maternal mortality (6, 7). Poor sanitation, lack of proper housing, and poor vector control measures are the reasons for most of these vector-borne diseases.

Another important group is nosocomial infections, the contribution of which has gone unnoticed until now. With the control of delivery-associated and community-acquired infections in the Western world, hospital-acquired infections caused by resistant organisms are increasing.

Although prospective studies on genital sepsis associated with childbirth are available, there is a dearth of information regarding infections that are unrelated to pregnancy (community acquired). Nosocomial infections, which are acquired after admission to a hospital, remain largely unquantified in the south Indian population. This study aims to define the spectrum of infectious diseases that directly or indirectly contribute to maternal death in a hospital setting.

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DOI: 10.5152/jtgga.2015.0134

Material and Methods

This study is a retrospective review of maternal death cases that occurred in the Christian Medical College, Vellore, India between January 2003 and December 2012. This tertiary-level teaching hospital caters to the large local population of Vellore and adjoining districts. A considerable number of women with high-risk pregnancy are referred to this center for advanced care during pregnancy and delivery from the whole of southern India and beyond. It is a privately owned faith-based institute where mostly lower-middle-class population is treated and a fair proportion (20%–30%) of patients is treated without cost for charity. The busy labor room has approximately eight beds for high-risk patients and 28 for low-risk patients, and there are eight beds in a high-dependency unit attached to the labor room complex. An operating room and blood bank facilities are available to support our high-output labor room, which is run by four to five postgraduate-level doctors with a proportionate number of junior doctors and staff nurses round the clock. Antenatal outpatient visits and admission and labor records are diligently maintained by on-duty staff and preserved by the medical records department. An online record of outpatient visit proceedings, investigations, and a detailed discharge summary is maintained against the patient's registration number. For this study, we identified and completed information regarding maternal death cases through a review of records from the labor room, intensive-care units, and outpatient database and discharge summaries. Maternal deaths were defined and classified according to International Classification of Diseases-Maternal Mortality (ICD-MM) (8). Deaths due to infections as the primary cause were classified into pregnancy-related infection or puerperal sepsis (endometritis, peritonitis, pelvic abscess, surgical site infection, and necrotizing fasciitis) and non-pregnancy-related or incidental infections, such as malaria, tuberculosis, and pneumonia (9). Deaths were considered to be associated with hospital-acquired infection when after a primary non-infectious cause, women developed signs of severe systemic infection later during the course of illness before death (9).

Twenty-five World Health Organization (WHO) near-miss criteria (10) were used to identify organ dysfunction that occurred during the period preceding maternal death. These 25 criteria belong to three categories, namely clinical criteria, laboratory criteria, and intervention-related criteria. Each system dysfunction is identified by the presence of at least one WHO near-miss criterion specific to that system (11). Information was collected in an Epi Info™ (CDC; Atlanta, USA) database using the WHO near-miss tool (11), and statistical analysis was performed on an Excel spreadsheet (Microsoft; Washington, USA). This study was approved by the Institutional Ethics Committee and a waiver for informed consent was provided because of the retrospective nature of the study. This study was not funded.

Results

During the 10 years of the study period, there were 212 registered maternal deaths in the institute with 98,139 total births and 95,384 live births between 2003 and 2012. During this period, there were 28,788 cesarean deliveries with an average cesarean section rate of 29.33%. The average perinatal mortality rate was 35.391 per 1000 live births. Only in 154 out of the 212 cases were the details provided in the records sufficient enough to extract useful information in addition to the final diagnosis. There were 84 maternal death cases that revealed severe systemic infection either as the primary cause of death or acquired during the course of illness. Table 1 shows the causes of maternal deaths that had signs of severe systemic infection. In 16.03% of maternal death cases, pregnancy-related infections were found to be the primary cause, which includes deaths due to sepsis following abortions. Metritis with pelvic cellulitis (11.79%) was the single most important cause of pregnancy-related infection. Septic abortions were observed in 3.37% of cases. Pregnancy-unrelated infections as a group were observed in 16.47% as the primary cause. Tuberculosis (4.7%), which included pulmonary tuberculosis (eight cases) and tubercular meningitis (two cases), was the most common incidental or pregnancy-unrelated infection associated with maternal mortality. This was closely followed by malaria, H1N1 influenza, and scrub typhus with six cases (2.8%) each. Only one mother died of AIDS-associated pneumonia. Nosocomial infections significantly contributed (7.07%) to the morbidity of mothers as a secondary cause, all of which were defined as ventilator-acquired pneumonia.

The mean age of the women who died of infections was 23.98±4.15 years, with more than 95% of the mothers below 30 years of age (Table 2). Approximately 60% of the women who died were pregnant for the first time, with fewer women (15.78%) having two or more prior deliveries. Over 83% of women who died were in their third trimester of pregnancy, with more than 53.94% of women beyond 36 weeks of pregnancy. On comparing mothers with severe infection with those without

Table 1. Infectious causes of maternal mortality

Pregnancy-related infection	34 (16.03%)
Metritis with pelvic cellulitis	25 (11.79%)
Necrotizing fasciitis	1 (0.47%)
Chorioamnionitis	1 (0.47%)
Septic abortion	7 (3.3%)
Pregnancy-unrelated/Incidental infection	35 (16.50%)
Tuberculosis	10 (4.7%)
H1N1 influenza	6 (2.8%)
Scrub typhus	6 (2.8%)
Malaria	6 (2.8%)
Dengue hemorrhagic fever	3 (1.40%)
Typhoid	1 (0.47%)
Herpes zoster	1 (0.47%)
HIV with Pneumocystis carinii pneumonia	1 (0.47%)
Orbital cellulitis	1 (0.47%)
Hospital-acquired infection	15 (7.07%)
Ventilator-acquired pneumonia	15 (7.07%)
Total	84 (39.62%)

Table 2. Baseline characteristics

Age	Total=84 (100%)
20 years or less	24 (25.57)
21–30 years	56 (66.66)
More than 30 years	4 (4.76)
Parity	Total=76 (100%)
0	46 (60.52)
1	18 (23.68)
2 or more	12 (15.78)
Gestational age at delivery	Total=76 (100%)
20 weeks or less	8 (10.52)
21–28 weeks	5 (6.57)
>28–36 weeks	22 (28.94)
More than 36 weeks	41 (53.94)

Table 3. Obstetric and perinatal outcomes

Mode of termination of pregnancy	Total=82 (100%)
Vaginal delivery	40 (48.78)
Cesarean delivery	29 (35.36)
Surgical abortion	3 (3.65)
Complete abortion	2 (2.43)
Medicated abortion	2 (2.43)
Died pregnant	5 (6.09)
Neonatal outcome	Total=75 (100%)
Live birth	48 (62)
Stillbirth	27 (36)
Early neonatal death	18 (25)

infection, the cesarean section rate in the former group was significantly higher (42% vs. 29.33%) (Table 3). There was a decline in the number of deaths due to septic abortion over the duration of the study period, with no such case reported in the last 3 years. Neonatal outcomes were poor, with 36% stillbirths and another 25% neonatal deaths in the next 7 days of life (Table 3). Table 4 shows the incidence of organ dysfunction according to the WHO near-miss criteria, where the incidence in maternal mortality cases with severe infection is compared with the incidence in the group without severe infection. Respiratory dysfunction (90.47%) and cardiovascular dysfunction (70.23%) remain the most common dysfunctions. Coagulation (52.38%), hepatic (38.09%), and renal dysfunction (33.33%) criteria were next in the order of high incidence. There were no significant differences in the incidence of various organ dysfunctions in the infection group versus the no-infection group except in the cases of respiratory ($p<0.009$) and hepatic dysfunctions ($p<0.005$). Severe infection leading to hysterectomy (uterine dysfunction) was observed in 7.14% of women who died because of severe infection. Admission to the intensive-care unit was observed in 86% of mothers, and 54% of women received some form of blood transfusion during their stay in the hospital. The average duration (median) of hospital stay was 8 days, with 4 and 14 days as the 25th and 75th percentiles, respectively, whereas the average duration from delivery to death was also 8 days, with 6 and 13 days as the 25th and 75th percentiles, respectively.

Discussion

South Asia, including India, has observed a 63% fall in maternal mortality over the last 20 years (12). Even with this positive trend, India has contributed an estimated 50,000 maternal deaths, which is approximately 72% of maternal deaths in South Asia in the year 2013. Direct causes of maternal death remain the most important causes, constituting approximately 50% of all maternal deaths (13). Obstetric hemorrhage, hypertensive disorders of pregnancy, puerperal sepsis, and abortion-related deaths are the most important direct causes (14). All direct causes of maternal mortality are considered preventable; however, pregnancy-related infections (puerperal sepsis) have the highest case fatality ratio among them (15). There are three major groups into which infections in pregnancy have been traditionally classified, namely pregnancy-related infection, pregnancy-unrelated or incidental infection, and nosocomial infection (9). We found that 16.07% (34/212) of maternal deaths were due to pregnancy-related infections in our study, which includes 3.37% deaths due to septic abortions. Being mainly related to the cleanliness of the birthing environment, a reduction in pregnancy-related infection could be achieved after the introduction of antisepsis and antibiotics. In a large multicenter study (12) of 7065 sites and 188 countries that evaluated the causes of maternal deaths from 1990 to 2013, there was a reduction in maternal deaths due to sepsis from 12% to 9%. The clean birthing practices campaign, (16) training programs for skilled birth attendants, and promotion of institutionalized deliveries (Janani Suraksha Yojana) are some of the measures in India that played a pivotal role in bringing the puerperal sepsis rate down (17). Having said that, several large-scale multicenter studies have revealed that there is as much as a 50-fold difference in puerperal sepsis rates across centers in Africa and Asia (18). This is because of the lack of a standard definition and under reporting of sepsis cases (19, 20). Most of the studies from low- and middle-income countries are hospital based, which is not a true representation of the community. There is further under reporting because of the fact that early discharge of patients occurs as a result of a shortage of hospital beds and because puerperal fever typically only occurs 24–72 h after delivery, when women have already been discharged.

Chuang et al. (21), in their study of 220 cases of puerperal sepsis, found bacteremia associated with Group A streptococcus (GAS) to be the most common clinical presentation, followed by endometritis, peritonitis, necrotizing fasciitis, and toxic shock syndrome, in that order. Bacteremia with GAS organisms without any foci was not encountered in our cases of maternal deaths because it is amenable to treatment by potent antibiotics, and thus, is not fatal. The proportion of other clinical presentations of genital sepsis is similar to that of Chuang et al. (21). Studies (5, 22, 23) have identified several obstetric risk factors for puerperal sepsis, such as pre-labor rupture of membranes, prolonged

Table 4. Organ dysfunction in maternal mortality cases (infected versus non-infected group)

WHO Organ Dysfunction Criteria	Incidence of organ dysfunction Infected group (t=84) versus non-infected group (t=68) Total incidence (% with 95% CI)		
Cardiovascular dysfunction shock, use of continuous vasoactive drugs, cardiac arrest, cardiopulmonary resuscitation, severe hypoperfusion (lactate >5 mmol/L or >45 mg/dL), or severe acidosis (pH<7.1)	70.23 (60.45–80.01)	82.35 (73.35–91.41)	p=0.083
Respiratory dysfunction acute cyanosis, gasping, severe tachypnea (respiratory rate >40 bpm), severe bradypnea (respiratory rate <6 bpm), severe hypoxemia (PaO_2/FiO_2 <200, O_2 saturation <90% for 60 min), or intubation and ventilation	90.47 (85.2–95.74)	76.47 (68.01–84.93)	p=0.009
Renal dysfunction oliguria non-responsive to fluids or diuretics, dialysis for acute renal failure, or severe acute azotemia (creatinine >3.5 mg/dL)	33.33 (24.87–41.79)	41.17 (31.35–50.99)	p=0.159
Coagulation dysfunction failure to form clots, massive transfusion of blood or red cells (5 units), or severe acute thrombocytopenia (<50,000 platelets/mL)	52.38 (43.42–61.34)	50 (40.03–59.97)	p=0.385
Hepatic dysfunction jaundice in the presence of pre-eclampsia, severe acute hyperbilirubinemia (bilirubin >6.0 mg/dL)	38.09 (29.37–46.81)	19.11 (11.27–26.95)	p=0.005
Neurological dysfunction prolonged unconsciousness/coma (lasting >12 h), stroke, status epilepticus/uncontrollable fits, or total paralysis	23.81 (16.17–31.45)	20.58 (12.52–28.64)	p=0.317
Uterine dysfunction hemorrhage or infection leading to hysterectomy	7.14 (2.52–11.76)	11.16 (4.88–17.44)	p=0.193

PaO_2: partial pressure of oxygen in artery; FiO_2: fraction of inspired oxygen; CI: confidence interval

labor, multiple vaginal examinations (more than five), obstetrical maneuvers, anemia, primiparity, and poor nutrition. However, the single most important factor associated with infection is operative delivery. In this study, the cesarean section rate in maternal mortality cases with infection was 42% compared with 29.33% in cases without infection. It was further found by Smaill et al. (23) that endometritis is associated more with emergency cesarean section than with elective surgery. Rising trends in obesity and gestational diabetes are increasingly contributing to puerperal sepsis in the form of surgical site infections (24).

Infections unrelated to pregnancy are community-acquired infections, such as pulmonary tuberculosis, malaria, HIV-AIDS, H1N1 influenza, and other endemic infections, such as dengue hemorrhagic fever and scrub typhus. Estimates of the contribution of these infections to indirect causes or indeterminate causes remain largely unknown (25). Unrelated infections as a group were found to have claimed 16.47% (35/212) lives in this study, which is similar to pregnancy-related infections. Among all these incidental causes, tuberculosis remains the most important cause of death in the Indian subcontinent. In an estimate from 1999 (26), death due to tuberculosis was observed in more women in their reproductive age group (15–45 years) than all causes of maternal mortality put together. If diagnosed early and treated with multi-drug antitubercular treatment, the outcome in infected women is as good as in women without the disease. In contrast, with late treatment or no treatment, there is a high incidence of fetal growth restriction, preterm labor, and perinatal loss, along with increased maternal mortality and morbidity (27, 28). After tuberculosis, malaria is the second most common infection resulting in pregnant women dying worldwide. In sub-Saharan Africa, almost 25% of maternal deaths can be attributed to malaria or malaria complicated by HIV-AIDS (29). Pregnant women are three times more likely to suffer from a severe disease and have a 50% increase in mortality associated with it (30, 31). Primiparae are more likely to suffer from adverse maternal outcomes due to severe anemia and cardiac failure during labor, whereas multiparous women have milder maternal symptoms but suffer from disproportionately high perinatal morbidity and mortality due to innate immunity and placental sequestration of malarial parasites (30, 32).

This institution witnessed six clustered cases of maternal deaths with polymerase chain reaction-positive H1N1 influenza during the pandemic, which started in 2009 and peaked in 2011. In India, the largest numbers of deaths were reported during the same period (33). Pregnant women are at an increased risk of severe respiratory morbidity and mortality and adverse perinatal outcomes (34-36). Late recognition, delay in initiating antiviral treatment, and the presence of comorbid conditions, such

as asthma and diabetes, were found to increase morbidity and mortality (37).

Scrub typhus is a zoonosis that is endemic to the Asia-Pacific region (38). It is poorly studied in the Indian subcontinent and its contribution to maternal death remains unmeasured. In a case series by Mahajan (39), five pregnant women diagnosed with scrub typhus were studied. One woman died of multi-organ failure. In this study, there were six maternal deaths attributable to scrub typhus during the 10-year period. The disease mainly presents as rash, fever, myalgia, and lymphadenopathy. Complications that develop after a week include jaundice, septic shock, pneumonitis, myocarditis, and meningoencephalitis (39).

There were four HIV-positive women who died during these 10 years in our hospital, but only one death could be attributed to an HIV-AIDS-related complication (Table 1). For Tamil Nadu (a southern state in India), which is considered to have a high prevalence of HIV, this finding appears biased (40). A possible explanation is that being a privately owned institute, there are fewer HIV-positive women registered in our hospital because the Indian government provides antiretroviral therapy and all delivery services to these mothers free of cost in public-sector hospitals. After the increase in coverage of antiretroviral therapy, there is reduced HIV-related mortality among pregnant women (41).

The concept of near miss has led to the appreciation of all events that follow the primary cause leading to severe maternal outcomes. In this context, we could focus on the fact that many patients who have obstetric hemorrhage or pregnancy-induced hypertension as the primary cause end up in an intensive-care unit for a long time and may develop a nosocomial infection. Ventilator-associated infections are the most serious among these, others being urinary tract infections, bedsores, etc. The contribution of hospital-acquired infections to morbidity and mortality specific to pregnancy is largely unknown. This study estimated that 7.07% (15/212) of women developed a nosocomial infection before they died. All of these had ventilator-associated pneumonia as a nosocomial infection in this study. With the increase in hospital deliveries, increasing use of antibiotics, and widespread availability of intensive-care unit facilities, nosocomial infections are on the rise and will continue adversely affecting outcomes unless kept in check.

Organ dysfunction criteria are used to access the severity of the maternal disease and prevent severe maternal outcomes (42). Cardiovascular and respiratory dysfunctions are the most commonly encountered organ dysfunctions: 70.23% and 90.47%, respectively, in mothers who died of severe infection. The organ dysfunctions that were significantly different from the non-infected group are respiratory dysfunction (90.47% vs. 76.47% $p<0.009$) and hepatic dysfunction (38.09% vs. 19.11% $p<0.005$). The first finding may be because of many infections specific to the respiratory system, such as tuberculosis and H1N1 influenza, whereas the second observation is because of more chances of multi-organ dysfunction in the puerperal infection group.

In conclusion, infections during pregnancy continue to be a major contributor to maternal mortality, although the proportions of causes are changing. Pregnancy-unrelated or incidental infections are as important, if not more so, as a contributor to maternal mortality compared with genital sepsis. Along with clean birthing practices and the use of prophylactic and therapeutic antibiotics, the fight has to be continued against tuberculosis and vector-borne diseases to avert these preventable maternal deaths. Awareness, primary prevention, early diagnosis, and treatment are required to control seasonal and epidemic diseases, such as malaria, H1N1 influenza, and dengue. Education, prevention of anemia, food fortification, and a midday meal program in schools will pay sustained dividends by ensuring that girls become stronger mothers of tomorrow.

Limitations of the study, records were deficient in being able to provide complete data. The diseases were diagnosed with methods that were available at the time and at times, on the clinical decision of the treating physician. Therefore, the classification of deaths according to a specific causative agent is less than accurate.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Christian Medical College Vellore, India.*

Informed Consent: *Exemption from informed consent was received from Ethics committee as it was a retrospective review of death records which took place over a decade.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - A.H., R.J.; Design - A.H., R.J.; Supervision - A.H., R.J., R.V.; Resource - A.H., R.V.; Materials - A.H., R.J., R.V.; Data Collection and/or Processing - A.H., R.J., R.V.; Analysis and/or Interpretation - A.H., R.J., A.H., R.V.; Writing - A.H., R.J.; Critical Reviews - A.H., R.J., R.V.*

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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Relationship between uterine natural killer cells and unexplained repeated miscarriage

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Abstract

Objective: To evaluate the relation between uterine killer (uK) cells and unexplained repeated miscarriage (RM).

Material and Methods: Eighty women with unexplained repeated miscarriage and missed miscarriage of current pregnancy were studied. Fetal viability and gestational age of the current pregnancy were confirmed by ultrasound, followed by suction evacuation to collect abortion specimens and uterine wall curettage to collect decidua specimens. Abortion specimens were collected for long-term monolayer cell culture and subsequent chromosome analysis using conventional G-banding. Decidua specimens were subjected to immunohistochemical staining using monoclonal antibodies specific to CD56+ and CD16+ expressed by uK cells.

Results: CD56+ CD16+ uK cells were found in 85% [68/80] of the studied decidua specimens of women with unexplained repeated miscarriage; 88.5% [54/61] had normal abortion karyotyping and 73.7% [14/19] had abnormal abortion karyotyping.

Moreover, 73.75% [59/80] of the studied women with a past history of early miscarriage had CD56+ CD16+ uK cells in their decidua specimens, and 66.25% [53/80] of studied women with a past history of late miscarriage had CD56+ CD16+ uK cells in their decidua specimens; the association between early and late miscarriage and CD56+ CD16+ uK cells in decidua specimens was significant.

Conclusion: CD56+CD16+ uK cells were predominant in the decidua specimens of the studied women with repeated miscarriage. A significant association was found between the presence of CD56+ CD16+ uK cells in the studied decidua specimens and unexplained repeated miscarriage.

(J Turk Ger Gynecol Assoc 2015; 16: 214-8)

Keywords: Obstetrics, uterine killer cells, repeated miscarriage

Received: 27 April, 2015 **Accepted:** 14 October, 2015

Introduction

Repeated miscarriage (RM) is defined as two or more failed pregnancies (confirmed by ultrasound or histopathological examination) and is known to affect approximately 0.5–1% of couples (1).

One miscarriage increases the risk of miscarriage in future pregnancy to 24%; this risk increases to 26% with two previous miscarriages and reaches 32% with three previous miscarriages; thus, women with two or more consecutive miscarriages merit meticulous study to detect the definite cause and possible treatment (2-4).

Various factors are implicated in the pathophysiology of repeated miscarriage. Fetal causes such as single gene or genomic imprinting defects account for 3.5–5% of the cases of repeated miscarriage; other fetal defects include fetal infections and developmental abnormalities (5). Maternal causes of repeated miscarriage include immunological causes, accounting for 30% of the cases, with anti-phospholipid antibody syndrome being the most common autoimmune cause (6, 7). Endocrine dysfunction accounts for 48.71% of the cases, while other maternal factors, including anatomical detects and subclinical endometrial infection, account for a minimal number of cases (8, 9).

Approximately 50% of repeated miscarriages are unexplained, with no definitive etiology. Several authors suggest the cause to be alloimmune rejection of the fetus (10).

Natural killer (NK) cells are immune system lymphocytes (11, 12). Uterine killer (uK) cells are short-lived lymphocytes found in uterine deciduas (13). Early in pregnancies, uK cells produce angiogenic factors and are believed to be important for implantation and development (13, 14).

Uterine killer cells have been linked to human reproductive disorders, including repeated miscarriage, implantation failure, fetal growth restriction, and preeclampsia (15, 16). These cells secrete cytokines and angiogenic factors, which are important for placental development and pregnancy establishment (16).

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DOI: 10.5152/jtgga.2015.0082

It has been found that 37.3% of patients who presented with repeated miscarriage had a mild to moderate increase in NK cells and that 14.7 % of women with repeated miscarriage had elevated levels of $CD56^+$ NK cells in peripheral blood (17, 18).
Other authors concluded that the cytotoxicity of NK cells is unrelated to the number of peripheral NK cells and that it can be estimated by NK cell markers such as killer inhibitory receptors (KIRs) or $CD16^+$ receptor expression (19).
Because more research is needed to establish the relationship between uK cells and human reproductive disorders (20, 21), this study was designed to evaluate the relationship between uK cells and unexplained repeated miscarriage.

Material and Methods

Eighty women with unexplained repeated miscarriage and missed miscarriage of current pregnancy were included for evacuation and curettage because of a current missed miscarriage (>8 weeks, diagnosed by ultrasound). The women were studied after proper counseling, consent, and approval of the ethical committee. Unexplained repeated miscarriage was defined as ≥2 previous miscarriages after <20 weeks' gestation. A thorough history was obtained and a thorough examination was performed for all studied women, followed by trans-vaginal ultrasound to confirm fetal the viability and gestational age of the current pregnancy by a sonographer who was blinded to the patients' data. Peripheral venous samples were collected from the studied women for oral glucose tolerance tests; thyroid stimulating hormone assays; prolactin, serum anticardiolipin, and lupus anticoagulant assays; as well as activated protein C resistance tests, Leiden factor V and prothrombin gene mutations, and protein C, S, and anti-thrombin III deficiency tests. Women with septic miscarriage, documented endocrinopathies (diabetes, thyroid disorders, or hyperprolactinemia), uterine anomalies, polycystic ovary syndrome, anti-phospholipid antibody syndrome, thrombophilia, abnormal karyotype in one or both parents determined by leukocyte culture, autoimmune disorders, history of hormonal contraception, and a history of intrauterine contraceptive device application within the last three months preceding current pregnancy were excluded from this study. Evacuation and curettage were performed for all women included in this study under general anesthesia using suction evacuation to collect abortion specimens after cervical dilatation, followed by uterine wall curettage to collect decidua specimens. Abortion specimens were collected in a special medium for long-term monolayer cell culture and subsequent chromosome analysis using conventional G-banding. Decidua specimens were subjected to immunohistochemical (IHC) staining using monoclonal antibodies specific to uK cells $CD56^+$ and $CD16^+$.

Reagents and materials used include the following:

1. Primary antibodies: Liquid monoclonal mouse antibody (MoAb) against $CD56^+$ and $CD16^+$ expressed on NK cells.
2. Universal Kits: Supersensitive immunodetection system (Biogenex Laboratories; San Francisco, USA), containing the following: a) negative control antibody; b) biotinylated anti-immunoglobulin for mouse antibody; c) label: streptavidine peroxidase complex; d) chromogen: 2.3 diaminobenzidine chromogen (DAB) solution, ready to use substrate buffer, and H_2O_2 substrate for use with liquid DAB chromogen and substrate buffer; e) blocking reagent to block endogenous peroxidase activity.
3. Lyophilized pepsin powder, phosphate buffer saline (PBS), counter stain (Mayer's hematoxylin), distilled water, and mounting media (Canada balsam).
4. Staining jars, microscopic positive charged slides, cover slips for slides, and immune-stainer.
5. Light microscope with 100x and 400x magnification.

IHC procedure: Decidua specimens were fixed in buffered formalin (not more than 24 hours) and embedded in paraffin wax; 3-micometer sections were mounted onto 3-aminopropyltriethoxysilane, (Sigma Chemical Co.; Poole, UK); then serial sections were stained for uK cells ($CD56^+$ and $CD16^+$) using antibody antigen-retrieval methods (22, 23).
Primary antibodies were incubated for 60 minutes for $CD56^+$ and for 120 minutes for $CD16^+$ at room temperature; the brown staining intensity of the reaction developed with 2.3 DAB containing 0.01% H_2O_2 was noted, and the sections were counterstained with hematoxylin, then dehydrated and mounted with distyrene, plasticizer, xylene (DPX) standard resin (Lamb Ltd.; London, UK), then examined by ordinary light microscopy. Appropriate positive controls (neuroblastoma for $CD56^+$ and tonsils for $CD16^+$) were used in each run to judge the effectiveness of the staining technique, and mouse immunoglobulin-G (Ig-G) antibodies were used instead of primary antibodies as negative controls.

Sample size and statistical analysis

The required sample size to produce statistically acceptable figure was 80 women, and this sample size was calculated using G Power software (Heinrich Heine Universität; Düsseldorf, Germany) for sample size calculation. Mean±SD was used to represent numerical values, while number (n) and percentage (%) were used to represent categorical values. Comparison between the variables was done using the Chi-square (x^2) test. A difference with $p<0.05$ was considered statistically significant.

Results

The mean age of the women included in this study was 29.6±6.39 years and the mean body mass index (BMI) was 26.9±4.5 kg/m^2. Karyotyping studies of the abortion specimens showed normal karyotyping in 76.25% [61/80] of the studied specimens and abnormal karyotyping in 23.75% [19/80] of the studied specimens (Table 1).
$CD56^+$ $CD16^+$ uK cells were found in 85% [68/80] of the studied decidua specimens of women with unexplained repeated miscarriage; 88.5% [54/61] had normal abortion karyotyping and 73.7% [14/19] had abnormal abortion karyotyping (Table 2).
73.75% [59/80] of the studied women with a past history of early miscarriage had $CD56^+$ $CD16^+$ uK cells in their decidua specimens and 66.25% [53/80] of the studied women with a past history of late miscarriage had $CD56^+$ $CD16^+$ uK cells in their decidua specimens; the association between early and late miscarriage and $CD56^+$ $CD16^+$ uK cells in the decidua specimens was significant (Table 3).

Table 1. Karyotyping analysis of the studied specimens

Variable	Number (n)	Percentage (%)
Normal female karyotype	58	72.5
Normal male karyotype	3	3.75
Abnormal karyotype	19	23.75
Triploidy	5	6.25
Tetraploidy	10	12.5
Aneuploidy	4	5.0
Total	80	100

Table 2. Relationship between karyotypes of abortion specimens and immunohistochemical results of decidua specimens

Variable	$CD56^+$ $CD16^+$ uterine killer cells n (%)
Normal karyotype (61 cases)	54 (88.5%)
Abnormal karyotype (19 cases)	14 (73.7%)
Total	68 (85%)

CD: classification determinant
CD receptors expressed on uterine killer cells.

Table 3. Relationship between CD56+ CD16+ uterine killer cells and miscarriage (early and late)

Variables	$CD56^+$ $CD16^+$ uterine killer cells n (%)
Number of early miscarriages	
1	13 (16.25%)
2	25 (31.25%)
3	12 (15%)
>3	9 (11.25%)
Total (80 cases)	59 (73.75%)
Number of late miscarriages	
0	4 (5%)
1-2	53 (66.25%)
Total (80 cases)	57 (71.25%)

CD: classification determinant
CD: receptors expressed on uterine killer cells.

Discussion

Early in pregnancies, uK cells produce angiogenic factors and are believed to be important for implantation and development. uK cells have been linked to human reproductive disorders, including repeated miscarriage, repeated implantation failure, fetal growth restriction and preeclampsia (15, 16); this study was designed to evaluate the relationship between uK cells and unexplained repeated miscarriage.

CD16 is expressed by most natural killer cells, neutrophils, and activated macrophages. CD56 is an isoform of the neural cell adhesion molecule and is expressed on natural killer cells, cytotoxic T lymphocytes, and neural-derived cells. Peripheral natural killer (pNK) cells have been found in both peripheral blood and endometrium. Although both pNK and uterine natural killer (uNK) cells express the surface antigen CD56, they are phenotypically and functionally different (11). Studies have shown that 90% of pNK cells express a $CD56^{dim}$ $CD16^+$ phenotype, while 80% of uNK cells express a $CD56^{bright}$ $CD16^-$ phenotype; the CD56 cells are known to have a regulatory function, while the CD16 cells have a cytotoxic function (24-26).

In humans, it has been proved that elevated circulating cytotoxic NK cells (not the count) increase the risk of miscarriage (27). Women <35 years old with unexplained repeated miscarriage were studied to minimize the risk of chromosomal abnormalities and miscarriages associated with advanced maternal age (28).

The BMI of women included in this study was 26.9±4.5 kg/m^2; this might be due to our selection criteria, as we excluded some risk factors that might predispose the women to RM, such as obesity (29, 30), diabetes mellitus, and thyroid disorders. Eighty abortion specimens were cytogenetically analyzed using tissue culture and conventional G-banding because comparative genomic hybridization (without culture) was not introduced in our institute until recently. Cytogenetic analysis using tissue culture and conventional G-banding has some limitations, including contamination, culture failure, and maternal cell growth (31). In this study, karyotyping studies showed normal karyotyping in 76.25% [61/80] of the studied abortion specimens and abnormal karyotyping in 23.75% [19/80] of the studied abortion specimens. A 29 to 57% rate of chromosomal abnormality was previously reported during analysis of miscarried tissue from women suffering RM (32-35), and the higher number of normal chromosomes studied in the miscarried tissue of women with RM confirms that there may be factors other than chromosomal abnormalities associated with RM (36).

$CD56^+$ $CD16^+$ uNK cells were found in 85% [68/80] of the studied decidua specimens of women with unexplained repeated miscarriage. Quenby et al. (16) reported that women with RM had significantly more uNK than controls, and Clifford et al. (37) also showed increased CD56+ uK cells in women with unexplained repeated miscarriage.

Increased expression of $CD56^+$ $CD16^+$ uNK was also reported in deciduas obtained after spontaneous miscarriage in women with a history of repeated miscarriage (38).

Quenby et al. (39) used IHC to investigate leukocyte populations in mid-luteal endometrial biopsies of 22 women suffering from RM compared to 9 women without RM, they found that $CD4^+$, $CD14^+$, $CD16^+$, and $CD56^+$ uNK cells were significantly higher in the RM group than in controls.

Lachapelle et al. (40) compared endometrial specimens from 20 women with RM with endometrial samples collected during the secretory phase from 15 fertile controls. Lachapelle et al. (40) found that the percentage of uK was similar in the two groups, although a greater percentage of $CD56^+$ $CD16^+$ uK was found in women with RM.

$CD56^+$ $CD16^+$ uNK cells were found in 85% [68/80] of the studied decidua specimens of women with unexplained repeated

miscarriage; 88.5% [54/61] had normal abortion karyotyping and 73.7% [14/19] had abnormal abortion karyotyping. This difference was statistically not-significant. Yamamoto et al. (41) reported the same findings when they studied uNK cells in decidua specimens of both chromosomally normal and abnormal missed miscarriages.

Although in this study we found that the expression of $CD56^+$ $CD16^+$ uNK cells in decidua specimens of women with RM is high, Yamamoto et al. (41) did not find overexpression of $CD56^+$ $CD16^+$ uK cells in the deciduas of studied women with missed abortions, because their study was limited to sporadic cases of missed abortions, not RM cases.

73.75% [59/80] of studied women with a past history of early miscarriage had $CD56^+$ $CD16^+$ uK cells in their decidua specimens and 66.25% [53/80] of studied women with a past history of late miscarriage had $CD56^+$ $CD16^+$ uK cells in their decidua specimens; the association between early and late miscarriage and $CD56^+$ $CD16^+$ uK cells in the decidua specimens was significant. The findings of this study suggest that $CD56^+CD16^+$ uK cells are predominant in the decidua of women with RM. Women refused to participate in the study and the use of tissue culture and conventional G-banding for cytogenic analysis and karyotyping of abortion specimens were limitations during this study. Further large case-controlled studies are needed to compare decidua specimens from RM cases with decidua specimens from normal cases without RM to establish the relationship between uK cells and human reproductive disorders and to improve future treatment for such cases.

Ethics Committee Approval: *Ethics committee approval was received for this study from the local ethics committee of Ain Shams University, Maternity Hospital.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - M.F., A.E., K.S.; Design - M.F., A.E., K.S.; Supervision - K.S., A.R., E.E.; Resource - I.A., K.S., A.R.; Materials - M.F., A.E., M.G.; Data Collection and/or Processing - I.A., M.F., A.E.; Analysis and/or Interpretation - I.A., M.F., A.E.; Literature Search - I.A., M.G., A.E.; Writing - I.A., M.G., A.E.; Critical Reviews - A.R., M.G., E.E.*

Acknowledgements: *The authors wish to thank all women participated in this study and Professor Nahla M. M. Awad, Assistant consultant of pathology, early cancer detection unit at Ain Shams maternity hospital, for her great participation in this research.*

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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Effects of anesthesia type on short-term postoperative cognitive function in obstetric patients following cesarean section

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Abstract

Objective: We aimed to compare the effects of general and spinal anesthesia on cognitive functions in pregnant patients undergoing elective cesarean section.

Material and Methods: Seventy-five American Society of Anesthesiology (ASA) I pregnant patients aged 18–40 years who were scheduled to undergo elective cesarean section were divided into three groups. Group sevoflurane (Group S) and Group desflurane (Group D) were administered general anesthesia, whereas Group regional (Group R) was administered spinal anesthesia. Hemodynamic variables, bispectral index, oxygen saturation were measured at baseline, after induction, spinal injection, and during the surgery. Extubation and eye opening time and Aldrete scores were recorded. Mini-mental state examination, Trieger dot test, and clock drawing test were performed one day before the surgery and repeated at the 1st, 3rd and 24th h postoperatively.

Results: There was no statistically significant difference among the groups in terms of demographic data and duration of surgery ($p>0.05$). Durations of anesthesia for Group S, Group R, and Group D were significantly different ($p<0.05$). Duration of anesthesia for Group R was significantly longer than for Groups S and D ($p<0.0001$). Aldrete recovery scores and total remifentanil consumption were significantly higher in Group D than in Group S ($p<0.05$). Extubation and eye opening times were significantly shorter in Group D than in Group S ($p<0.01$). According to TDT, statistical significance was found among Group S, Group R, and Group D at the 3rd and 24th h postoperatively ($p<0.05$), and there was a statistically high significant difference in Groups S and R ($p<0.0001$).

Conclusion: We concluded that general anesthesia with sevoflurane or desflurane and spinal anesthesia had no effects on cognitive functions in patients undergoing cesarean operation. (J Turk Ger Gynecol Assoc 2015; 16: 219-25)

Keywords: Sevoflurane, desflurane, general anesthesia, spinal anesthesia, cognitive function, cesarean section

Received: 16 April, 2015 **Accepted:** 15 August, 2015 **Available Online Date:** 02 November, 2015

Introduction

Postoperative cognitive dysfunction (POCD), a common brain complication following surgery and anesthesia, is characterized by the impairment of recent memory, concentration, language comprehension, and social integration. While the diagnosis of delirium requires detection of symptoms, the diagnosis of POCD requires preoperative neuropsychological testing (baseline) and a determination that defines how much of a decline is called cognitive dysfunction (1). POCD may occur after any type of surgery and at any age but is most often associated with cardiac surgery and in patients over the age of 60 years (1, 2).

Although anesthetics have effects on organs and systems, their main impact is on the central nervous system, and this situation leads to affect the cognitive functions which is an upper brain activity in varying degrees and periods after general anesthesia. Rapid recovery of patients who have been under general anesthesia and their returning to their pre-anesthesia condition mentally are major goals for anesthetists (3, 4). It takes time for psychomotor functions to return to the preoperative levels after the anesthesia is ended (5). It has been demonstrated that after being exposed to anesthetics, psychomotor and cognitive functions are impaired for 10–12 h. This impairment can last for 1-2 days, and these symptoms may even occur with the smallest anesthetic administration (6, 7). Furthermore, in the post-anesthetics period, memory is more affected in young patients, whereas the mental organizations are more affected in elderly patients (3, 8).

The choice of an anesthetic method for cesarean operations depends on the reason for the procedure, its degree of urgency, and on the preference of both the patient and anesthetist. There is no anesthetic method ideal for cesarean operations. The anesthetist must choose a method that they believe is the safest and most comfortable for the mother, has the least depressant effect on the newborn, and ensures optimal operat-

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DOI:10.5152/jtgga.2015.15073

ing conditions for the surgery. Although there are studies that suggest that general anesthesia and insufficient postoperative pain control play a role in the development of early POCD (9, 10), there are other studies asserting that the type of anesthesia (general or regional) does not affect the formation and development of POCD (11).

A study focusing on the comparison of POCD after cesarean operations using spinal anesthesia and general anesthesia has not been found in literature. Therefore, this study aims to compare the effect of sevoflurane, desflurane, and spinal anesthesia on intraoperative hemodynamics, recovery, and early cognitive functions in patients who will undergo elective cesarean operation.

Material and Methods

Following the approval of the Faculty Ethics Committee (No: Jan 2013/311), 75 pregnant women who will undergo elective cesarean operation, are aged ≥18 years, have ASA I physical status, are in the full term, and whose consents were taken were included in the study. Emergency patients, multiple and pre-term pregnancies, growth retardations, patients known to have congenital malformation, patients known to have neurological or psychiatric diseases affecting the central nervous system (CNS) and cognitive functions, patients who were taking medications affecting CNS, patients with malnutrition and dehydration, patients known to have low levels of vitamin B12 and folic acid, patients with alcohol or any substance addictions, and patients aged >40 years were excluded from the study.

Patients included in the study were divided in three random groups: as undergoing general anesthesia with sevoflurane (Group S, n=25), undergoing general anesthesia with desflurane (Group D, n=25), and undergoing regional anesthesia via spinal injection (Group R, n=25). It was ensured that from arrival at the operating room to the end of the procedure, all pregnant women lay on their left side at 15°. To determine the levels of cognitive functions of patients, the Trieger dot test (TDT), mini-mental state examination (MMSE), and clock drawing test (CDT) have been performed one day before the procedure. In patients taken to the operating room, peripheral vascular access was established with the 18 gauge cannula, and 500 cc of 0.9% NaCl solution was administered until anesthesia induction. Electrocardiogram, peripheral oxygen saturation (SpO_2), and non-invasive arterial pressure monetarization (Datex-Ohmeda S/5 Anesthesia Delivery Unit, Bromma, Sweden) were performed, and none of the patients were pre-medicated.

Systolic artery pressure (SAP), diastolic artery pressure (DAP), mean arterial pressure (MAP), SpO_2, heart rate (HR), and bispectral index (BIS) (Drager infinity kapa, Drager medical systems Inc., Denvers, USA) levels were monitored, and the measured values were recorded as baseline values. In the general anesthesia groups, these measurements were recorded after general anesthesia induction, after intubation, in every 5 min from the beginning and the end of the procedure, during extubation, and 5 min after extubation. In general anesthesia groups, in addition to these measurements, the measurements of intraoperative end tidal sevo/des % with 5-min intervals, mean end tidal sevo/des % at the end of the case, and total consumed remifentanil (Ultiva flk®, GlaxoSmithKline, İstanbul, Turkey) dosage and end tidal carbon dioxide measures were also recorded.

In all three groups, patients were administered pre-oxygenation for 3–5 min with 100% oxygen. In Groups S and D, for anesthesia induction, 0.5 μg/kg remifentanil was administered as a bolus for 30 s, and then 0.2 μg/kg/m remifentanil infusion was continued and induction was completed with 4 mg/kg thiopental (Pental flk®, İE Ulagay İlaç, İstanbul, Turkey) and 1 mg/kg succinylcholine (Lysthenon amp®, Fako İlaç, İstanbul, Turkey). For maintenance of anesthesia, Group S was administered 0.5 MAC sevoflurane, 50% O_2+50% N_2O; and Group D was administered 0.5 MAC desflurane, 50% O_2+50% N_2O. Cricoid pressure was applied to patients along with anesthesia induction. After maintaining relaxation, endotracheal intubation was performed, and cricoid pressure was discontinued by inflating the cuff. Patients were ventilated with a tidal volume of 8-10 mL/kg and respiratory rate of 10-12/min to achieve an $EtCO_2$ of 30-34 mmHg. 10–12/m respiration frequency to provide end tidal CO_2 30–34 mmHg. A 20% increase in MAP and/or >90 pulse/m HR or >60 BIS levels of the patient after the baby was born was considered as light anesthesia, and remifentanil dosage was increased by 25%. When the increase in remifentanil dosage was insufficient, the dosage of general anesthetic agent was increased. A 20% decrease in the MAP value and/or <50 pulse/m HR and/or <40 BIS value was considered as deep anesthesia, and remifentanil dosage was decreased by 25%. When the decrease in remifentanil dosage was insufficient, the dosage of general anesthetic agent was decreased. Neuromuscular transmission was monitored in patients. Patients were administered succinylcholine as a neuromuscular blocking agent, and neuromuscular transmission (NMT) (Infinity Trident®, Drager, Germany) measures were obtained with a single-twitch method. When the twitch had recovered to 0.20 of its control value 0.1 mg/kg atracurium (Dematrac®, Dem İlaç, İstanbul, Turkey) was administered. During the procedure, anesthetic and analgesic requirements were adjusted to maintain MAP and HR values at a ±20% limit of the baseline value, and the BIS value between 40 and 60. After the baby was born, 10 iu of oxytocin were slowly intravenously administered. Further, 10–20 iu of oxytocin were added to 1000 mL isotonic. General anesthetic agent was discontinued at the start of skin closure while remifentanil infusion was terminated at the completion of fascia closure. To provide postoperative analgesia, 2 mg/kg tramadole (Contramal®, Abdi İbrahim İlaç, İstanbul, Turkey) and 20 mg tenoxicam (Oksamen®, Mustafa Nevzat İlaç, İstanbul, Turkey) were intravenously administered after the baby was born. At the end of the procedure, when the TOF ratio (ratio of forth twitch to the first) returned to at least 0.9, the patients were extubated. Then the patients were followed in the postanesthesia care unit. In groups undergoing general anesthesia, Aldrete recovery scores were recorded in the 2^{nd} and 5^{th} minutes. In the groups undergoing general anesthesia, the duration of anesthesia was recorded as the time between the beginning of anesthesia induction and extubation, and extubation time was recorded as the period between termination of all anesthetic medicine and the time when extubation was possible. For individuals in Groups S and D, in addition to extubation times, spontaneous eye opening times at the end of

the procedure were also recorded. The duration of the surgery was recorded as the period between the first skin incision and last skin suture.

In the spinal anesthesia group, Group R, the levels of SAP, DAP, MAP, SpO_2, HR, and BIS for all pregnant women were monitored, and the measured values were recorded as baseline. In Group R, these measurements were recorded after spinal injection with 5-min intervals between the beginning and end of the procedure. Skin disinfection for pregnant women in Group R was performed in the sitting position with the antiseptic solution, and the subarachnoid space was penetrated between the L3-L4 or L4-L5 gap with a 25G atraumatic spinal needle from midline. After free, clean cerebrospinal fluid flow was observed, 10–12 mg bupivacaine (10 mg for patients shorter than 165 cm and 12 mg for patients taller than 165 cm) was administered for 10–15 s. The level of sensorial blockade was determined with the pinprick test, while the level of motor blockade was determined with the Bromage scale. The interval between starting of the intrathecal injection and the time when no pain was felt in the T4 dermatome was regarded as the sensorial blockade starting time. The interval between the end of intrathecal injection and the time when Bromage was 2-3 was regarded as the motor blockade starting time. The maximum level of sensorial blockade, time when sensorial blockade reached the T4 dermatome level (starting time of sensorial blockade), motor blockade starting time, motor blockade at 5 min (Bromage score), and regression time of sensorial blockade to the T10 dermatome level were recorded. Surgery was allowed when sensorial blockade reached the T5-T4 dermatome level. In the spinal anesthesia group, 5–10 mg ephedrine was intravenously administered in case significant hypotension developed (initial SAP below 20–30%). In case of bradycardia (45 or less pulse per minute) atropine was intravenously administered, and patients who were administered with atropine were excluded from the study. After the baby was born, 10 units of oxytocin were slowly intravenously administered; 10–20 units of oxytocin were added to 1000 mL isotonic. The Duration of surgery was recorded as the period between the first skin incision and the last skin suture. For individuals in Group R, the duration of anesthesia was recorded as the time between the beginning of spinal anesthesia and the last skin suture. Patients in Group R were also administered 2 mg/kg tramadole, and 20 mg tenoxicam was intravenously administered at the end of the surgery.

In case the visual analogue scale (VAS) value of individuals in all the three groups was 4 or above, additional analgesia with 1 mg/kg tramadole was intravenously administered. Thus, it was aimed to have the VAS values of 4 or less for patients. Because of the possibility of postoperative pain affecting cognitive functions, patients with postoperative 1st and 3rd hour VAS values over 4, despite the administration of additional analgesia, were excluded from the study.

The evaluation of the newborn was performed by a pediatrician with Apgar scores in the 1st and 5th minutes after the birth. In all the three groups, the starting of anesthesia-time of birth, uterus incision-time of birth and whether the newborn required resuscitation or not (ambu or intubation) were recorded.

To evaluate cognitive functions of individuals in all the three groups, MMSE, TDT, and CDT tests were performed again in the postoperative 1st, 3rd, and 24th h. Patients were monitored for adverse effects, such as nausea and vomiting, desaturation, and hypotension.

Statistical analysis

We accepted a type I error of 0.05 and type II error of 0.80 for detecting a true difference. A 0.5 or greater difference in the independent variables was considered clinically significant. An estimate of standard deviation in independent variables was 1. Therefore, we calculated that a minimum of 23 patients were required in each group to obtain 5% type 1 error and an 80% power of detecting a difference of 0.5 or more. For each group, 25 patients were included to compensate for the possible dropouts. The power calculation was performed with nQuery Advisor Version 7.0 (Statistical Solutions, Saugus, MA, USA).

In this study, data obtained were evaluated with SPSS 20.0 packet program. Frequencies and percentile distributions of data were stated. According to the normality test, differences among groups were analyzed, and for comparison of two groups Mann–Whitney U Test was used for variables that were not normally distributed.. For comparisons of three groups Bonferroni-corrected Kruskal–Wallis H test was used for variables that were not normally distributed; $p<0.05$ was accepted as statistically significant.

Results

Seventy-five pregnant patients who planned to undergo elective cesarean operation under general and spinal anesthesia and who fell into the ASA I risk group were included in the study.

Height, weight, and the durations of surgery do not demonstrate a significant difference in terms of anesthesia methods ($p>0.05$) (Table 1). When the durations of anesthesia between the groups were analyzed, the duration of anesthesia for patients in Group R was found to be significantly longer than that in Group D ($p<0.05$) (Table 1).

When the HR values of groups S, R, and D were compared, HR in 10th, 15th, 20th, and 25th minutes for patients in Group R was found to be significantly higher than that those in Group S ($p<0.05$), HR in 20th and 25th minutes for patients in Group R was found to be significantly higher than that of patients in Group D ($p<0.05$), and extubation HR for patients in Group D was found to be significantly higher than that of patients in Group S ($p<0.05$) (Figure 1).

In terms of MAP, post-induction MAP in the 5th, 25th, and 30th minutes of patients in Group D was found to be significantly higher than that of patients in Group R, and MAP in 5th minute of patients in Group S was found to be significantly higher than that of patients in Group R ($p<0.05$). There were no statistically significant difference among the groups in terms of the baseline; post-intubation; 10th, 15th, and 20th minute; and extubation and post-extubation 5th-minute MAP values ($p>0.05$) (Figure 2).

Post-induction 5th, 10th, 15th, 20th, 25th, and 30th-minute BIS values of patients in Group R were found to be significantly higher than those in the other two groups ($p<0.05$). Extubation and post-extubation 5th-minute BIS values of patients in Group D were found to be significantly higher than those in Group S ($p<0.05$).

Table 1. Patient characteristics, intraoperative and postoperative variables

Groups	Group S (n=25)	Group D (n=25)	Group R (n=25)
Age (year)	29.12±6.36	28.44±6.38	26.56±4.65
Weight (kg)	73.20±8.35	75.48±9.07	72.44±8.31
Height (cm)	162.8±4.96	161.47±4.85	161.84±3.91
Gestational Age (week)	37.56±0.87	37.44±0.87	37.24±1.23
Duration of anesthesia (min)	32.68±2.98	31.52±2.63	36.04±6.92*
Duration of surgery (min)	29.80±2.58	28.52±2.87	30.48±4.39
Remifentanil Consumption (μg)	371.44±58.62	461.20±79.33	-
Extubation time (min)	6.84±1.43$^{\alpha}$	2.64±1.63	-
Eye opening time (min)	8.08±1.71$^{\beta}$	3.76±1.74	-
Aldrete Recovery Score 2nd min	7.64±0.49#	8.16±0.47	-
Aldrete Recovery Score 5th min	8.96±0.54$^{\epsilon}$	9.36±0.70	-

Values are shown as Mean±SD.
*p=0.047 (Comparison between Group R and Group D)
$^{\alpha}$p<0.001 (Comparison between Group S and Group D)
$^{\beta}$p<0.001 (Comparison between Group S and Group D)
#p=0.01 (Comparison between Group S and Group D)
$^{\epsilon}$p=0.022 (Comparison between Group S and Group D)

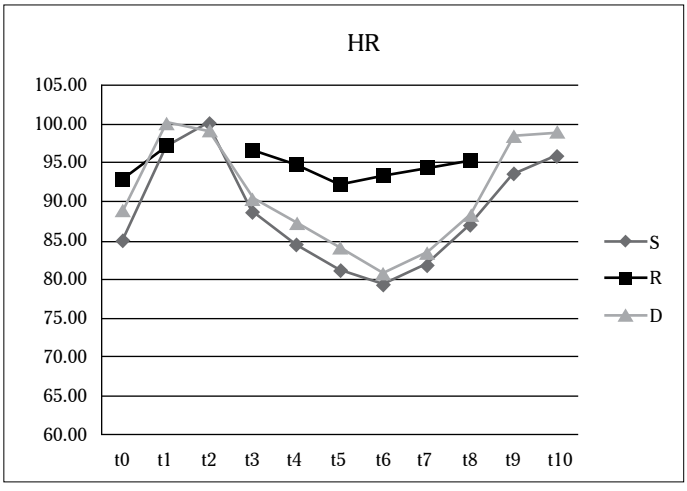

Figure 1. Heart rate (HR) of the patients (Mean±SD)

There was no statistically significant difference between postintubation BIS values of Groups S and D (p>0.05) (Figure 3).

According to the 1st minute Apgar scores of patients and times of birth, the 1st minute Apgar score and time of birth (how many seconds after anesthesia induction) of patients in Group R were significantly higher than those of patients in Groups S and D

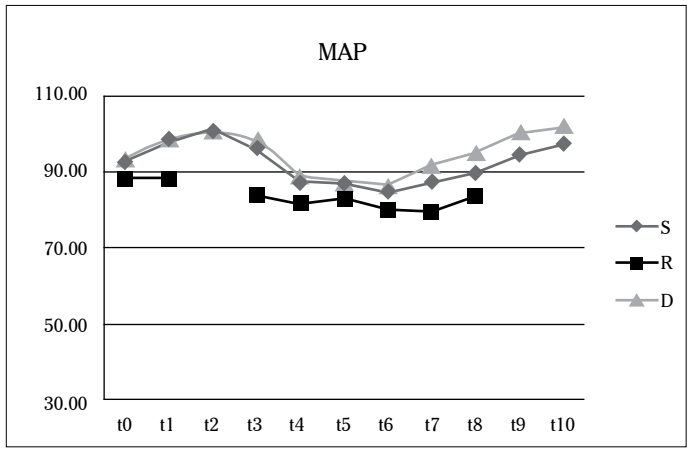

Figure 2. Mean arterial pressure (MAP) of the patients (Mean±SD)

Table 2. Characteristics of operation, APGAR Scores of newborns

Groups	Group S (n=25)	Group D (n=25)	Group R (n=25)
Parity	2.00±0.8	1.60±0.76	1.72±0.89
Gestational Age (week)	37.56±0.87	37.44±0.87	37.24±1.23
APGAR 1st min	7.24±0.61	7.24±0.88$^{\alpha}$	7.75±0.68*$^{\alpha}$
APGAR 5th min	8.80±0.53	9.20±0.50	9.00±0.41
Time of birth after uterine incision (min)	0.63±1.19	0.65±0.20	0.59±0.17
Time of birth after anesthesia induction (min)	4.97±1.03	5.24±1.08	10.41±0.01$^{\beta}$

Values are shown as Mean±SD.
*p=0.034 (Comparison between Group R and Group D)
$^{\alpha}$p<0.01 (Comparison between Group R and Group S)
$^{\beta}$p<0.001 (Comparison between Group S, Group D and Group R)

(p=0.034, p<0.01, respectively) (Table 2). There were no statistically significant difference in terms of age, parity, gestational week, 5th minute Apgar score, and how many seconds after uterus incision time of birth is between the groups S, R, and D (p>0.05) (Table 2). Total remifentanil dosage and Alderete 2nd and 5th minute scores were found to be significantly higher for patients in Group D than those of patients in Group S, and extubation time and eye opening times were found to be significantly higher for patients in Group S than those of patients in Group D (p<0.05) (Table 1).

There were no statistically significant differences among groups S, R, and D in terms of preoperative, postoperative 1st hour, postoperative 3rd hour, and postoperative 1st day MMSE and CDT values (p>0.05). In terms of TDT values, postoperative 3rd hour and postoperative 1st day TDT values were significantly different between Group S and Group R patients, and postoperative 3rd hour and 1st day TDT values of patients in Group S were found to be significantly higher than those of patients in Group R (p<0.05) (Table 3) (Figure 4-6).

Table 3. Cognitive function tests of the patients

Groups	Group S (n=25)	Group D (n=25)	Group R (n=25)
MMSE			
Preoperative	24.72±2.34	24.04±2.57	23.88±2.91
Postoperative 1st hr	24.36±2.16	24.12±2.76	24.44±2.58
Postoperative 3rd hr	24.92±2.16	24.80±2.72	24.48±2.03
Postoperative 1st day	25.00±2.10	24.76±2.68	24.60±2.32
TDT			
Preoperative	4.60±2.71	4.32±2.88	3.96±2.78
Postoperative 1st hr	10.48±3.57	10.24±4.02	7.96±3.79
Postoperative 3rd hr	10.80±3.88	8.12±4.80	5.84±3.30*
Postoperative 1st day	9.04±4.16	6.84±4.23	5.40±3.18α
CDT			
Preoperative	2.00±1.01	2.24±1.05	2.36±1.04
Postoperative 1st hr	1.32±0.85	1.64±0.99	1.68±0.95
Postoperative 3rd hr	1.72±0.89	1.92±1.08	2.12±1.01
Postoperative 1st day	1.84±0.85	1.92±1.08	2.20±1.00

MMSE: mini mental state examination test; TDT: trieger dot test; CDT: clock drawing test
Values are shown as Mean±SD.
*p<0.0001 (Comparison between Group R and Group S)
αp=0.006 (Comparison between Group R and Group S)

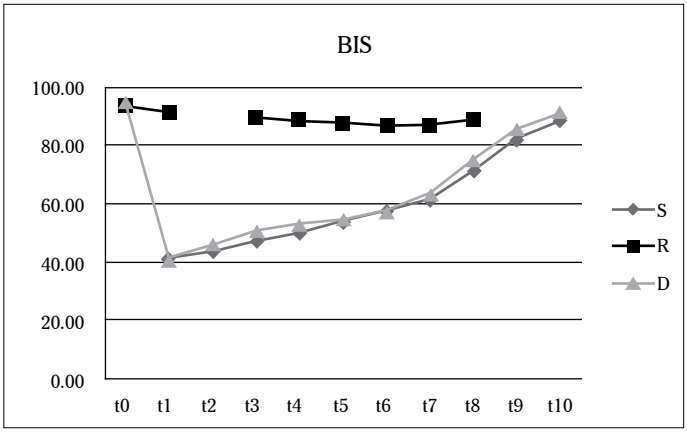

Figure 3. Bispectral index (BIS) values of the patients (Mean±SD)

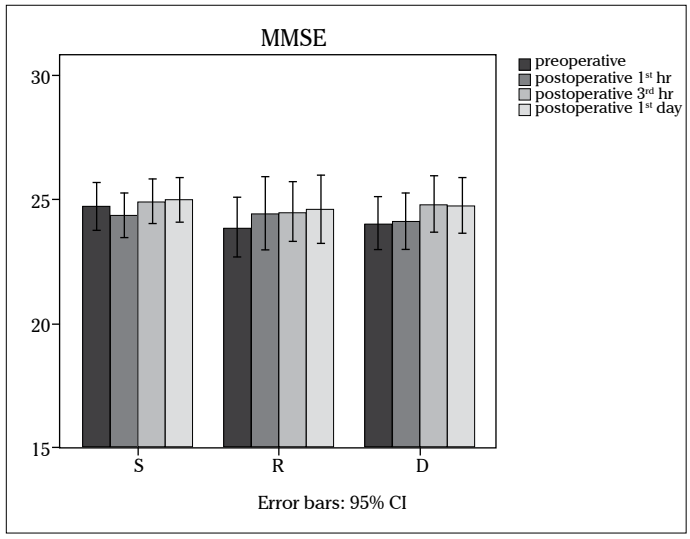

Figure 4. Mini-mental state examination (MMSE) of the patients (Mean±SD)

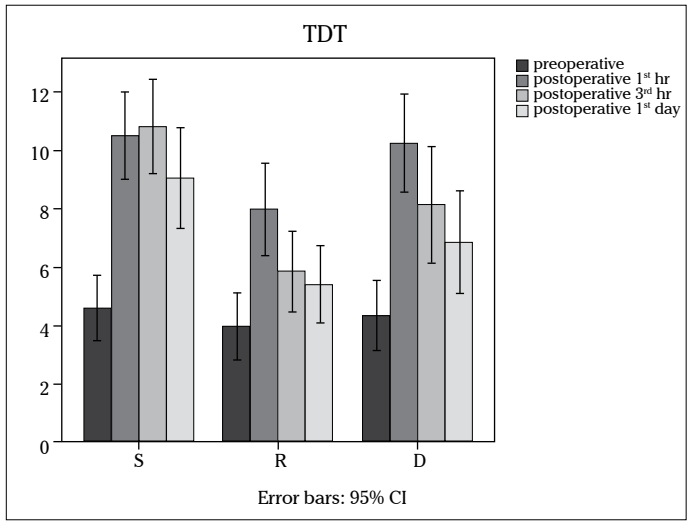

Figure 5. Trieger dot test (TDT) of the patients (Mean±SD)

Discussion

Although there are many studies evaluating the effect of anesthesia on POCD, the effect of anesthesia technique on POCD is unclear (9). In our study, we aimed to compare the effects of general anesthesia methods involving inhaled sevoflurane and desflurane agents and regional anesthesia applied via spinal anesthesia during elective cesarean operations on cognitive functions. In the literature, there is no study analyzing the relationship between anesthesia techniques applied during cesarean operations and early period POCD. Although it was found that general and spinal anesthesia have no negative effects on early period POCD, spinal anesthesia was found to be superior in the comparison between the groups that received general anesthesia with sevoflurane and regional anesthesia.

There are many studies suggesting that POCD may be prevented by performing regional anesthesia in suitable surgical operations, whereas some studies demonstrate that there is no significant difference between regional and general anesthesia with regard to POCD development (9, 11). Following cesarean operation, which is one of the obstetrical practices, POCD may theoretically develop, but a sufficient number of clinical studies evaluating the effect of anesthesia technique, psychological, nutritional, and stress status of mother on the incidence are required (12). We think that the occurrence of cognitive dysfunctions following cesarean operations performed under general anesthesia may be decreased using regional anesthesia more widely. Thus, both mother and baby can be protected from the possible harmful anesthetic effects of volatile and intravenous agents; unnecessary

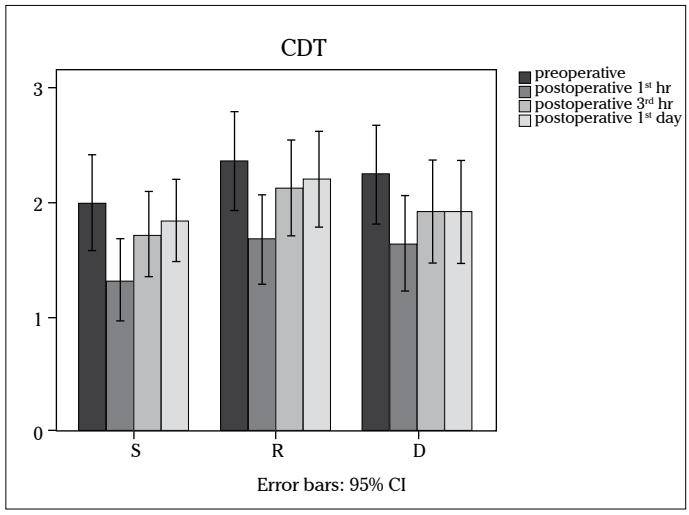

Figure 6. Clock drawing test (CDT) of the patients (Mean±SD)

separation of mother and baby is avoided by means of longer postoperative analgesia and absence of pain, somnolence, and malaise, which will be advantageous for patients.

Although there are many tests used to evaluate the cognitive functions, MMSE is widely used in studies because of its proven validity and safety in the Turkish population, applicability under both outpatient clinic and bedside conditions, and absence of uncomfortable and embarrassing aspects for the patient and physician. It is a 30-point test evaluating orientation, recording memory, attention and calculation, recall, and language (2, 13, 14). MMSE score between 24 and 30 indicates intact cognitive functions, whereas scores 23 and below suggest impaired cognitive functions (13). In our study, we detected no difference between general and spinal anesthesia groups in terms of MMSE scores. It is known that all neuropsychological tests used in the evaluation of cognitive functions can be learned, which manifested itself as higher scores of memory (word tests) and mathematical thinking tests for some cases when compared with initial values. In addition to this, we believe that preoperative anxiety and depression may also be responsible for lower scores obtained at first evaluation. Larsen et al. (15) reported no difference between sevoflurane and desflurane groups in terms of postoperative TDT values. In the study by Chen et al. (16), it was reported that there was no significant difference between desflurane and sevoflurane groups with regard to MMSE values, and initial MMSE values were achieved by the end of the first postoperative day in all patients. In our study, we also found no difference between desflurane and sevoflurane groups and also between these groups and spinal anesthesia group with regard to MMSE.

In contrast to our study, Hole et al. (17) and Chung et al. (18) revealed that early postoperative cognitive dysfunction was more severe in the general anesthesia group when compared with the regional anesthesia group. Similarly, Papaioannou et al. (19) also reported that elderly patients who received general anesthesia demonstrated apparently more severe cognitive dysfunction. Williams- Russo et al. (20) suggested that the type of anesthesia did not affect the degree or mode of POCD in elective orthopedic surgery patients. Similarly, Berggren et al. (21) and Ghoneim et al. (22) suggested that general anesthesia and central block did not affect postoperative cognitive functions, whereas Wu et al. (23) reported that central block did not decrease POCD incidence when compared with general anesthesia. Tzabar et al. (6) declared that the incidence of cognitive dysfunction on postoperative 3rd day following ambulatory surgery was apparently higher in the general anesthesia group when compared with the local anesthesia group.

We think that the type of anesthesia has no effect on POCD, particularly in short-time surgeries and young patients, but regional anesthesia may decrease the occurrence of known risk factors of POCD, such as respiratory complications, postoperative pain, and patient-originated results by contributing to intraoperative anesthesia and postoperative analgesia.

It is observed that the difference between the sevoflurane group and spinal anesthesia group in terms of TDT was significant, and TDT values of the sevoflurane group were significantly higher than those of the spinal anesthesia group. We believe that this result was achieved because pregnant women who received spinal anesthesia were more successful in the drawing TDT test because they were less sedated and more stable compared with the ones who received general anesthesia. Hence, it was found that the type of anesthesia had no effect on POCD, general anesthesia did not increase the development of early onset POCD measured by changes in MMSE scores, and regional anesthesia had no negative effects on cognitive functions when compared with general anesthesia.

Although sevoflurane does not cause tachycardia, high MAC value of desflurane or an acute increase in the inspired concentrations of desflurane causes tachycardia (24). Similar to the study of Nathanson et al. (25), our study also revealed that HR values were higher in the desflurane group when compared with the sevoflurane group, but this difference was insignificant. We think that this occurs because of the activation of sympathetic system caused by desflurane. Furthermore, we think that higher HR values of regional anesthesia group may be because of the anxiety caused by wakefulness of the patients receiving spinal anesthesia who are aware of all surgical procedure. It is also known that SpO_2 value below 80% also causes impairment of cognitive functions (26). None of our cases involved in the study had hypoxia, which may be one of the possible positive effects on cognitive functions.

It is known that various inhaled anesthetic agents may cause different BIS values in patients even when they are applied in the same end-tidal concentrations providing the same potency (27). In their study, Farag et al. (28) revealed that the depth of anesthesia maintains continuity of cognitive functions during the postoperative period. They reported that patients with low intraoperative BIS values had less impairment in cognitive functions during postoperative 4–6 weeks. In the study by Zhang et al. (29), it was suggested that BIS values of the groups who received general anesthesia and those who received spinal anesthesia were similar, but because the degrees of cognitive function impairments were different, the depth of anesthesia may not affect POCD.

In our study, higher BIS values of the spinal anesthesia group compared with the general anesthesia group was an expected result, whereas extubation and post-extubation 5th-minute BIS values of the desflurane group were significantly higher than those of the

sevoflurane group. We think that this is because of the rapid recovery related to low solubility of desflurane in blood and tissues. The limitation of this study includes the evaluation of cognitive functions in the early postoperative time period evaluated the POCD only early time period. It would be better if the cognitive tests were repeated in the first week and a month after the operation with a large number of patients.

In conclusion, this study reveals that general anesthesia with sevoflurane, general anesthesia with desflurane, and regional anesthesia via spinal injection had no deleterious effects on cognitive functions that were evaluated using MMSE, TDT, and CDT in patients undergoing cesarean operation. Therefore, we think that all three methods may be safely used in elective cesarean operations. We also think that further studies with a large number of patients evaluating the effects of general and regional anesthesia methods on cognitive functions in young and middle aged patients undergoing cesarean section and comparing different cognitive tests are required.

Ethics Committee Approval: *Ethics committee approval was received for this study from Necmettin Erbakan University Ethical Committee.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - H.B., C.A.; Design - H.B., C.A.; Supervision - H.B.; Resource - H.B., C.A., O.S.; Materials - H.B., C.A., O.S.; Data Collection and/or Processing - C.A., O.S., K.G.; Analysis and/or Interpretation - H.B.; Literature Search - C.A., O.Ş.; Writing - C.A., H.B.; Critical Reviews - H.B.*

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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The evaluation of risk factors for failed response to conservative treatment in tubo-ovarian abscesses

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Abstract

Objective: The aim of our study is to assess the risk factors for medical treatment failure and to predict the patients who will require the surgical therapy as well as to predict the factors affecting treatment success.

Material and Methods: This was a cross-sectional study including 76 women with tubo-ovarian abscesses (TOA) who were either conservatively or surgically treated and were admitted to two gynecology units over a 4-year period. The demographic characteristics of the patients, gynecologic and obstetric histories, size and localization of abscesses were recorded. Gentamicin plus clindamycin treatment protocol was implemented for all patients. Ampicillin treatment was added in three patients with the positive culture of Actinomyces. Response to treatment was evaluated after 48–72 h. Patients who fail to respond to medical treatment required surgery or percutaneous drainage. We compared clinical and laboratory factors between the groups.

Results: In surgery group, patients were significantly older than the others (44.9±5.4 versus 39.1±7.6 years). Fifty-six patients (74%) responded to antibiotics and 20 of the patients required surgical intervention. Patients treated with antibiotics were hospitalized for an average of 6.32±2.8 days versus 12.75±5.6 days for those who required surgery (p=0.021). Patients who were surgically treated had a mean size of TOA of 67.9±11.2 mm versus 53.6±9.4 mm for those treated with antibiotics alone (p=0.036). There were no significant differences between groups in laboratory parameters, except for initial white blood cell (WBC) counts. The complications of surgery included in descending order of frequency blood transfusions, surgical wound infections, bowel injury, and bladder injury.

Conclusion: An increased size of pelvic mass, higher initial WBC counts, advanced age, and smoking were all associated with failed response to conservative treatment. It is important to identify the risk factors to distinguish patients who will respond to antibiotic therapy and those who will need a surgical treatment. Thus, the required early intervention can result in a reduction in the morbidity associated with TOA.

(J Turk Ger Gynecol Assoc 2015; 16: 226-30)

Keywords: Antibiotic therapy, pelvic abscess, surgery, tubo-ovarian abscesses

Received: 01 July, 2015 **Accepted:** 06 August, 2015 **Available Online Date:** 02 November, 2015

Introduction

Tubo-ovarian abscesses (TOA) are characterized by a walled-off inflammatory mass involving adnexa. It is a consequence of advanced pelvic inflammatory disease (PID) in almost all cases, including diverticulitis, appendicitis, inflammatory bowel disease, and gynecologic or obstetric surgery, and rarely occurs after pelvic surgery (1). Risk factors for TOA are similar to those of PID such as multiple sexual partners, presence of intrauterine devices, history of PID, and immunosuppression (1).

Most of the women with TOA are at their reproductive ages; thus, the primary aim of management is to be as conservative as possible. TOA still carry a risk of mortality, but its incidence dramatically decreased because of the new generation of antibiotics and tools for early diagnosis. However, the morbidity associated with TOA remains significant with complications including infertility, ectopic pregnancy, chronic pelvic pain, pelvic thrombophlebitis, and ovarian vein thrombosis (2). The optimal treatment regimen for TOA is less clear. Treatment modalities include intensive antibiotic therapy, minimally invasive drainage procedures, invasive surgery, or a combination of these interventions. Although TOA mostly respond well to medical treatment, approximately 20%–25% of patients require surgical procedures, including laparotomy or laparoscopy with drainage of abscess, unilateral or bilateral salpingo-oophorectomy, and hysterectomy (3). However, surgery for TOA is often technically difficult and associated with complications.

The aim of our study is to predict the patients who will require surgical therapy as well as the factors affecting treatment success. This study may contribute to create objective criteria to predict the need for operational intervention.

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DOI:10.5152/jtgga.2015.15123

Material and Methods

This was a cross-sectional study including 76 women with TOA who were either treated conservatively (group 1) and surgically (group 2) and were admitted to two gynecology units over a 4-year period. The study was subject to local ethics committee's approval, and written informed consent was obtained from patients who participated in this study. All authors and the study protocol have complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects. In this study, some of the following tests were conducted in case of a suspicion of TOA: gynecologic examination, physical examination, erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, C-reactive protein value (CRP), and ultrasound and microbiological studies from vaginal swab sampling. TOA was diagnosed in patients with abdominal pain, cervical mucopurulent discharge, cervical-adnexal tenderness on examination, and one or more of minor criteria such as fever, leukocytosis, and presence of ultrasonographic (Logiq and Voluson, General Electric, Tiefenbach, Austria) findings such as a complex mass with irregular walls, partitions, and internal echoes. The demographic characteristics of the patients, gynecologic and obstetric histories, and the size and localization of abscesses were recorded. Specimens from vagina were cultured in aerobic, anaerobic, and Thayer–Martin mediums. Pap smear test was performed for all patients.

Gentamicin was administered to all the patients [loading dose intravenous (2 mg/kg of body weight), followed by a maintenance dose (1.5 mg/kg) every 8 h] (Genta; I.E Ulagay Medical, Istanbul, Turkey) plus clindamycin (900 mg IV every 8 h) (Cleocin; Pfizer Inc., New York, USA) treatment protocol according to the recommendation of the Center for Disease Control and Prevention (4). Ampicillin (Duocid; Pfizer Inc., New York, USA) treatment was administered to three patients with the positive culture of *Actinomyces*. Response to treatment was evaluated after 48–72 h. Patients who fail to respond to medical treatment required surgery or percutaneous drainage. The criteria used to determine the failure of treatment were as follows: 1) persistent fever (>38.2°C), 2) enlarging pelvic mass, 3) persistent or increased abdominopelvic tenderness, 4) persistent or further elevation of WBC count, and 5) signs of sepsis such as arterial hypotension [systolic blood pressure (SBP) of <90 mmHg, mean arterial pressure of <70 mmHg, or an SBP decrease up to >40 mmHg in adults or less than two standard deviations below the normal value based on age], arterial hypoxemia [arterial oxygen tension (PaO_2)/fraction of inspired oxygen [FiO_2] of <300), acute oliguria (urine output of <0.5 mL/kg/h for at least 2 h despite adequate fluid resuscitation), creatinine increase up to >0.5 mg/dL or 44.2 μM/L, coagulation abnormalities [international normalized ratio (INR) of >1.5 or activated partial thromboplastin time [aPTT] of >60 s), thrombocytopenia (platelet count of <100,000 μL, tachypnea, respiratory rate of >20 breaths/min, heart rate of >90 beats/min or more than two standard deviations above the normal value depending on age, and ileus (absent bowel sounds) (5). All patients were followed up for 6 months to evaluate recurrence.

Data were analyzed by the SPSS 17.0 software (SPSS Inc. IBM, Chicago, IL, USA), and descriptive data were expressed as mean±standard deviations (SDs) as well as range. Chi-square and Fischer exact tests were used to analyze categorical variables and the Mann–Whitney U test was used to compare the clinical parameters of women between the groups. P values of <0.05 were considered to be statistically significant. Receivers operating characteristics (ROCs) were used to determine the threshold for age, TOA size, and WBC count.

Results

We classified the patients under medical treatment (group 1) and medical plus surgical treatment (group 2) groups. The mean age of the patients in this study was 40.34±7.4 years. There was only one postmenopausal patient. In the surgery group, patients were significantly older than those in the other group (44.9±5.4 versus 39.1±7.6 years). The other characteristics of the groups are given in Table 1. Forty-five patients (59%) had intrauterine devices when TOA was diagnosed. Fifty-six patients (74%) responded to antibiotics and 20 patients required surgical intervention (Figure 1). Patients treated with antibiotics were hospitalized for an on average of 6.32±2.8 days versus 12.75±5.6 days for those who required surgery (p=0.021).

Vaginal cultures of 50 patients were negative; the remaining results are shown in Table 2. There was no significant difference between groups in laboratory parameters, except for initial WBC counts (Table 3). Patients treated with antibiotics alone had lower mean WBC count than those who required surgery (11.2±2.6 versus 16.3±6.5; p=0.03). The mean CRP level was higher in the surgery group than that in the medical treatment group. However, the difference was not significant (p=0.4). Furthermore, there was no difference in ESR levels between the groups (64.5±31.1 versus 62.5±27.2; p=0.84).

Patients who were surgically treated had a mean size of TOA of 67.9±11.2 mm versus 53.6±9.4 mm for those treated with antibiotics alone (p=0.036). We decided on performing

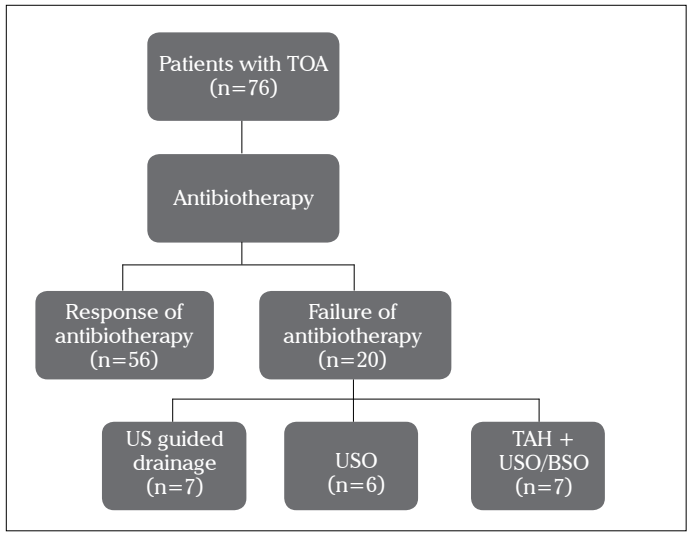

Figure 1. The flowchart showing the treatment modalities of patients with tubo-ovarian abscesses

TOA: tuboovarian abscess, TAH: total abdominal hysterectomy, USO: unilateral salpingooophoerectomy, BSO: bilateral salpingo-oophoerectomy, US: ultrasound

Table 1. The demographic data of the patients and comparison between the two groups

	Group 1 (n=56)	Group 2 (n=20)	p
Age (years)	39.1±7.6 (19–50)	44.9±5.4 (31–52)	0.042
Gravida	3.4±2.0 (0–11)	4.2±1.6 (2–7)	0.247
Parity	2.3±1.0 (0–5)	2.7±1.1 (1–5)	0.246
Abortion	0.47±0.23 (0–6)	0.50±0.29 (0–2)	0.939
Smoking	18 (47.3%)	8 (66%)	0.035
Education (>8 years)	27 (54%)	13 (50%)	0.891
IUD present	33 (59%)	12 (60%)	0.888

IUD: intrauterine device
Data are given as mean±standard deviations (range within parentheses) or percentages (frequencies within parentheses).
Group 1: patients treated conservatively, Group 2: patients treated surgically.

Table 2. The distribution of patients with positive vaginal culture among groups

	Antibiotic therapy alone (n=19)	Antibiotic therapy+Surgical intervention (n=7)
Gardnerella vaginalis	4	2
Candida albicans	3	_
Actinomyces israelii	3	_
Peptostreptococcus and *Bacteriodes*	3	2
Peptostreptococcus and *Veillonella*	2	2
Peptostreptococcus	2	1
Escherichia coli	1	_
Staphylococcus aureus	1	_

Table 3. The comparison of laboratory parameters between two groups

	Group 1 (n=56)	Group 2 (n=20)	p
WBCs count (/mm^3)	11.2±5.1 (3.8–22.7)	16.3±6.5 (5.1–23.9)	0.03
ESR (mm/h)	64.5±31.1 (6–118)	62.5±27.2 (16–100)	0.84
CRP (mg/L)	48.6±38.9 (6–96)	59.5±39.3 (6–96)	0.41

WBCs: white blood cells; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein
Data are given as mean±standard deviations (range within parentheses).
Group 1: patients treated conservatively, Group 2: patients treated surgically.

surgical intervention after an average of 2.1±0.9 days from the start of medical treatment. A total of 74% of the patients were successfully treated with antibiotic therapy alone. When surgical intervention was needed, seven hysterectomies (performed with unilateral or bilateral salpingo-oophorectomy), six unilateral salpingo-oophorectomies, and seven ultrasound-guided drainages were performed. Surgically treated patients had more complications. The most common complication encountered was blood transfusions in seven patients followed by surgical wound infections in three patients; one patient had bowel injury and the other had bladder injury. In the 6-month follow-up period, we did not observe any recurrence in both the surgical and medical treatment groups.

On analysis of the ROC curve, an age cut-off of 42.3 years was found to be optimal for predicting the need of surgical intervention with a sensitivity of 70% and a specificity of 60.7%. A cut-off size of 5.7 cm for TOA was found to be optimal for predicting the need for surgical intervention with a sensitivity of 65% and a specificity of 80%. A cut-off value of 13.6 for WBC count for predicting the need for surgical intervention was found with a sensitivity of 60% and specificity of 53.5% (Table 4).

Discussion

Despite the decrease in the mortality of TOA in the last decades after using broad-spectrum antibiotics as well as early diagnosis and improvement of imaging techniques, the rupture of abscess is still associated with the risk of mortality. Although the optimal treatment of TOA still remains unclear, conservative therapy should be chosen as the treatment modality as it is mostly seen during the reproductive period.

Medical management with broad spectrum antibiotics is generally considered as the initial management of TOA. When antibiotic therapy fails, surgical treatment is indicated. There remains some controversy concerning the extent and timing of operation that is appropriate for the patient requiring surgical intervention. Most of the patients with TOA are candidates for medical management. Success rates of medical treatment have been reported between 67% and 75% (6, 7). In our study, surgical treatment was necessary for 20 patients (26%). Success of medical treatment is associated with the following factors: age, abscess size, bilateral adnexal involvement, laboratory parameters (8). Failure of the medical treatment is increased in patients who had abscesses with a size of >5 cm; this may be because of the decreased penetration of antibiotics into the abscess cavity (9-11). Dewitt et al. (12) also found that TOA size is associated with important outcomes including more complications and longer duration of hospitalization as well as an increased need for surgery or drainage in patients with large abscesses as opposed to those with smaller abscesses. They found a 43% failure rate for abscesses with a size of >8 cm. In our study, 18 of 20 (90%) patients who were surgically treated had abscesses with a size of >5 cm; the mean size of abscess was larger in the surgical treatment group.

A previous study showed that the recurrence rate of TOA following treatment with antibiotics was higher than that after surgical intervention (12). However, in our study, there had been no re-

Table 4. Statistical analyses for prediction of the surgery following the determination of presumed thresholds

	Thresholds	Sensitivity	Specificity	ACC	PPV	NPV	LR+	LR-
Age (years)	>42.3	70%	60.7%	63.1%	39%	85%	1.78	0.49
Size (cm)	>5.7	65%	80%	76.3%	54.1%	86.5%	3.25	0.43
WBCs count (/mm^3)	>13.6	60%	53.5%	55.2%	31.5%	78.9%	1.29	0.74

ACC: accuracy; PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; WBCs: white blood cells

currence of TOA during the 6-month follow with regard to both medical and surgical therapies.

Karasu et al. (13) reported that the mean age of the patients who were successfully treated with parenteral antibiotics was significantly lower than that of the patients who did not respond to medical treatment (37.4 versus 43.5 years). They found 40 years as the cut-off age for medical treatment with a sensitivity of 60% and a specificity of 65%. Greenstein et al. (14) also reported that older age was associated with significantly higher risk of surgery. Our results were similar to previous studies and patients who were surgically treated were older than the others. Fifteen of 20 (75%) patients who were surgically treated were aged >40 years. Patients for whom fertility was no longer a matter of concern facilitated our decision in favor of surgical intervention. We did not detect a malignant tumor associated with abscess, despite a prior study that reported malignant tumors in 13% (2/15) of TOA patients aged ≥40 years.

Osser et al. (15) suggests that the use of intrauterine device (IUD) doubles the risk of PID. Sweet and Gibbs (16) reported that the co-occurrence of TOA with IUD was approximately up to 20%–54%. Our data also indicate an association rate of 59%, which is compatible with that reported in the literature.

Previous studies reported that the prolonged duration of hospitalization was required in patients with TOA who underwent drainage or surgery (12, 13, 17). Similarly, we found that the mean duration of hospitalization was significantly longer in patients who were surgically treated.

Laboratory evaluation in patients with TOA mostly reveals leukocytosis, increased ESR, and elevated CRP levels. Several authors have investigated the relationship between laboratory findings and the need for surgery. In a study, a higher CRP level was identified as an important indicator for surgery. However, the lymphocyte and WBC counts in the same study were not significantly elevated (17). Güngördük et al. (18) showed that CRP and ESR levels were higher in surgically treated patients. In another study, CRP and ESR levels were found to be good predictors for medical treatment failure. In this study, they found the cut-off value for CRP and ESR levels as 6.7 mg/L and 50 mm/h, respectively (13). Unlike these studies, a significant difference was not observed in CRP levels and WBC count between the favorable or poor prognosis group in the study by Topçu et al. (19). In our study, we found that there was no significant difference in CRP and ESR counts between the groups. We did not observe a relationship between these parameters and medical treatment failure. On the other hand, the initial WBC counts were significantly different between medically and surgically treated patients.

The small population sample was the limitation of our study. The results need to confirm with a larger study population. Also, prospective cohort studies will be needed to demonstrate an independent relationship across clinical and laboratory parameters as well as decision of surgical intervention.

In conclusion, increased size of pelvic mass, higher initial WBC counts, advanced age, and smoking were all associated with failed response to conservative treatment. At present, antibiotics are the mainstay of treatment for TOA. In some patients, medical treatment must be combined with operational intervention. It is important to identify risk factors to discriminate patients who will respond to antibiotic therapy or need a surgical treatment. Thus, the required early intervention can be provided to reduce morbidity associated with TOA.

Ethics Committee Approval: *Ethics committee approval was received for this study from Etlik Zübeyde Hanim Women's Health Research Hospital.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - F.K., M.A.A., İ.A.; Design - S.E.Y., M.Ö.A., İ.A.; Supervision - F.K., A.Y.; Resource - Y.Y., B.T.; Materials - İ.A., Y.Y., B.T.; Data Collection and/or Processing - İ.A., M.Ö.A., A.Y.; Analysis and/or Interpretation - S.E.Y., F.K., M.A.A.; Literature Search - M.Ö.A., B.T., S.E.Y.; Writing - İ.A., S.E.Y., M.Ö.A.; Critical Reviews - M.A.A., F.K.*

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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Prevalence and risk factors of anemia among pregnant women attending a high-volume tertiary care center for delivery

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Abstract

Objective: The aim of this study was to identify the prevalence of anemia and predisposing factors contributing to anemia in pregnant women prior to delivery.

Material and Methods: A retrospective case-control study was conducted on 1221 women who delivered between 37 and 42 weeks of gestation between July 2014 and January 2015. Data on the subjects' socioeconomic and demographic characteristics, pregnancy outcomes, and hemoglobin levels within 24 h prior to delivery were collected. The study population was divided into two groups on the basis of the presence of anemia within 24 h prior to delivery. Anemia was defined as a hemoglobin level of <11 g/dL. The prevalence of pre-delivery anemia was estimated, and antenatal predictors of anemia were determined using multivariate logistic regression analysis.

Results: The prevalence of anemia in women attending our center for delivery was 41.6% [95% confidence interval (CI) =38.84–44.37]. After multivariate logistic regression analysis, parity >3 [odds ratio (OR) =1.82, 95% CI=1.24–2.96, p=0.002], illiterate (OR=2.23, 95% CI=1.35–3.45, p=0.001) and primary educational level (OR=2.01, 95% CI=1.28–3.39, p=0.008), household monthly income per person <250 Turkish liras (OR=2.34, 95% CI=1.49–3.89, p<0.001), first admission at second (OR=1.63, 95% CI=1.24–2.81, p=0.006) and third trimester (OR=2.45, 95% CI=1.41–4.06, p<0.001), number of antenatal visits <5 (OR=1.45, 95% CI=10.5–2.11) and 5–10 (OR=1.3, 95% CI=1.03–2.09), duration of iron supplementation <3 months (OR=2.62, 95% CI=1.51–4.17) and 3–6 months (OR=1.68, 95% CI=1.13–2.91), and occurrence of preeclampsia (OR=1.55, 95% CI=1.03–2.1, p=0.041) were independently associated with anemia.

Conclusion: Socioeconomic determinants constitute most of the anemia cases and, hence, should be considered as major risk factors of anemia in women attending for delivery at term. (J Turk Ger Gynecol Assoc 2015; 16: 231-6)

Keywords: Anemia, delivery, perinatal outcome, pregnancy, socioeconomic factors

Received: 14 April, 2015 **Accepted:** 15 July, 2015 **Available Online Date:** 02 November, 2015

Introduction

Anemia in pregnancy is one of the most common preventable causes of maternal morbidity and poor perinatal outcome. The main causes of anemia during pregnancy involve deficiencies of key nutrients, infections, and parasitic diseases (1). Among these etiologic factors, iron deficiency is often identified as the primary contributor to anemia in pregnancy. In Turkey, routine iron supplementation to all pregnant women has been advised since 2005 (2). Despite the implementation of the program for a decade, anemia in pregnancy still remains as one of the major health problems in the country with a prevalence of 32.6% (3).

In developing countries, demographic, cultural, and socioeconomic factors could affect the occurrence of anemia in pregnancy. In addition, previous studies have found a significant association between maternal anemia and adverse perinatal outcomes such as placenta previa, placental abruption, preterm birth, and low birth weight (4, 5). The determination of these factors will help to provide valuable information for the identification of the "at-risk" group and also for the implementation of interventions to reduce anemia. However, these studies regarding the etiology of anemia in pregnancy were conducted with anemic women prior to conception or during the first trimester of pregnancy (4, 5). There is a need for studies exploring the prevalence of anemia and predisposing risk factors for anemia observed at the time of delivery.

Pregnant women with anemia, those going into labor and delivery, have the highest potential to encounter complications related to anemia and transfusion (6-8). A modest blood loss at delivery may not impair the hemodynamic response of women with normal hemoglobin levels but may be too hazardous for anemic women (5, 7, 9). In addition, there may not be enough time for clinicians to normalize the hemoglobin

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DOI:10.5152/jtgga.2015.15071

levels of delivering women, particularly in places where transfusion facilities are limited.

Consequently, in this study we aimed to determine the prevalence of anemia and identify the factors contributing to anemia in pregnant women attending our center for delivery at term.

Material and Methods

This study was conducted retrospectively with women who had delivered at a high-volume tertiary care center between July 2014 and January 2015. Our study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, and the research protocol was approved by the Ethics Committee of our center. Informed consent was obtained from all participants. The study population consisted of singleton pregnancies that delivered between 37 and 42 weeks of gestation and were monitored in the obstetric unit of our center after delivery. Chronic diseases leading to anemia such as renal, cardiac, and lung diseases and hemoglobinopathy were excluded. The minimum sample size required for this study was estimated by assuming a confidence interval of 95%, a 5% margin of error, and a prevalence of 32.6% for anemia among pregnant women. Accordingly, the minimum sample size required for the study was 333. A total of 1221 women were enrolled in the study, and all of them were recruited to increase the power of the study.

Before discharge, all the women who had delivered were interviewed using a questionnaire to collect their socioeconomic and demographic data. Furthermore, the following information was obtained from the computerized medical record system of our hospital: hemoglobin value within 24 h prior to delivery, maternal age, number of parity and abortus, body mass index before pregnancy, weight gain during pregnancy, educational level, occupational status, household monthly income per person, smoking habit, alcohol consumption, number of antenatal care visits, number of ultrasonography examinations done during pregnancy, gestational age at first admission, gestational age at delivery, and duration of iron and folic acid supplementation. Household monthly income per person was calculated as the total household monthly income divided by the total number of family members living together. Adverse perinatal outcomes were recorded as dichotomous variables (yes or no) and included antenatal bleeding, hypermesis gravidarum, placenta previa, gestational diabetes, preeclampsia, intrauterine growth restriction, and congenital anomalies. We categorized patients into two groups according to the presence or absence of anemia within 24 h before the onset of delivery. Anemia was defined as a hemoglobin level of <11 g/dL according to the World Health Organization criteria (3). The prevalence and antenatal predictors of anemia in pregnant women attending our center for delivery was determined.

According to our protocol, an oral dose of 30 mg/day iron was prescribed to all non-anemic pregnant women, and if anemia was diagnosed, the iron dose was increased to 60–120 mg/day until the anemia was treated. Similarly, periconceptional 0.4 mg of folic acid supplementation was recommended for all women planning a pregnancy and those in their first trimester of gestation.

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous and categorical data were expressed as mean±standard deviation and the number of patients, respectively. The means of the continuous variables were compared using t-test between the two groups after checking that the variables were normally distributed. The distribution of categorical variables was examined using chi-square statistics. Risk factors and perinatal outcomes associated with anemia were determined using multivariate logistic regression modeling with backward elimination. Odds ratio (OR) and their 95% confidence intervals (CI) were calculated. $P<0.05$ was considered statistically significant.

Results

A total of 1221 women attended our center for delivery during the study period, among which 508 (41.6%, 95% CI=38.84–44.37) had a hemoglobin level <11 g/dL and 713 (58.4%, 95% CI=55.6–61.12) had a level of ≥11 g/dL. The socioeconomic and demographic characteristics for groups with normal and low hemoglobin level are shown in Table 1. Maternal age >35 years (OR=1.47, 95% CI=1.04–2.08, p=0.029), body mass index ≥30 (OR=1.72, 95% CI=1.06–2.79, p=0.027), parity >3 (OR=2.38, 95% CI=1.74–3.26, p<0.001), illiterate (OR=2.09, 95% CI=1.31–3.35, p=0.002) and primary educational level (OR=2.67, 95% CI=1.90–3.74, p<0.001), absence of occupation (OR=1.48, 95% CI=1.06–2.09, p=0.021), household monthly income per person <250 Turkish liras (TL) (OR=4.94, 95% CI=3.37–7.25, p<0.001) and 250–500 TL (OR=2.58, 95% CI=1.81–3.68, p<0.001), weight gain during pregnancy <10 kg (OR=1.56, 95% CI=1.09–2.24, p=0.016), number of antenatal visits <5 (OR=2.49, 95% CI=1.63–3.81, p<0.001) and 5–10 (OR=1.71, 95% CI=1.34–2.17, p<0.001), admission to antenatal care at second (OR=1.90, 95% CI=1.45–2.49, p<0.001) and third trimester of gestation (OR=1.93, 95% CI=1.44–2.58, p<0.001), and duration of iron supplementation <3 months (OR=2.53, 95% CI=1.81–3.53, p<0.001) and <3–6 months (OR=2.09, 95% CI=1.60–2.72, p<0.001) were significantly associated with anemia at the time of delivery. The perinatal outcomes associated with anemia are presented in Table 2. Antenatal bleeding (OR=2.09, 95% CI=1.02–4.28, p=0.039) and preeclampsia (OR=2.68, 95% CI=1.13–6.37, p=0.02) were associated with an increased risk of anemia. There were no other significant differences between the groups in demographic characteristics and perinatal outcomes.

To further identify the predictors of anemia within 24 h before delivery, multiple logistic regression analysis was performed to control for potential confounders (Table 3). Among the risk factors, parity >3 (OR=1.82, 95% CI=1.24–2.96, p=0.002), illiterate (OR=2.23, 95% CI=1.35–3.45, p=0.001) and primary educational level (OR=2.01, 95% CI=1.28–3.39, p=0.008), household monthly income per person <250 TL (OR=2.34, 95% CI=1.49–3.89, p<0.001), number of antenatal visits <5 (OR=1.45, 95% CI=10.5–2.11) and 5–10 (OR=1.3, 95% CI=1.03-2.09), admission to antenatal care at second (OR=1.63, 95% CI=1.24–2.81, p=0.006) or third trimester of gestation (OR=2.45, 95%

Table 1. Association between clinical characteristics and anemia in women attending our center for delivery

	Hb<11 g/dL n (%)	Hb≥11 g/dL n (%)	OR (95% CI)	p
Maternal age (y)				
<20	42 (40.8)	61 (59.2)	1.05 (0.69–0.61)	0.823
20–29	239	365	1	
30–34	147 (28.9)	204 (28.6)	1.1 (0.84–1.44)	0.484
>35	80	83	1.47 (1.04–2.08)	0.029
BMI (kg/m^2)				
<18.5	29 (37.7)	48 (62.3)	0.93 (0.57–1.51)	0.764
18.5–24.9	288 (39.4)	443 (60.6)	1	
25–29.9	153 (44.9)	188 (55.1)	1.25 (0.96–1.62)	0.09
≥30	38 (52.8)	34 (47.2)	1.72 (1.06–2.79)	0.027
Parity				
0	152	282	1	
1–3	211	318	1.23 (0.95–1.60)	0.121
>3	145	113	2.38 (1.74–3.26)	<0.001
Abortion				
0	356	523	1	
1-2	142	176	1.18 (0.91–1.53)	0.198
>2	10	14	1.05 (0.46–2.39)	0.920
Educational level				
Illiterate	60	56	2.09 (1.31–3.35)	0.002
Primary	255	348	2.67 (1.90–3.74)	<0.001
Secondary	127	180	1.38 (0.95–2.0)	0.091
Higher	66	129	1	
Occupational status				
No	448	595	1.48 (1.06–2.09)	0.021
Yes	60	118	1	
Household monthly income/person (TL)				
250	195	148	4.94 (3.37–7.25)	<0.001
250–500	265	385	2.58 (1.81–3.68)	<0.001
>500	48	180	1	
Weight gain (kg)				
<10	71	65	1.56 (1.09–2.24)	0.016
10–18	357	510	1	
>18	80	138	0.83 (0.61–1.13)	0.228
Smoking				
No	488	697	1	
Yes	20	16	0.56 (0.29–1.09)	0.085
Alcohol				
No	499	710	1	
Yes	11	7	0.45 (0.17–1.16)	0.09

No. of admissions to antenatal care				
<5	58	44	2.49 (1.63–3.81)	<0.001
5–10	232	257	1.71 (1.34–2.17)	<0.001
>10	218	412	1	
No. of USG				
<5	41	76	0.72 (0.48–1.09)	0.109
5–10	295	392	1	
>10	172	245	0.93 (0.73–1.19)	0.583
Gestational age at first admission (days)				
First trimester	157	327	1	
Second trimester	202	221	1.90 (1.45–2.49)	<0.001
Third trimester	151	163	1.93 (1.44–2.58)	<0.001
Gestational age at delivery (weeks)	38.2±2.18	38.4±1.94		
Iron supplementation (months)				
<3	116	109	2.53 (1.81–3.53)	<0.001
3–6	264	300	2.09 (1.60–2.72)	<0.001
>6	128	304	1	
Folic acid supplementation				
None	215	302	1.27 (0.87–1.84)	0.211
First trimester	238	313	1.36 (0.93–1.96)	0.107
Periconceptional	55	98	1	

BMI: body mass index; CI: confidence interval; Hb: hemoglobin; OR: odds ratio; TL: Turkish liras; USG: ultrasonography

Table 2. Perinatal outcomes associated with anemia in women attending our center for delivery

	Hb<11 g/dL n	Hb≥11 g/dL n	OR (95% CI)	p
Antenatal bleeding	19	13	2.09 (1.02–4.28)	0.039
Hyperemesis gravidarum	11	14	1.10 (0.50–2.45)	0.806
Gestational diabetes	12	15	1.13 (0.52–2.43)	0.764
Preeclampsia	15	8	2.68 (1.13–6.37)	0.02
IUGR	11	16	0.96 (0.44–2.09)	0.92
Placenta previa	9	12	1.05 (0.44–2.52)	0.92
Congenital anomalies	19	25	1.07 (0.58–1.96)	0.823

CI: confidence interval; Hb: hemoglobin; OR: odds ratio; IUGR: intrauterine growth restriction

CI=1.41–4.06, p<0.001), duration of iron supplementation <3 months (OR=2.62, 95% CI=1.51–4.17) and 3–6 months (OR=1.68, 95% CI=1.13–2.91), and occurrence of preeclampsia (OR=1.55, 95% CI=1.03–2.1, p=0.041) were independently associated with anemia.

Discussion

This study revealed that the prevalence of anemia in women within 24 h before delivery was 41.6%, which is higher than the estimated average prevalence rate of 32.6% documented by World Health Organization (WHO) for our country (3). This high prevalence of anemia among pregnant women in this study may be explained by the distribution of socioeconomic status of the population. This estimation of WHO for Turkey was acquired from community-based surveys. However, the rate in our study was derived from the population, which was mainly composed of women with lower socioeconomic status. Another noteworthy point is the variation in the gestational age at the time of measurement. Contrary to previous studies, the hemoglobin values of the women in our study were evaluated in the third trimester of pregnancy, in which fetal growth and red blood cell expansion increases the prevalence of anemia (4, 5). Additionally, in this study, it is demonstrated that pre-delivery anemia was related to parity, educational level, household monthly income per person, number of hospital admissions, gestational age at the first admission, duration of iron supplementation, and preeclampsia.

Results in our study showed that pregnancies with parity more than 3 were 1.8 times more likely to have anemia than those with a parity ≤3. Higher parity was documented in a number of studies as a cause of anemia in pregnancy (10, 11). In contrast, Ezugwu et al. (12) did not find any significant difference among nullliparous, multiparous, and grand multiparous groupings with respect to maternal anemia. However, low proportion of grand multiparous women (3.7%) in their study participants might have pushed the contribution of parity to statistically insignificant levels. Possible explanation to the high prevalence of anemia among grand multiparous women is that these

Table 3. Multivariate logistic regression analysis of risk factors and anemia

	OR	95% CI	p
Maternal age>35	1.23	0.92–1.61	0.223
BMI≥30 (kg/m^2)	1.19	0.89–1.63	0.102
Parity>3	1.82	1.24–2.96	0.002
Unemployment	1.20	0.89–1.57	0.121
Educational level			
Illiterate	2.23	1.35–3.45	0.001
Primary	2.01	1.28–3.39	0.008
Household monthly income/person (TL)			
<250	2.34	1.49–3.89	<0.001
250–500	1.74	0.98–3.51	0.071
Weight gain<10 kg	1.13	0.88–1.57	0.145
No. of admissions to antenatal care			
<5	1.45	1.05–2.11	0.012
5–10	1.30	1.03–2.09	0.028
Gestational age at first admission			
Second trimester	1.63	1.24–2.81	0.006
Third trimester	2.45	1.41–4.06	<0.001
Iron supplementation (m)			
<3	2.62	1.51–4.17	<0.001
3–6	1.68	1.13–2.91	0.001
Antenatal bleeding	1.34	0.94–3.47	0.212
Preeclampsia	1.55	1.03–2.10	0.041

CI: confidence interval; OR: odds ratio; BMI: body mass index; TL: Turkish liras

women might have got pregnant with low levels of nutrients due to the depletion of reserves of the mother in prior pregnancies and lactation periods.

Women with low educational level and household monthly income per person were detected to be significantly more vulnerable to anemia than others. Confirming this observation, Ndukwu and Dienye (13) reported an inverse relationship between the prevalence of anemia and socioeconomic status. In addition, the severity of anemia was also found to be inversely related to educational status and family income (14). This is not surprising considering the fact that women who were poorly educated and had financial constraints might suffer the deleterious effects of poor nutrition and not have early access to health services.

Women who were taking iron supplements for less than 3 months and 3–6 months had 2.62 and 1.68 times the risk of anemia at term, respectively. Similar observations were made in several studies that documented a reduction in the prevalence of anemia at the end of pregnancy after routine supplementation of iron to pregnant women (15, 16). On the other hand, a study from the United States did not demonstrate any effect of prenatal prophylactic iron supplementation on the overall prevalence of anemia (17). The possible reason why an association was not observed in the previous study is that they carried out the study with patients who had adequate iron stores. In addition, the power of that study was affected due to the lack of follow up (17). Therefore, for anemia intervention to be most effective, it is important that women should attend antenatal clinics in the first trimester of their pregnancies. In this study, only 17% of women had their first antenatal care visit in the first trimester, and hence, most pregnant women missed anemia interventions.

Another finding is that more than half the women with anemia (57.1%) had 10 or less antenatal care visits. In other words, the women who were admitted for antenatal care less than 10 times during the pregnancy had significantly higher prevalence of anemia than those that were admitted 10 times or more during the pregnancy. A multi-country randomized control trial conducted by WHO showed that essential interventions can be provided over four visits at specified intervals, at least for healthy women (18). Contrary to this report, our study showed that women with antenatal visit numbers between 5 and 10 were also associated with anemia before delivery. The reason for this relation may be explained by the fact that the women in our study could have underlying medical problems accompanying anemia, which increased the antenatal visit number. Moreover, it is possible that some hospital admissions resulted from reasons other than antenatal care such as prescription or maternal anxiety. Furthermore, a systematic review including a total of over 60,000 women compared the effects of reduced antenatal care visits (4–9 visits) with standard care (13–14 visits) (19). In that study, the reduced visit model was not associated with significant increases in postpartum anemia (Relative risk=0.88, 95% CI=0.75–1.03) (19). Similarly, we think that the impact of antenatal visit number on maternal anemia in our study mainly resulted from the gestational age at the initiation of antenatal care. An earlier gestational age at first admission will increase the total antenatal care visits at the end of pregnancy and will also prevent the depletion of iron stores because of early supplementation.

After multivariate logistic regression analysis, the association between anemia and preeclampsia still remained significant. It is already known that 10–20% of women with severe preeclampsia could progress to hemolysis, elevated liver enzymes, and low platelet syndrome, which is characterized by microangiopathic hemolytic anemia (20). On the other hand, maternal anemia and iron deficiency during the first trimester of pregnancy were demonstrated to cause subsequent development of preeclampsia through the stimulation of cortisol releasing hormone and alterations in the peripheral gas exchange of placental villi (21).

Because of the retrospective design of this study, whether anemia preceded the preeclampsia or vice versa could not be verified. The limitation of our study was that we could not identify potential confounding variables such as folic acid deficiency because of the retrospective design of the study. Although we excluded some of the reasons of anemia including hemoglobinopathies and chronic inflammatory diseases, folic acid deficiency could not be assessed in the study as a causative factor for anemia during pregnancy. However, the prevalence of anemia in pregnancy secondary to folate deficiency was known to be approximately 3% (22). Hence, we thought that this incidence is too low to cause a significant alteration in the evaluation of our findings. Another limitation is the failure to identify the causes of anemia in pregnant women who received routine antenatal supplementation. Data in the medical records of women did not allow us to conclude about all etiologies of persistent anemia. The possible explanation why some pregnant women did not benefit from supplementation is that most of them could have been suffering from deleterious effects of undiagnosed medical disorders and were possibly anemic before pregnancy. Therefore, iron and folic acid supplementation is an important part of anemia control program, but supplements should be viewed as one of the several tools in the battle against anemia. In conclusion, our study provides evidence about the underlying factors for anemia among pregnant women attending our center for delivery at term. Based on the results of this study, identification of pregnant women with these factors is a worthy consideration for the reduction of anemia during and after delivery. We recommend that socioeconomic determinants, which cause limited access to adequate food and antenatal care, constitute most of the anemia cases and hence, should be recognized as major risk factors for anemia in women who are going to deliver. In many developing countries, pregnant women start antenatal care in the second or third trimester due to the belief that antenatal care is curative rather than protective. Therefore, those women should be encouraged to begin antenatal care early after conception to allow adequate time for restoring iron stores.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Tepecik Training and Research Hospital.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - C.E.T., A.E.; Design - C.E.T., A.E., U.S.; Supervision - C.E.T., M.Ö., C.G.; Resource - B.Ç., M.K., M.B.E.; Materials - B.Ç., M.K., M.B.E.; Data Collection and/or Processing - B.Ç., M.K., M.B.E.; Analysis and/or Interpretation - A.E., U.S., C.G.; Literature Search - U.S., C.G.; Writing - C.E.T., A.E., C.G.; Critical Reviews - U.S., M.Ö.*

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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Effects of altitude changes on Doppler flow parameters for uterine, umbilical, and mid-cerebral arteries in term pregnancy: A pilot study

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Abstract

Objective: We hypothesized that maternal and fetal circulations may be affected by moderately high altitudes. Therefore, we compared the differences in maternal and fetal Doppler flow parameters in women with term pregnancy living at a moderately high altitude (1890 m in Erzurum) with those of women living at the sea level (31 m in İstanbul).

Material and Methods: Eighty women (n=40, for each group) with full-term and singleton pregnancies underwent Doppler waveform analysis, and the pulsatility and resistance index values for the uterine, umbilical, and mid-cerebral arteries were recorded. Also, sex, birth, and placental weights during delivery were obtained from the medical records.

Results: Similar mean placental weight values were found at the sea level compared with the moderately high altitude ($p>0.05$). The mean birth weight values were found to be lower at the moderately high altitude than those at the sea level ($p<0.05$). The pulsatility and resistance index values for the umbilical and mid-cerebral arteries were found to be similar between the groups ($p>0.05$). However, the pulsatility and resistance index values for both the right and left uterine arteries were higher at the sea level than those at moderately high altitude ($p<0.05$, for all).

Conclusion: Moderately high altitude does not affect fetal vascular Doppler parameters. However, it appears to increase the uterine artery blood flow bilaterally, and these alterations in the bilateral uterine artery blood flow may be associated with a physiological adaptation to high altitude.

(J Turk Ger Gynecol Assoc 2015; 16: 237-40)

Keywords: Doppler, ultrasound, fetus, altitude, pregnancy

Received: 02 July, 2014 **Accepted:** 30 June, 2015 **Available Online Date:** 02 November, 2015

Introduction

Respiratory, cardiovascular, and hematological adaptations (an increase in blood viscosity, a decrease in carbon monoxide diffusion capacity, an increase in cerebral arterial blood flow, a reduction in blood volume, and a decline in cardiac output) occur in individuals living at high and moderate altitudes. These adaptations are associated with the changes in oxygen uptake and transport that occurs in response to hypoxemia (1, 2).

The Doppler flow parameters of the umbilical, mid-cerebral, and uterine arteries have been investigated to assess the fetal well-being and maternal risk for preeclampsia (3-5). The altitude was found to be independently associated with the birth weight restriction and adverse perinatal outcomes (6). Moreover, the frequency of preeclampsia, gestational hypertension, and other pregnancy-related complications were reported to be higher in women living at a high altitude (3600 m) than women living at a low altitude (300 m) (7). Kumtepe et al. (8) demonstrated a greater incidence of eclampsia among women living at high altitudes (>1500 m).

There are studies in the literature comparing the effect of altitude on maternal and fetal Doppler flow parameters; however, these studies have conflicting results (9-15). For instance, Galan et al. (14) reported no uteroplacental or fetal vascular Doppler velocimetry differences between moderate highlanders and lowlanders, whereas Krampl et al. (11) reported a lower impedance of uterine artery blood flow in women living at a high altitude than at the sea level. We therefore hypothesized that maternal and fetal circulations may be affected by moderately high altitudes. Therefore, we aimed to detect the differences in the pulsatility index (PI) and resistance index (RI) values for uterine, umbilical, and mid-cerebral arteries in women with term pregnancy living at a moderately high altitude (1890 m in Erzurum) with those of women living at the sea level (31 m in İstanbul). In addition, we compared the differences in birth and placental weights between moderately high and low altitudes.

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DOI:10.5152/jtgga.2015.15134

Material and Methods

This descriptive study was approved by the Ethics Committee of Atatürk University, Medical Faculty, Erzurum, Turkey, and written informed consent was obtained from all participants. Between February 1, 2014 and July 20, 2014, women between 20 and 40 years of age with full-term pregnancies (≥37 gestational weeks) admitted to the Obstetric Department of two institutes (Nenehatun Hospital, Erzurum Turkey: 1890 m above the sea level and Bezmialem Vakif University, İstanbul, Turkey: 31 m above the sea level) for the control were enrolled in this study. Initially, the last menstrual period was questioned, and ultrasonographic evaluation was performed to confirm the gestational age and to detect fetal abnormalities. Patients with a body mass index ≥30 kg/m^2, multiple pregnancies, complicated pregnancies (e.g., preeclampsia, fetal malformation, gestational diabetes mellitus, and placenta praevia), smoking, chronic illnesses (e.g., hypertension, diabetes mellitus), and no certain menstrual history were excluded from the study. Moreover, women were excluded if they were not of the ethnic Turkish background and if they did not permanently reside at the altitude.

To minimize the inter-operator variability, all ultrasonographic scans were performed by the same operators at each altitude between 10.00 and 12.00 PM, and three consecutive measurements were calculated in the absence of fetal movements or breathing movements. Ultrasound devices had simultaneous real-time, color-mode, and Doppler-mode capability, and all measurements were performed using the equipment pulse Doppler 5 MHz trans-abdominal probe (Mindray, Schenzen, China and Voluson 730 Pro, GE Healthcare Technologies, Milwaukee, Wisconsin, USA). In all cases, the uterine artery (UA) on each side was visualized at the point just distal to the crossover with the iliac artery. The umbilical artery (UmbA) was examined on a free loop of the umbilical cord, and the mid-cerebral artery (MCA) was visualized in a transverse axial view of the fetal head. Sociodemographic information (age, body mass index, parity, gestational week) and PI and RI indices for UA, UmbA, and MCA were recorded. In addition, sex, birth, and placental weights during delivery were obtained from the medical records.

A power analysis for this study was calculated based on the work of Galan et al. (14) using Russ Lenth's Power and sample size calculation application (15). We aimed to detect a mean difference between the two groups, at least a 1.5 standard deviation (SD) on Doppler index values. Accordingly, we determined that the number of patients required in every group was 30, based on the power of 80% at 5% significance level.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software 12.0 (SPSS Inc., Chicago, Illinois, USA) and expressed as mean±SD; $p<0.05$ was considered significant. The Kolmogorov–Smirnov test was used to test the normality of the variables. If data was not normally distributed, the comparisons were determined using Mann–Whitney's U-test. Comparisons were determined using the independent samples t-test when the data was normally distributed, and Fisher's exact test was used to compare the percentage values.

Table 1. Clinical characteristic of patients living at the sea level and moderately high altitude

	Moderately high altitude group (n=40)	Sea level group (n=40)	p
Age (years)	28.45±3.86	30.07±4.54	0.089
Body mass index (kg/m^2)	29.55±3.34	29.40±3.93	0.855
Parity	2 (1–6)	2 (1–6)	0.632
Mean gestational age (weeks)	38.96±1.19	39.01±1.02	0.858
Gender of fetus (Female/Male)	26/14	28/12	0.785

Data were expressed as mean±SD or median (min-max)

Table 2. Comparison of Doppler flow parameters and mean birth and placental weights between the groups

	Moderately high altitude group (n=40)	Sea level group (n=40)	p
Mean birth weight (g)	3389.87±331.63	3603.12±446.90*	0.018
Mean placental weight (g)	609.90±78.36	593.86±8.90	0.389
Right uterine artery PI	0.67±0.29	0.93±0.64*	0.026
Right uterine artery RI	0.40±0.09	0.47±0.08*	0.003
Left uterine artery PI	0.69±0.28	0.99±0.54*	0.003
Left uterine artery RI	0.43±0.10	0.51±0.09*	0.001
Umbilical artery PI	0.81±0.27	0.77±0.16	0.432
Umbilical artery RI	0.54±0.10	0.52±0.73	0.364
Mid-cerebral artery PI	1.53±0.63	1.42±0.39	0.339
Mid-cerebral artery RI	0.79±0.29	0.72±0.10	0.340

*p<0.05; compared with the moderately high altitude group.
PI: pulsatility index; RI: resistance index

Results

During the study period, 120 women (65 in Erzurum and 55 in İstanbul), of whom 90 agreed to participate, met the inclusion criteria for the study. Forty-five women in each group underwent Doppler waveform analysis. Of these, 10 women were lost to follow-up before delivery and were therefore excluded from the study. Thus, 40 women in each group were included in the final statistical analyses. There were no differences in the clinical characteristics between the groups (Table 1). Doppler flow parameters and mean birth and placental weights in the groups are presented in Table 2. The mean placental weight values were similar between the groups ($p>0.05$). The mean birth weight values were found to be lower at the moderately high altitude than those at the sea level ($p<0.05$). Both groups had similar PI and RI values for the umbilical and mid-cerebral arteries ($p>0.05$). However, the PI and RI values for both the right and left uterine arteries were higher at the sea level than those at the moderately high altitude ($p<0.05$, for all) (Table 2).

Discussion

It has been known that high altitudes lead to respiratory, cardiovascular, and hematological changes (such as hyperventilation, polycythemia, pulmonary vasoconstriction, and an increase in the systemic blood pressure) in individuals (1, 2). In this study, we compared the maternal and fetal Doppler flow parameters in women with term pregnancy living at a moderately high altitude with those of women living at the sea level. We found lower bilateral uterine artery PI and RI values at the moderately high altitude than those at the sea level. However, we found no differences between the groups in terms of Doppler parameters for the umbilical and mid-cerebral arteries. Moreover, we reported lower birth and similar placental weight values at the moderately high altitude compared with those at the sea level.

Uterine artery Doppler flow parameters have been used to assess the risk of developing preeclampsia and fetal growth restriction (16-18). The incidence of pregnancy-related complications such as preeclampsia and eclampsia was found to be higher in women living at a high altitude than that in women living at a low altitude (7, 8). We observed lower PI and RI indices in both the right and left uterine arteries at the moderately high altitude than those at the sea level. Similar to our results, Krampl et al. (11) reported lower uterine artery blood flow impedance at a high altitude than that at the sea level. In a study by Galan et al. (14), Doppler parameters for the uterine artery were found to be similar between moderately high altitude (1609 m) and sea level (40 m). However, the altitude difference was 1859 m in our study, whereas it was 1569 m in their study (14). In addition, they were unable to compare the ethnic backgrounds among their study population. On the other hand, our study population was selected from a single type of ethnic population. Nevertheless, the cause for these different Doppler findings in our study is unclear. Julian et al. (13) found a greater uterine artery diameter and volumetric flow at a low altitude than at a high altitude. Moreover, they found an elevation in the maternal circulating vasoconstrictor endothelin relative to the vasodilator nitric oxide metabolite levels at a high altitude. These findings may explain the reason why the uterine artery RI and PI decrease at a high altitude.

We found lower birth weight at a high altitude compared with that at the sea level. In addition, we found similar placental weight values between the groups. Similar to our results, Krampl et al. (19) compared ultrasound fetal size at a high altitude and sea level. They reported smaller fetal biometry measurements and lower estimated fetal weight values at a high altitude than those at the sea level. Furthermore, a decline in the birth weight with an average of 102 g per 1000 m elevation was reported by Jensen et al. (20). Consistent with our results, Yung et al. (21) reported a significant reduction in the birth weight in the high-altitude group, and placental weight values were also similar between the groups in their study. Besides, Galan et al. (22) reported that the reduced birth weight of the newborns at a high altitude is the result of a reduction in fetal subcutaneous fat tissue and not lean mass.

It is unclear why a decrease in fetal birth weight occurs at a high altitude without any change in placental weight. A recent study (21) revealed the presence of endoplasmic reticulum stress, protein synthesis inhibition, and slowed proliferation in the placenta in response to chronic hypobaric hypoxia. On the other hand, Keyes et al. (23) reported that both high altitude and hypertensive complications are independently associated with the reduction in the birth weight. In another study (24), a significant increase in the reactive oxygen species production in uterine arteries and pressure-dependent uterine arterial myogenic tone was found in pregnant sheep exposed to high-altitude (3801 m) hypoxia for 110 days compared with the sea level (300 m). These changes may contribute to the low birth weight at high altitudes. At the same time, Reshetnikova et al. (25) analyzed 10 normal term placentas each collected at three different altitudinal levels. They found no differences in the villous and capillary surface areas or capillary length; however, they found a significant increase in capillary volume at a high altitude. They concluded that the placenta is capable of adapting to hypobaric hypoxia to increase its functional capacity for gaseous exchange.

Our findings are in agreement with those of Schwartz et al. (9), who studied 42 normal Colorado residents (1600 m in Denver and 3100 m in Leadville) longitudinally from 20 to 36 weeks gestation. They found a lowered birth weight at a high altitude than at a moderate altitude, but they reported no significant differences in the Doppler indices of the umbilical and mid-cerebral arteries. Julian et al. (13) found no differences at any time in the umbilical or mid-cerebral arteries Doppler flow parameters at low altitude than high altitude. Indeed, the fetus of pregnant sheep acclimatized to long-term hypoxia had similar increases in the cerebral blood flow and cerebral tissue oxygenation to the fetus at a low altitude (26). Contrary to our results, Krampl et al. (12) observed higher umbilical artery PI values at a high altitude than at the sea level. They suggested that the increase in the viscosity and umbilical artery vasoconstriction due to altered endothelial function and increasing production of vasoconstrictor substances such as endothelin or serotonin or increased placental lipid peroxide production may cause the changes in feto-placental circulation at a high altitude.

Our study has two limitations. First, the same operators performed all Doppler studies, but different ultrasound machines were used in our study. Second, our study has a relatively small patient population.

In conclusion, we found lower birth weight and uterine artery PI and RI indices at the moderately high altitude than at the sea level. We found similar placental weight values and Doppler parameters for the umbilical and mid-cerebral arteries among the groups. We suggested that altitude appears to increase the uterine artery blood flow bilaterally, and these alterations in bilateral uterine artery blood flow may be associated with a physiological adaptation to high altitudes. Large prospective clinical studies, including placental pathological examination, are required to evaluate the effects of the altitude on maternal and fetal Doppler flow parameters and fetal development.

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Author Contributions: *Concept - A.N.A., G.B., B.D.; Design - A.N.A., S.K.K., İ.G.; Supervision - A.N.A., G.B., İ.G.; Resource - G.B., S.K.K., İ.G.; Materials - A.N.A., G.B., S.K.K.; Data Collection and/or Processing - A.N.A., G.B., S.K.K.; Analysis and/or Interpretation - A.N.A., S.K.K., İ.G.; Literature Search - A.N.A., B.D., İ.G.; Writing - A.N.A., G.B., S.K.K.; Critical Reviews - A.N.A., B.D., İ.G.*

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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Abdominal anatomy in the context of port placement and trocars

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Abstract

Although the anatomy of the human being has not changed, technical developments in operating materials and methods demand a simultaneous development in operative management. Developments in electronic and optical technologies permit many gynecological operations to be performed laparoscopically. One fundamental distinction between any other operating method and laparoscopy is the hurdle that the initial entry, whether with a needle, cannula, or trocar, is mostly performed blind. However, there is a risk that blind entry may result in vascular or organ damage. One of the difficulties associated with entry complications is that any damage may not be immediately recognized, leading to major abdominal reparative surgery, and at worst, a temporary colostomy. Therefore, the technical and operative quality of laparoscopic surgery begins with port placement and trocars. Visual access systems are available but are not yet widely used. The aim of this review was to introduce the different port placement and trocar systems as well as their correct and professional usage in correlation with the abdominal functional anatomy. (J Turk Ger Gynecol Assoc 2015; 16: 241-51)

Keywords: Gynecological endoscopy, port placement, entry technique, complication prevention, pneumoperitoneum, functional gynecological anatomy

Received: 29 July, 2015 **Accepted:** 12 October, 2015

Introduction

Extra-abdominal relevant anatomy

Anatomical basis: The anterior abdominal wall has four muscles that are penetrated at all entries: rectus abdominis, external obliquus abdominis, internal obliquus abdominis, and transversus abdominis.

Although the penetrating areas are variable in laparoscopy, the usual trocar placement uses similar inserting areas. Therefore, it is obligatory for any surgeon to be experienced in the anatomy of the abdominal wall and its consecutive relevant anatomical structures.

There are no significant vascular structures that need to be respected upon insertion of the subumbilical trocar. Solely, strict attention has to be given to holding to the median line to avoid any accidental damage to paramedian structures.

There are two arteries in the superficial abdominal wall that should be visualized. Damage to these arteries should be avoided because even superficial incisions can lead to severe bleeding that requires the conversion from laparoscopy to laparotomy. Both vessels can be visualized by diaphanoscopy (Figures 1-4). Trocar placement is performed, dependent on the corresponding internal site, at a 90° angle to the abdominal wall once the aiming point has been located. The superficial epigastric artery arises from the femoral artery approximately 1 cm below the inguinal ligament through the fascia cribrosa, turns upward in front of the inguinal ligament, and then ascends while spreading out between the two layers of the superficial fascia of the abdominal wall, nearly as far as the umbilicus. The circumflex iliac superficial artery originates from the femoral artery close to the superficial epigastric artery. After perforating the fascia lata, it runs parallel to the inguinal ligament and laterally to the iliac crist while spreading into smaller branches (1).

Places for trocar insertion

The laparoscope and optic trocar should be inserted, whenever possible, in the subumbilical region using a semilunar or straight incision (Figure 1). Only if trocar placement is not possible, e.g., due to severe adhesions or large intra-abdominal tumors, are alternative entry sites negotiated, e.g., above the umbilicus or Palmer's point (Figure 5), as a precursor entry site.

The placement for the working trocars depends on the operation. If the operative focus is located in the pelvis and no large tumor is expected to be touching the umbilical region, the two working trocars can be inserted in the lower abdominal wall in a vessel-free area, as confirmed by diaphanoscopy. Any auxiliary trocar can be placed in the midline suprasymphysically or left of the midline. A maximum distance between the optic trocar and the working trocars should be

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DOI: 10.5152/jtgga.2015.0148

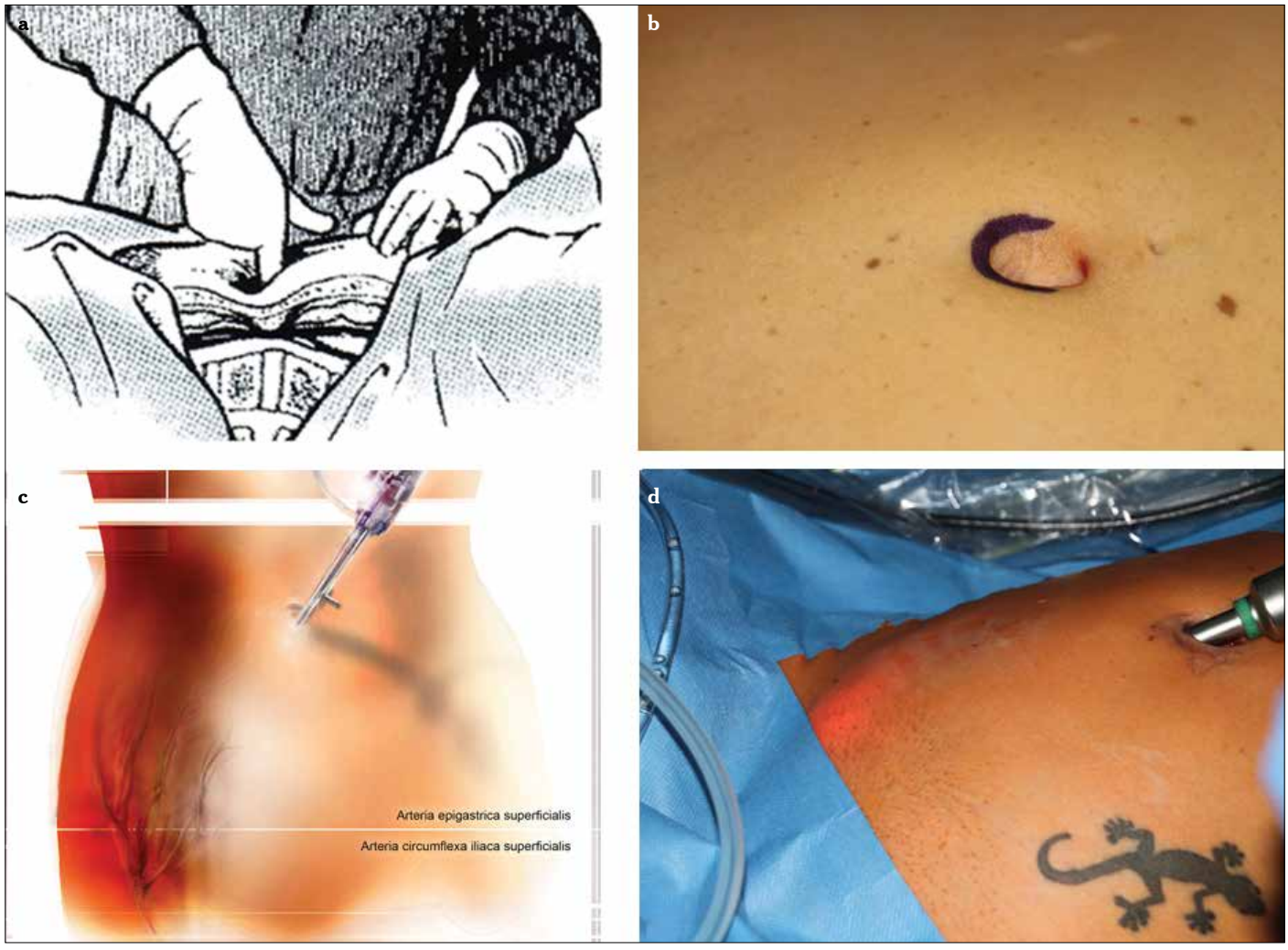

Figure 1. a-d. Typical palpation point in the subumbilical region. The fingertip is pointing to the promontorium. Subumbilical incision and local palpation demonstrate the short distance from the skin to the spine (a), diaphanoscopy illuminates the region of insertion of the ancillary trocars while demarcating the superficial epigastric artery and the circumflex iliac superficial artery (b-d)

achieved. Furthermore, the working trocars should not be in a cranial-caudal line but should be slightly shifted. Apart from the obliterated urachus and the bladder in the lower region, no remarkable anatomical structures were found (Figure 6).

Intra-abdominal relevant anatomy through the eye of the trocar

Anatomical landmarks

It is important to identify the different landmarks of the abdominal wall. Beginning in the midline, the plica umbilicalis mediana contains the obliterated urachus and requires no further attention besides a hoisted bladder, e.g., after caesarian section. Moving laterally, the paired plica umbilicalis medialis contains the obliterated umbilicalis artery in the ligamentum umbilicale mediale, which carries fetal blood through the umbilical cord to the placenta before it obliterates after birth and is therefore hazard-free too. The next step leads to the plica umbilicalis lateralis with the integrated vasa epigastrica inferiors. The inferior epigastric artery originates at the inguinal ligament of the external iliac artery. It cuts along the subperitoneal tissue ventrally and then moves upwards in an oblique manner—alongside the medial edge of the anulus inguinalis profundus. Subsequently, it perforates the fascia of the musculus transversus abdominis and climbs upwards between the musculus rectus abdominis and the rectus wall, thereby moving ventrally of the linea arcuata. Above the umbilicus, it divides into many small branches that anastomose with the superior epigastric artery. In contrast to the superficially spreading vascular branches, the inferior epigastric artery cannot be visualized by diaphanoscopy.

Places for trocar insertion

Once the cutaneous region has been determined from the outside with the aid of diaphanoscopy, the safe distance to the plica umbilicalis lateralis can be verified by palpation. The correct point of insertion is generally approximately two thumbs medial of the spina iliaca anterior superior (Figures 2, 3). Being distant to the plica, the trocar is placed at a 90° angle and pushed forward until the tip of the trocar can be seen with the laparoscope and then pushed toward the least delicate organ, which is the uterus (Figure 4) (2, 3).

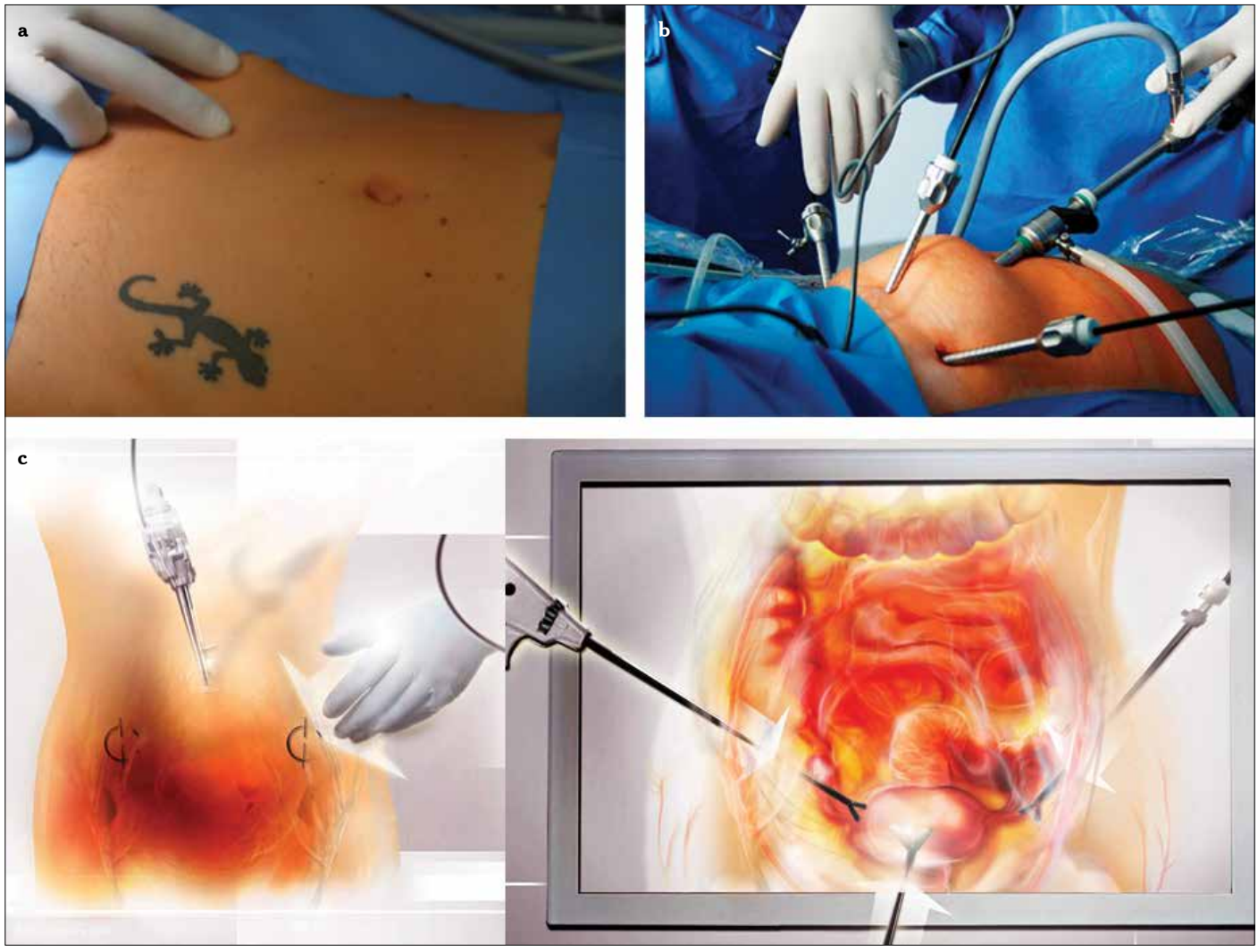

Figure 2. a-c. Point of insertion from the outside (two thumbs medial of the anterior superior spine), at a 90° angle to the surface with penetration of all abdominal wall layers (a), trocar insertion site lateral to the plica umbilicalis lateralis (b), overview after insertion of the laparoscope and three ancillary trocars (c), graphical illustration of (a) and (b)

Different types of ports and trocars

Development of ports and trocars

The word "trocar" is of French origin and is derived from *trois* (three) + carré (edge). A trocar is a medical instrument with a mostly sharply pointed end, often three-sided, that is used inside a hollow cylinder (cannula) to introduce ports into the abdomen.

Types of ports and trocars

Trocars are available in different sizes, from 3 mm to 12 mm and larger. In standard procedures, the optic trocar is placed in the lower part of the umbilicus, and its size varies depending on the operative procedure. For easy procedures, such as diagnostic laparoscopies or adnectomies, a 5 mm optic trocar is generally sufficient and provides enough light and precision. More pretentious procedures demand a brighter light source and a better picture, which is provided by a 10 mm optic trocar. Standard procedures use two working trocars on each side of the lower abdomen for secondary instruments, and 5 mm ports are generally sufficient. Smaller trocars, up to 3 mm, can be used for unproblematic procedures. Trocar entries for the laparoscope and the instruments can be dilated to 12 mm or larger, e.g., if a morcellator has to be utilized or larger tumors need to be extracted through an endoscopic bag (4).

Disposable trocars

With the decrease in production costs, disposable trocars have become popular in many countries. The advantage of a disposable material is that the tips are always sharp; therefore, less manual energy is necessary for the trocar insertion. The disadvantages are the higher expenses and the environmental stress.

Reusable trocars

Reusable trocars are available with two different types of inserting tips: pyramidal and conical. Today, the most popular tip is the pyramidal because this tip is sharper than the conical one. Sharpness, therefore, is the most important factor in the closed-entry technique.

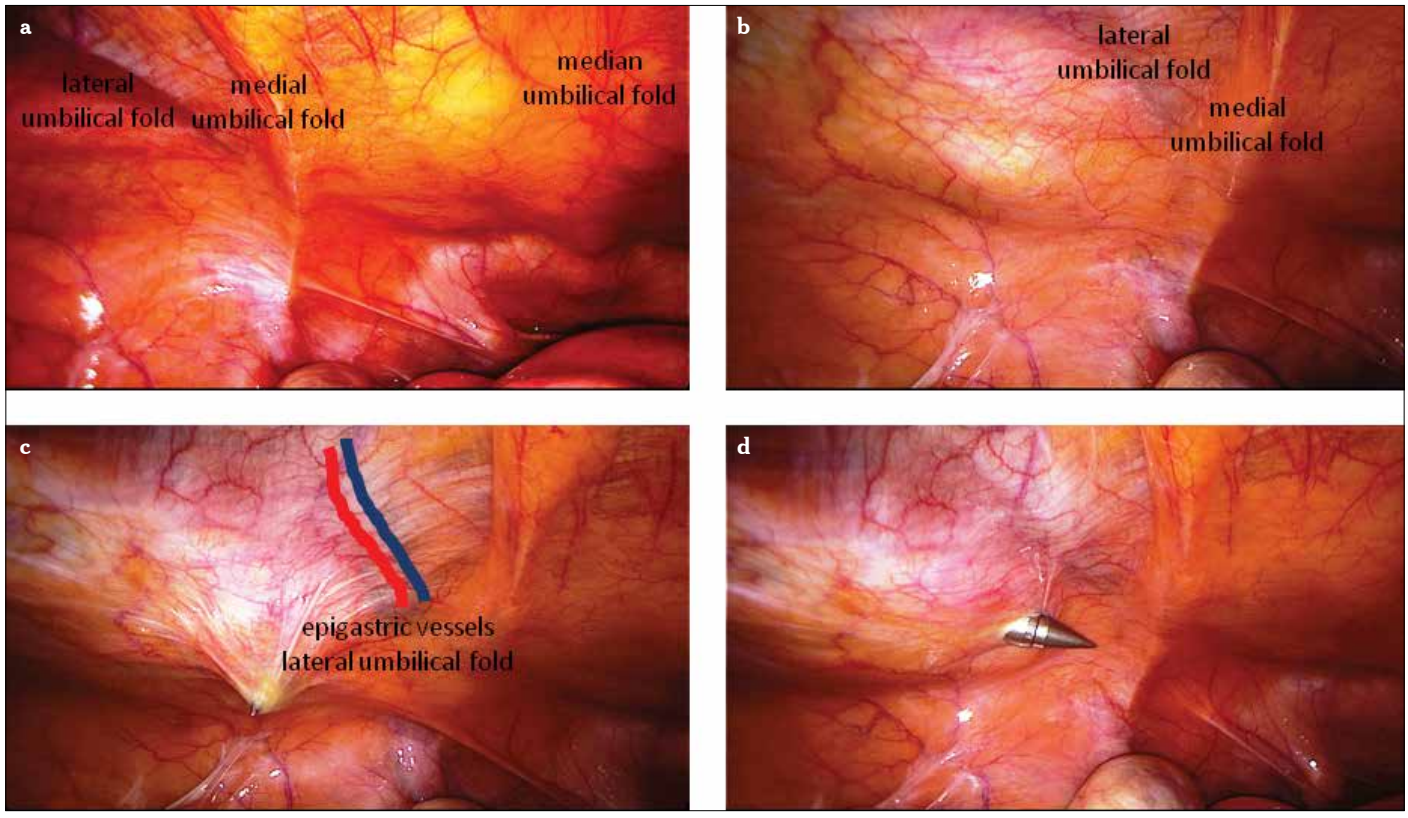

Figure 3. a-c. Overview of the abdominal wall from the interior (a), the plica umbilicalis mediana contains the obliterated urachus, the plica umbilicalis medialis contains the obliterated umbilical artery, and the plica umbilicalis lateralis contains the inferior epigastric vessels. Palpation with the index finger from the outside under laparoscopic view (b), entry with a sharp trocar strictly lateral to the inferior epigastric vessels (c-d)

In economic terms, reusable trocars seem to be more cost-effective than disposable instruments. However, the disadvantages of reusables are the time required for cleaning and sterilizing and the necessity of frequent sharpening and technical service (4, 5).

Single-site surgery

During the past few years, the field of laparoscopy has undergone several changes, and continuous efforts have been made to improve the morbidity and cosmesis of laparoscopic surgery, with a special focus on the miniaturization of equipment, the evolution of robotic surgical units, and reduction of the port size and number. Laparoendoscopic single-site surgery (LESS) is a term that covers a spectrum of surgical techniques targeted toward performing laparoscopic surgery by consolidating all the ports into only one surgical incision. Although early results are encouraging, this should not disguise the technical difficulties associated with performing a LESS procedure. LESS is challenging due to the lack of port triangulation (which leads to the clashing of laparoscopic instruments), two-dimensional view, poor ergonomic position for the surgeon, and an overall steep learning curve for suturing.

LESS has been utilized to successfully perform a large number of procedures and has also been utilized in general surgery. It was first reported for gynecological procedures in the 1970s for laparoscopic tubal ligations. However, this procedure did not initially gain popularity because of the technical challenges involved. In the last decade, technological advances in flexible optical and coagulation devices have allowed the performance of more advanced procedures, such as total LESS hysterectomy and pelvic/aortic lymphadenectomy. In this period, several reports have been published demonstrating the feasibility and reproducibility of this approach for benign and malignant uterine disease.

The benefits of single-site surgery seem superior to those reported for standard laparoscopy, including faster recovery, lower postoperative analgesic requirements, and better cosmetic results.

Instruments

The single trocar is inserted via a skin incision of approximately 1.5–2 cm within the umbilical scar and may have different numbers and sizes of ports for the insertion of the instruments [Single-port entries: Single Port Laparoscopic System (Covidien; Dublin, Ireland), LESS technique (Olympus Europa; Hamburg, Germany), X-CONE (Karl Storz; Tuttlingen, Germany)].

Special optics dedicated to single-port surgery facilitates an excellent intra-abdominal visualization and ensure no conflict between the camera and the instruments outside the patient. For this purpose, systems with 5 mm HD telescopes, either with a long and flexible external arm and a 30 degree optic or with a flexible internal tip and a 0 degree optic, are available.

The 5-mm working instruments are inserted into the remaining ports. These may include graspers, a bipolar coagulator,

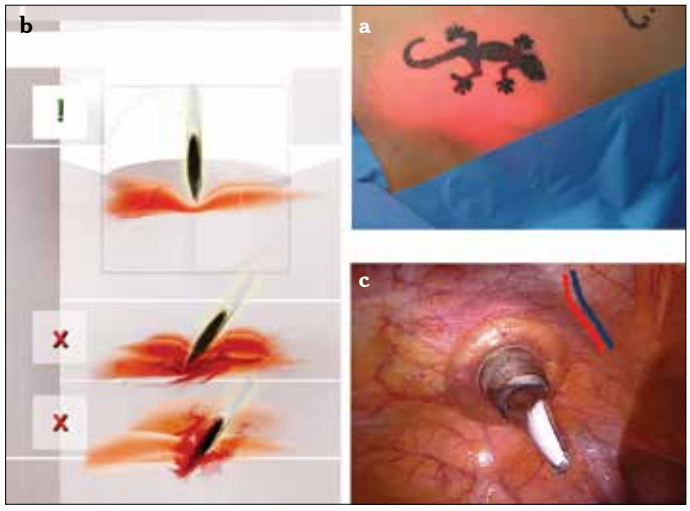

Figure 4. a-c. Diaphanoscopy illuminates the region of insertion of the ancillary trocars while demarcating the superficial epigastric artery and the circumflex iliac superficial artery (a), vision is dependent of the thickness of the abdominal wall. Point of insertion from the outside (two thumbs medial of the anterior superior spine), at a 90° angle to the surface with penetration of all abdominal wall layers. Direct entry of the ancillary trocars can avoid severe bleeding in the subcutaneous tissue (b), single-use trocar is situated strictly lateral to the plica umbilical lateralis and strictly lateral of the inferior epigastric vessels (c)

cold scissors, a suction/irrigation device, and a multifunctional device which grasps, coagulates, and transects simultaneously. To facilitate surgical maneuvers and prevent clashing between instruments and the surgeon's hands, the combination of one 33-cm-long instrument with a 43-cm-long straight instrument is recommended. Alternatively, one double-bended and one straight instrument can be adopted (Figure 7).

Entry techniques

Brief manual of port placement

The technique of entering the abdominal cavity can be separated into three different approaches:

1. The open technique;
2. The classical closed technique via a Veress needle;
3. The modified closed technique via direct trocar insertion.

Most gynecologists use the classical closed-entry technique, whereas most general surgeons still use the open (Hasson) method. Only a minority of surgeons use the modified closed technique.

Creation of the pneumoperitoneum

The most critical moments in laparoscopy, independent of operating competence, are the creation of the pneumoperitoneum and insertion of the primary trocar whether by Veress needle (Figure 8) and trocar insertion under sight or blindly or by minilaparotomy (Hasson technique).

Patients with a higher than average risk for complications in between the first steps include the following:

- **Obese patients**. The thicker abdominal wall decreases tactile sensation, and the insertion of the Veress needle is thus more difficult. At minilaparotomy, vision is likewise restricted so that the section is often more than small, and the risk for organ damage and postoperative complications, such as wound infection or hernia formation, is higher because of the limited overview and the larger wound. Once the trocar is inserted, the required insufflation pressure is set.
- **Very thin patients**. The distance between the umbilicus and the main vessels is no more than 2 cm because the abdominal wall lies very close to the retroperitoneal situated structures. To prevent the wrong insertion angle, the inserting instrument has to be at a 45° angle to the back of the patient and, after elevating the abdominal wall, at a 90° angle to the wall surface. Before needle or trocar insertion, the anatomic route of the major vessels can be identified by palpating the pulse of the vessel track.
- **Patients with previous laparoscopies or laparotomies**. A history of previous abdominal operations significantly increases the risk for omental or bowel adhesions to the abdominal wall. In the case of scars or a history of previous operations, an alternative entry site or entry method has to be considered (Figures 5, 6).
- **Patients with previous failed insufflations**. Previous preperitoneal insufflation is associated with an artificial space that extends all the way to the peritoneal cavity and makes the entry for the Veress needle or for any other entry method difficult. An alternative insufflation site should be considered (6).

Open-entry technique

The open-entry technique was invented by Harrith Hasson in 1974 and is still used extensively worldwide as the direct alternative to the closed-entry technique. The open-entry technique is favored by general surgeons, although its advantages over other entry techniques cannot clearly be proven. This entry technique begins with opening the peritoneal cavity prior to CO_2 insufflation. After performing a minilaparotomy in the subumbilical region, the optic trocar is placed intraperitoneally under sight (Figure 6) (6-16).

Advantages and disadvantages of open laparoscopy (the Hasson technique)

This technique supersedes the blind puncture of the abdominal cavity by either the Veress needle or a sharp trocar.

The open technique is less likely to cause major vessel injury, and if any injury does occur, it is easier to recognize and to repair at the same time. The technique has an equivalent risk of bowel and vessel injury as the closed technique; however, the operative process to dissect the different layers of the abdominal wall can be quite time consuming. Furthermore, open access leads to irritating air leaks because of the large incision, particularly in obese patients. Additionally, because of the larger skin incision and faster surgical preparation, the open technique is associated with a higher rate of wound infection. Several randomized trials and a Cochrane analysis have not indicated a significant safety advantage to either technique (7-11, 17-19).

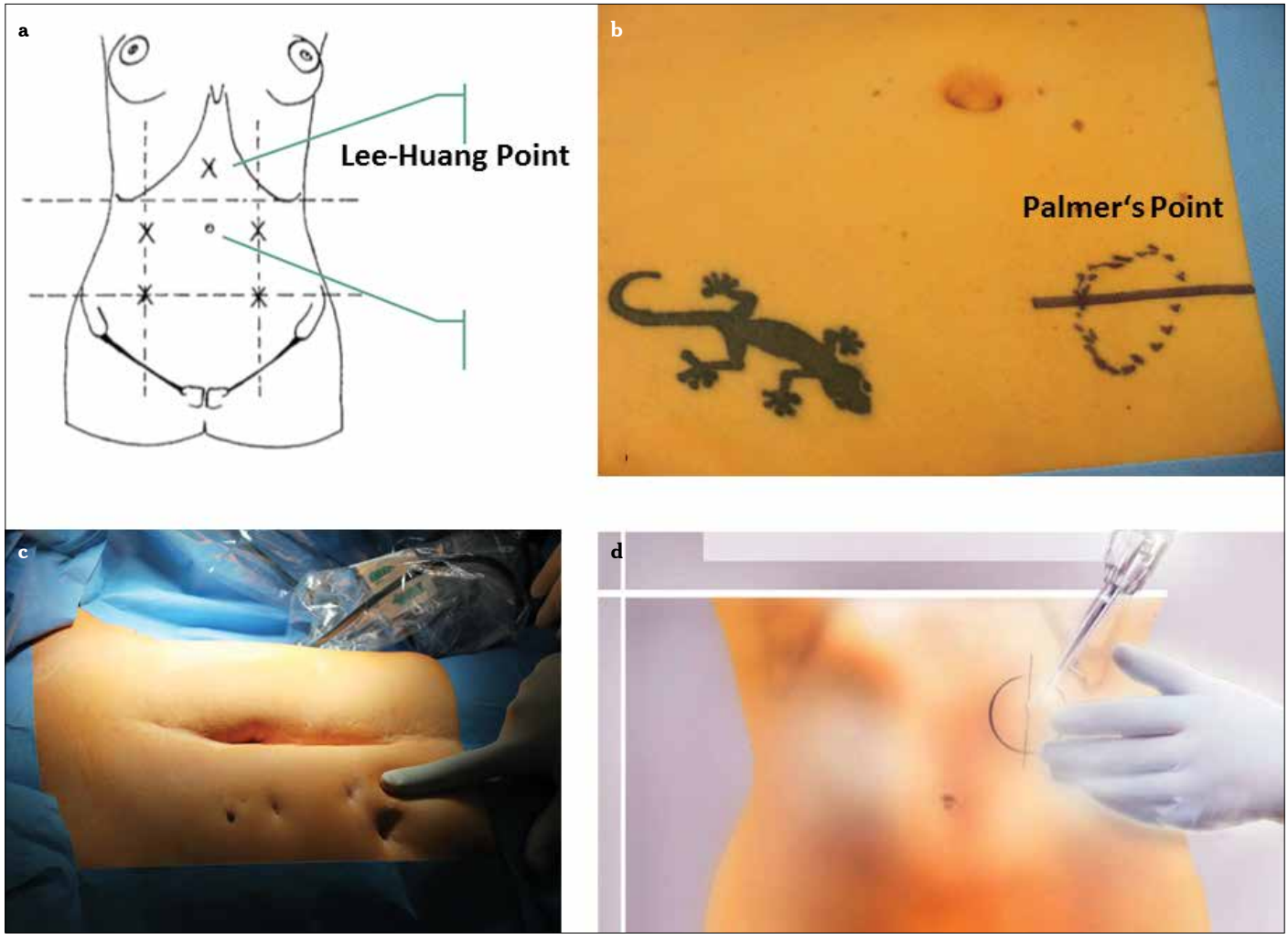

Figure 5. a-d. Depiction of the alternative entry site. For the large uterus, particularly at or above the level of the umbilicus, the Lee-Huang point is recommended for video laparoscopy (a), Palmer's Point, it is situated in the midclavicular line approximately 3 cm below the costal margin (b-d)

Closed-entry technique

Veress needle technique and CO_2 gas

To insert the Veress needle, the operating table needs to be in a horizontal position. The Trendelenburg tilt is performed after having created the pneumoperitoneum. The most common site for the Veress needle entry is the umbilical area. The skin incision in the lower part of the umbilicus is between 0.5 cm for the use of a 5 mm optic trocar and 1.5 cm for the use of a 10 mm optic trocar. The incision is made horizontally with an 11-scalpel blade after carefully lifting the skin underneath, so that the risk of damaging organs lying under the peritoneum in extremely thin patients is minimized. As the wall layers are at their thinnest at this level, a deep incision might enter the peritoneal cavity. Before incising the skin, it is recommended to palpate the aorta in its course and to identify the iliac bifurcation. This allows the abdomen to be inspected and palpated for any extraordinary masses (20).

All instruments need to be checked before use. The Veress needle needs to be tested to check that the valve springs and that the gas flow is between 6 and 8 mmHg. A sharp needle with a good spring action is necessary. Disposable needles fulfill these criteria. For the insertion of the primary trocar, the patient is still in the flat position. In this position, the insertion of the primary instrument at a 45° angle toward the uterus is associated with the lowest risk of damaging the major vessels running retroperitoneally downwards. Before inserting the instrument, the abdominal wall is lifted (Figure 8). The abdominal wall can either be lifted medially with one hand or with two hands on both sides depending on the obesity of the patient. In obese patients, the inserting angle is close to 90°, whereas in thin patients, the angle is close to 45°. If the first entry attempt fails, a second attempt is made before choosing an alternative entry site. Before placing the Veress needle, different safety checks should be performed to guarantee the lowest risk of complication:

a. Needle flow: To ensure flawless insertion of the Veress needle, the manometer should be set to a maximum resistance of 4–6 mm Hg with a gas flow rate of 1 L/min. If the resistance is high, there is some obstruction inside the Veress needle.
b. Palpation of aorta: If the abdominal aorta can be palpated directly below the umbilicus, the bifurcation must be situ-

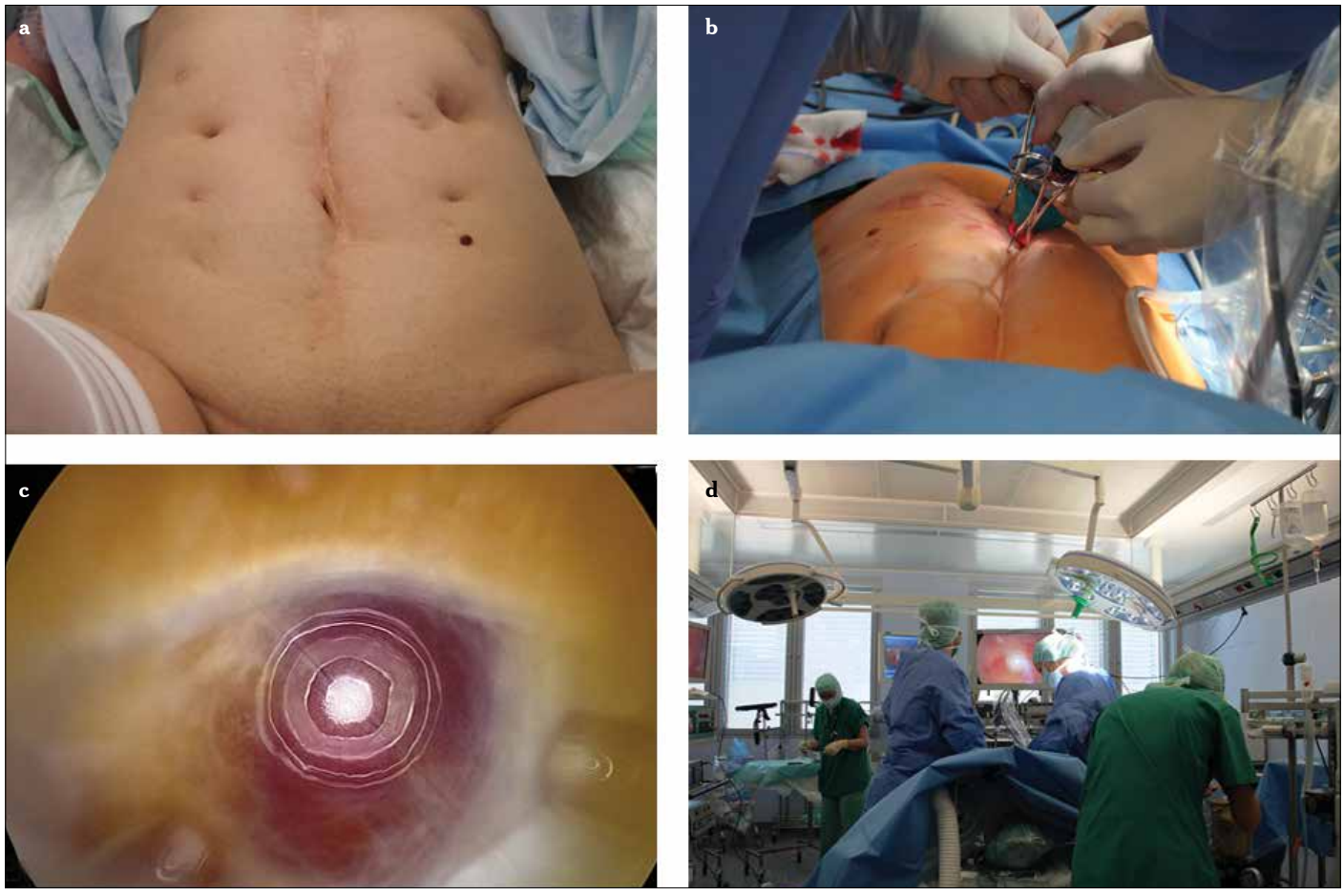

Figure 6. a-d. Entry under view in a case of previous peritonitis after repeated laparotomy, including the left epigastric area (a-d) Endopath™, or EndotipTM are disposable and reusable entry ports to enter under view (c-d)

ated further toward the lower pelvis. It cannot be injured by oblique insertion of the Veress needle. If the bifurcation is felt above the umbilicus, perpendicular insertion after lifting of the anterior abdominal wall is recommended.

c. Most times, two clicks can be heard. The first click is heard after perforation of the muscle fascia and the second click after perforation of the peritoneum. Three clicks might be heard above the linea arcuata. The proper needle placement is ensured by keeping the Veress needle between the thumb and index finger.

d. Aspiration test: Injection of 5–10 mL of normal saline solution results in negative aspiration if the Veress needle is correctly placed and blood-tinged aspirate or aspirate with intestinal contents if the needle is placed in a blood vessel or intestine

e. Hanging drop test and "fluid in flow": With the Veress needle placed in the abdominal cavity, lifting the abdominal wall creates a negative intra-abdominal pressure. A drop of water is then positioned on the open end of the Veress needle. If the needle is correctly positioned, the water should disappear down the shaft. The drop is only sucked in if the intra-abdominal pressure is negative. For "fluid in flow," a 5 mm syringe is filled with a saline solution. The piston is removed; the syringe is connected to the Veress cannula and by lifting the abdominal wall, the saline solution level drops rapidly as it enters into the free abdominal cavity.

f. Before insufflation with CO_2 gas begins, the initial pressure must be below 9 mmHg to confirm the correctly placed needle. The initial gas pressure (<9 mmHg) reflects the correct intraperitoneal Veress needle placement, although this pressure is not a precise reflection of the intraperitoneal pressure. The Veress needle is connected to the insufflator and the pressure is measured continuously as the needle traverses the various layers of the abdominal wall. A pressure below 9 mmHg confirms the correct needle placement.

Any movement of the needle after placement must be avoided as this may convert a small needlepoint injury into a complex and threatening tear. After ensuring that the Veress needle has been positioned correctly, the insufflation of CO_2 gas is started. CO_2 gas is used because room air is not soluble in blood and may cause an air embolism if it is pumped into a blood vessel accidentally. Before starting the intra-abdominal insufflation, the gas hose is flushed with approximately 1 L of CO_2 gas to purify any room air. The initial intra-abdominal insufflation pressure should not exceed 10 mmHg and is started with only 1 liter of CO_2 gas flow per minute. Once a good gas flow and an appropriate

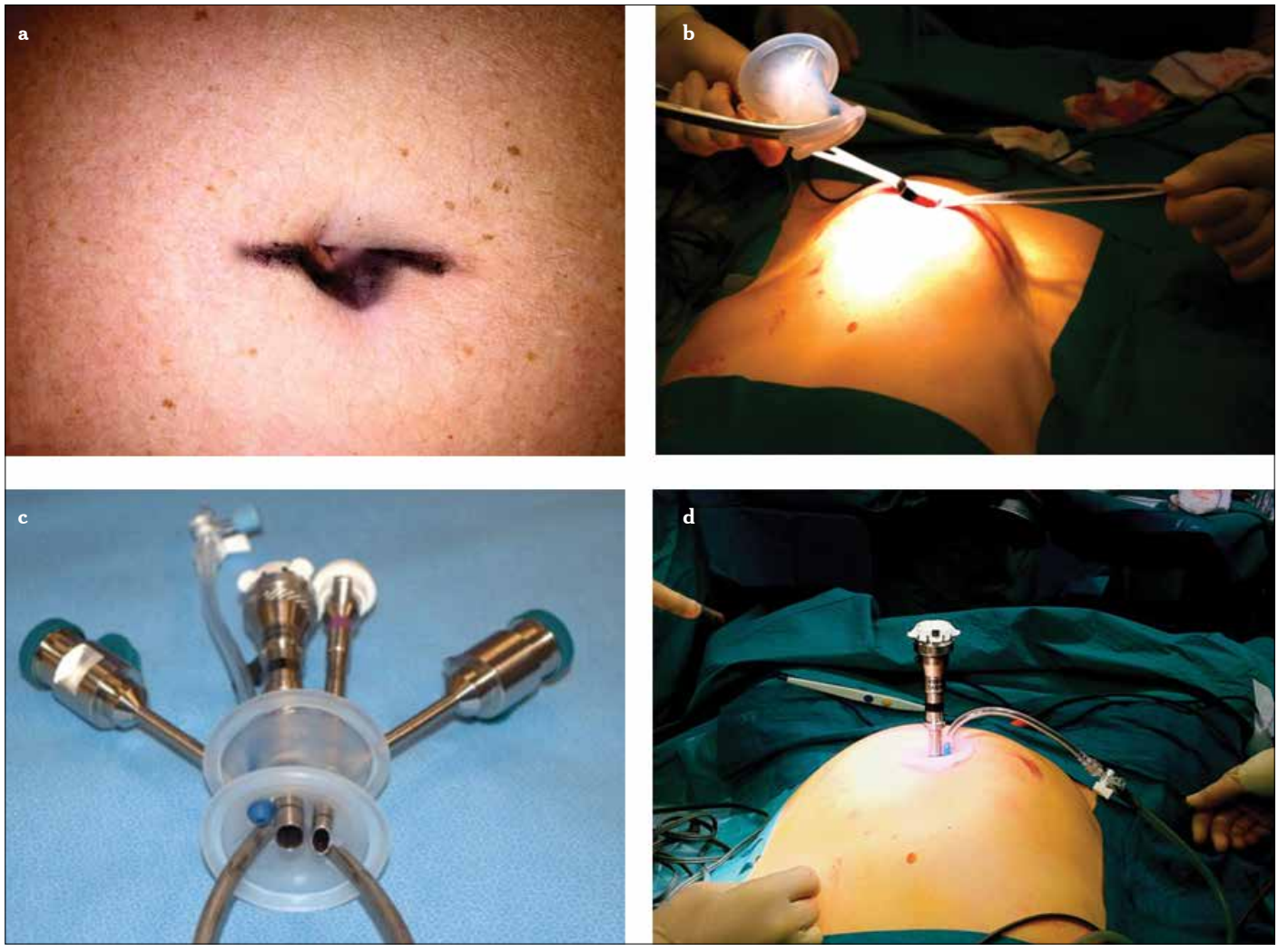

Figure 7. a-d. Omega-shape umbilical incision (a), single-site port introduction with the "folded" port-clamping technique using an atraumatic clamp (b), single-port cluster (c), pneumoperitoneum induction after the umbilical placement of the single-site port and positioning of the 12-mm lubricated trocar for the 30-degree scope (d)

pressure have been achieved, the influx can be raised so that 2–3 liters of CO_2 gas can be insufflated per minute until 3–6 liters are insufflated, depending on the patient's size and obesity.

g. After an insufflation volume of approximately 300 mL, the percussion of the liver region confirms the loss of liver dullness. This sign indicates the intra-abdominal insufflation and the distribution of the gas in the whole abdominal cavity.

After having created the pneumoperitoneum in the usual manner, the abdominal pressure should be built up to 18–25 mmHg before inserting the primary trocar as this maximizes the distension of the abdominal wall from all underlying structures. Once the layers of the abdominal wall are compressed, trocar incision becomes easy and the risk of injury minimal as the inflated distance between the abdominal wall and intra-abdominal structures further reduces the risk of damage. The distension pressure should be reduced to 12–15 mmHg for ventilation reasons once the trocar placement has been verified. During gas insufflation, symmetric distension of the lower abdomen and the disappearance of liver dullness can be observed. Once the insufflation pressure reaches 20–25 mmHg, the distension of the abdominal wall should be sufficient for safe insertion of the trocar. This can be tested by the:

h. Aspiration and Sounding test (after CO_2 insufflation): CO_2 is aspirated in a syringe containing 20 mL of normal saline solution and the result examined. When the tip lies free in the abdominal gas, CO_2 bubbles are visible in the normal saline solution during respiration, indicating the position in the free abdominal cavity. When planning a Z insertion, the aspiration must be performed horizontally toward the right or left and caudally depending on the preparation.

The 5 mm trocar is then placed in a Z technique superficially and brought through the abdominal wall orthogonally.

i. Hiss phenomenon: After successful perforation of the anterior abdominal wall with the primary trocar, a soft hissing sound is produced as a result of negative pressure in the abdominal cavity.

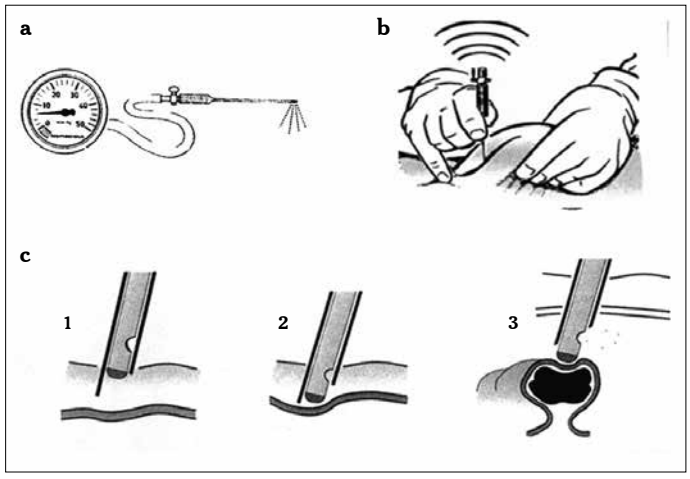

Figure 8. a-c. Veress needle and insufflation pressure (a), lifting of the abdominal wall, insertion angle is 45° (b), entry of the Veress needle through the abdominal wall (c1), the sharp tip penetrates the skin and fascia (c2), after piercing the peritoneum, the blunt tip springs forward due to the release of resistance and thereby avoids organ (bowel) damage (c3)

The correct position of the trocar can be checked with a 5 mm optic in cases of small operations or the entry site can be dilated to 10 mm after validation that there are no remarkable adhesions. Panoramic viewing reveals any pathological changes in the vicinity of the abdomen, e. g., in the intestines, liver, gallbladder, or spleen (7, 11, 21-23).

It is recommended to use heated and humidified CO_2 gas for insufflation. Various types of small machines can be attached to the electronic pneuautomatic to fulfill this purpose. The advantage of heated, moist CO_2 gas can best be illustrated with an egg. If you spray the egg white and yellow yolk with a continuous flow of heated CO_2 gas (37°C), they dry out. If you spray them with cold CO_2 gas, they dry out. If you spray them with cold, moist CO_2 gas, they dry out. However, if you spray them with heated, moist CO_2 gas, they retain their original composition (24-26).

Entry under vision

Entering the abdominal cavity under vision is more popular among general surgeons than among gynecologists, although its use is increasing among gynecologists. Trocar insertion is performed with direct vision trocars that are available as single use or reusable instruments (Figure 6). Entry under vision can either be reached directly or after creation of pneumoperitoneum with the Veress needle.

The 5 or 10 mm laparoscope is placed directly into the trocar sheath so that the trocar end can be seen and followed. The trocar is then pushed with a twisting motion stepwise into the peritoneal cavity. Each layer of the abdominal wall is visualized and registered as the trocar is moved in.

Beside disposable trocars, there is one non-disposable port system for this technique. It features a reusable, stainless steel-threaded cannula with no sharp components and requires no trocar. This port system can be screwed in without any physical effort. Using the Endotip™ (Karl Storz; Tuttlingen, Germany), a 0° telescope is put into the trocar behind the aperture so that the whole circumference can be seen. The cannula is then rotated clockwise with low force with finger/wrist action. The trocar is inserted into the skin and fascia incision and held perpendicularly to the supine patient with the non-dominant hand. The cannula is then rotated clockwise with the dominant hand applying minimal downward force (27). Only a little force is needed to engage and transpose upwards the various layers as it burrows through the either hyperdistended or soft and flat abdominal wall. Under continuous gentle rotation of the optic trocar, it passes through the sequential layers subcutaneous fat, anterior rectus sheath, preperitoneal fat, and peritoneum. The different layers are easy to differentiate, and the peritoneum is pierced only after it has been ascertained that the abdominal wall is free of adherent bowel. (18, 23, 28, 29).

Endopath™ (Ethicon, Johnson & Johnson; New Brunswick, NJ, USA) has a cannula-integrated thread design that provides greater abdominal wall retention and minimal trocar slip-outs. It is compatible with a wide range of instruments (4.7–12.9 mm). The bladeless tip separates rather than cuts along tissue fibers, pushing tissues and vessels away. Visualization through a plexiglas cannula eliminates blind entry by enabling visualization of the tissue layers during insertion. The design requires a lower peak instrument insertion and extraction force.

Both methods have in common a laparoscope that is inserted into the trocar, and once the trocar has penetrated the subcutis, it advances through the abdominal wall layers stepwise under permanent monitoring. By this method, bowel damage or blood vessel injury may be avoided.

Vaginal instruments

Uterus manipulator

There are a number of different uterine manipulators (Figure 9). The use of a uterine manipulator is controversial. The auxiliary function of manipulating the uterus during the procedure is not required for small operations with a good operative access, e.g., adnexal surgery. The indication for the use of a uterine manipulator has to be taken carefully as its use is associated with a certain intraoperative risk for injury of the cervix, uterine cavity, and other neighboring structures. On the other hand, the proper application of a uterine manipulator helps to improve vision and therefore provides better surgical preconditions (6).

Advancements in laparoscopic entries

Termination of the laparoscopic procedure

After completion of the operation, the laparoscope should be used to check on the way out that there has not been a through-and-through injury of bowel adherent under the umbilicus by visual control during port and laparoscope removal. All ancillary ports are removed under direct vision to ensure that there is no unrecognized hemorrhage and if there is one, it can be treated immediately. Prior to the removal of the instruments, a last inspection of the abdominal cavity is essential to ensure the absence of bleeding and retroperitoneal hematoma. Also, the area under the optic trocar has to be inspected for any

Figure 9. a-ı. Hohl manipulator (Storz) (a), Dionisi uterine manipulator (Storz) (b), Mangeshikar uterine manipulator (Storz) (c), RfQ uterine manipulator (d), Clermont-Ferrand uterine manipulator (Storz) (e), Braun uterine manipulator (f), Koninckx uterine manipulator (Storz) (g), Tintara uterine manipulator (Storz) (h), Donnez uterine manipulator (Storz) (ı)

unrecognized bleeding from this place of insertion. Once the working trocar on the left and possibly in the midline are taken out, the peritoneal gap is coagulated from the trocar in the right lower abdomen. The peritoneal gap of this trocar is coagulated bipolarly after the trocar has already been taken out and the gap is closed on the way out. Fascial incisions of the ancillary trocars larger than 5 mm should be sutured to prevent hernia formation. A single stitch with an absorbable polyfilar suture 3-0 is applied under direct view using the pneumoperitoneum and the laparoscope to prevent peritoneal involvement or even injury of the omentum or bowel. At the end of the operation, the patient is returned to the horizontal position to avoid brisk vascular changes. The pneumoperitoneum is then released slowly by opening the inserting valve. The laparoscope is taken out in the horizontal position to avoid a possible aspiration of air, which is responsible for shoulder pain in the postoperative period. Before removing the optic trocar, reinsertion of the laparoscope and removal of the trocar under sight are performed so that the stepwise closure of the abdominal wall in a reverse Z technique is guaranteed. Thus, the fascial incision does not have to be sutured as it is functionally closed (30, 31).

A certain amount of gas or irrigation fluid remaining in the abdominal cavity can be tolerated. This gas may irritate the peritoneum and the patients may experience discomfort and minor pain in the shoulder area for up to 2 weeks after the operation (6, 7, 32).

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - I.A., L.M.; Design - I.A., M.E., L.M.; Supervision - N.M., G.N.; Resource - I.A., N.M.; Materials - M.E., L.M.; Data Collection and/or Processing - M.E., N.M., G.N.; Analysis and/or Interpretation - I.A., G.N., N.M.; Literature Search - I.A., L.M., M.E.; Writing - I.A., M.E.; Critical Reviews - G.N., N.M., M.E.*

Acknowledgements: *We thank Mathias Podlovics for all the graphic designs. We also thank Dawn Rüther for editing the manuscript and Thoralf Schollmeyer for his valuable contributions to the manuscript.*

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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The laparoscopic management of Swyer syndrome: Case series

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Abstract

Swyer syndrome, also known as 46 XY pure gonadal dysgenesis, is a rare endocrine disorder. Affected individuals are phenotypically female with female genitalia, normal Mullerian structures, absent testicular tissue, and a 46 XY chromosomal constitution.

We report a series of eight cases of Swyer syndrome, of which six were managed by laparoscopic gonadectomy. The two other cases had to undergo an exploratory laparotomy in view of their presentation with adnexal masses. Two of the girls were siblings. The chief presenting complaint was primary amenorrhea. Four girls also presented with a history of poor development of secondary sexual characters. The average age at presentation was 16.19±2.85 years. The average height was 158.33 ±4.63 cm, and the average weight was 49.33±8.44 kg. Breast development was either Tanner 2 or 3 in four girls, whereas three girls had a Tanner 1 underdeveloped breasts. Axillary and pelvic hair was sparse in all the girls. The vagina was well canalized in all the girls. Hormonal evaluation revealed hypergonadotropic hypogonadism with a mean follicle-stimulating hormone (FSH) level of 95.81 mIU/L and a mean luteinizing (LH) level of 24.15 mIU/L. Imaging analysis revealed the presence of a small uterus in all the cases, except one. Bilateral ovaries were either not visualized or streak gonads were present. Adnexal mass was detected in two of the six cases with raised carcinoembryonic antigen (CA) 125 levels in one case. Genetic analysis revealed a karyotype of 46 XY in six girls, 46 XY/45 X in one, and the culture repeatedly failed in one girl. Because of the risk of malignancy, bilateral gonadectomy was performed in all cases. Histopathological analysis revealed that three of the six cases had dysgerminoma. The patients have been started on hormone replacement therapy. Laparoscopy is a minimally invasive modality for the definitive diagnosis and treatment of cases with Swyer syndrome. An early diagnosis of Swyer syndrome is possible during workup for primary amenorrhea before they present with adnexal masses. (J Turk Ger Gynecol Assoc 2015; 16: 252-6)

Keywords: Swyer syndrome, laparoscopic gonadectomy, gonadoblastoma

Received: 20 April, 2015 **Accepted:** 23 August, 2015 **Available Online Date:** 02 November, 2015

Introduction

Swyer syndrome is a condition characterized by the presence of an unambiguously female phenotype and Mullerian structures in the presence of a "y" line (XY karyotype), as was first described by Jim Swyer in 1955. The Swyer syndrome XY female should be differentiated from the other conditions of the XY female, such as testicular feminization, because they have different implications on the current management and future reproductive function. Over a period of 2 years, we managed eight cases of Swyer syndrome, of which, five patients were managed by laparoscopic bilateral gonadectomy.

Laparoscopy provides a minimally invasive approach for the management of these cases if detected at an appropriate time.

Case Series

Over a period of 2 years, eight young patients with Swyer syndrome were diagnosed and treated.

An informed consent was taken for case review and reporting. All the cases presented to the outpatient department with complaints of primary amenorrhea, and four patients also presented with a history of poor development of secondary sexual characters.

The average age at presentation was 16.19±2.85 years. The average height was 158.33±4.63 cm, and the average weight was 49.33±8.44 kg. Breast development was either Tanner 2 or 3 in 4 cases, whereas three cases had a Tanner 1 underdeveloped breasts. Axillary and pelvic hair was sparse in all the patients.

On abdominal examination, one of the patients had a 5×4 cm palpable mass in the lower abdomen, whereas the rest had no significant abdominal findings.

On vaginal examination, the vagina was well canalized in all cases, and the uterus could not be palpated. Two of the cases had palpable adnexal masses, of which, one had bilateral adnexal masses (7×5 cm right and 5×5 cm left adnexa), whereas the others had a fixed mass in the pelvis.

A hormonal analysis was performed in all the cases, and it revealed a hypergonadotropic state, confirming gonadal dysgenesis, which is a feature of the Swyer syndrome.

Ultrasound examination revealed a small uterus with non-visualization of ovaries in all the cases.

Out of the eight patients, six had a 46 XY karyotype. One patient had a mosaic [45 X (40%); 46 XY (60%)] karyotype, and the culture for karyotype failed twice in one of the patients but was included in the series because the clinical presentation was strongly suggestive.

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DOI:10.5152/jtgga.2015.15061

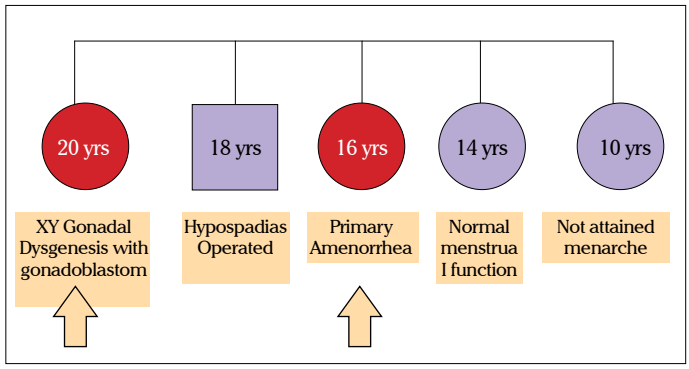

Figure 1. The pedigree chart of familial Swyer syndrome siblings

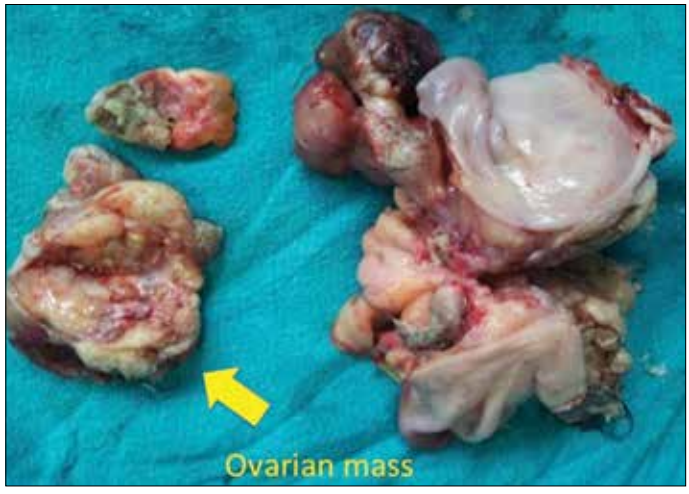

Figure 2. Tumor deposit on the uterus with a 6×5 cm right ovarian mass in one of the young girls with Swyer syndrome presenting with an adnexal mass

Out of these cases, two were siblings who had a familial Swyer syndrome, whereas the rest of them were sporadic occurrences (Figure 1). One of the two siblings of familial Swyer syndrome had a history of laparotomy and left salpingotomy of an ovarian tumor at the age of 11 years. A review of the records revealed it to be dysgerminoma stage 1a.

The principals of management included laparoscopic gonadectomy, appropriate management of adnexal masses in the specific cases, followed by hormone replacement therapy (HRT).

Five out of the eight cases underwent laparoscopic gonadectomy. All of them had streak gonads. In one of the patients who had previously undergone left oophorectomy for a dysgerminoma, the right streak gonad also revealed a dysgerminoma on histopathological examination.

Exploratory laparotomy with ascitic fluid cytology with total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH + BSO) with infracolic omentectomy was performed in one of the cases with bilateral adnexal masses and raised carcinoembryonic antigen (CA 125) and lactate dehydrogenase (LDH) levels. Intraoperatively, there was a 6×4 cm tumor deposit on the uterus with a 6×5 cm right ovarian mass (Figure 2). On the left side, there was a streak gonad. Bilateral tubes and omentum were unremarkable. Histopathological examination revealed a dysgerminoma in the right ovary.

The other patient with an adnexal mass also underwent an exploratory laparotomy with bilateral gonadectomy along with the removal of the rudimentary uterus, tubes, and infracolic omentectomy. The gonadal mass was also histopathologically characterized as dysgerminoma (Figure 3).

The clinical presentation and management of the six cases with non-familial Swyer syndrome has been summarized in Table 1, 2 and those of familial Swyer syndrome siblings has been summarized in Table 3, 4.

All the patients were put on HRT post-surgery. The two young cases with adnexal masses were registered in a cancer clinic, and they are under follow up.

Discussion

Swyer syndrome, 46 XY complete gonadal dysgenesis, is an uncommon entity occurring in a ratio of 1: 80,000 in the general population (1). In our case series, we managed five out of the eight cases with laparoscopic surgery. Laparoscopy provides a minimally invasive approach for the management of these cases if detected on time before they present with germ cell tumors. Michala et al. (1) reported a case series of 29 women with Swyer syndrome. The method of gonadectomy has not been specified in that series. However, because the period of study included even those diagnosed before 1990s, laparoscopy as a method of management might not have been uniformly available. In the current perspective, however, the role of laparoscopy cannot be overemphasized.

The age at diagnosis is an important determinant in the management of Swyer syndrome because of the risk of gonadal malignancy, initiation of adequate HRT for the induction of puberty, and for the improvement of bone mineral density. The average age at diagnosis in our series was 19.8 years compared with 17.2 years in the series by Michala et al. (1).

The diagnosis of Swyer syndrome is made around the time of puberty when the child who has been reared as a female fails to achieve menarche and has delayed development of secondary sexual characters. In our series, all the cases had primary amenorrhea, and 4 out of the 8 cases also had a delayed development of secondary sexual characters. Two of the young cases also had adnexal masses at the time of diagnosis. The presence of adnexal masses precludes a laparoscopic management as laparotomy with staging would be warranted.

The characteristic feature that differentiates Swyer syndrome from another disorder of XY females such as androgen insensitivity syndrome (AIS) is the higher propensity for malignant transformation. In Swyer syndrome, both internal and external genitalia are female, and there is hypergonadotropic hypogonadism, whereas in AIS, the external genitalia are female, internal are male, and they have hypogonadotropic hypogonadism.

In our series of eight patients, two had presented with ovarian masses with amenorrhea, and the histopathology revealed dysgerminoma. In the series of 29 cases, 1.32% had dysgerminoma and 14% had gonadoblastoma. Han et al. (2) described a case of dysgerminoma diagnosed in a dysgenetic gonad of a 21-year-old patient with Swyer syndrome, who presented with primary

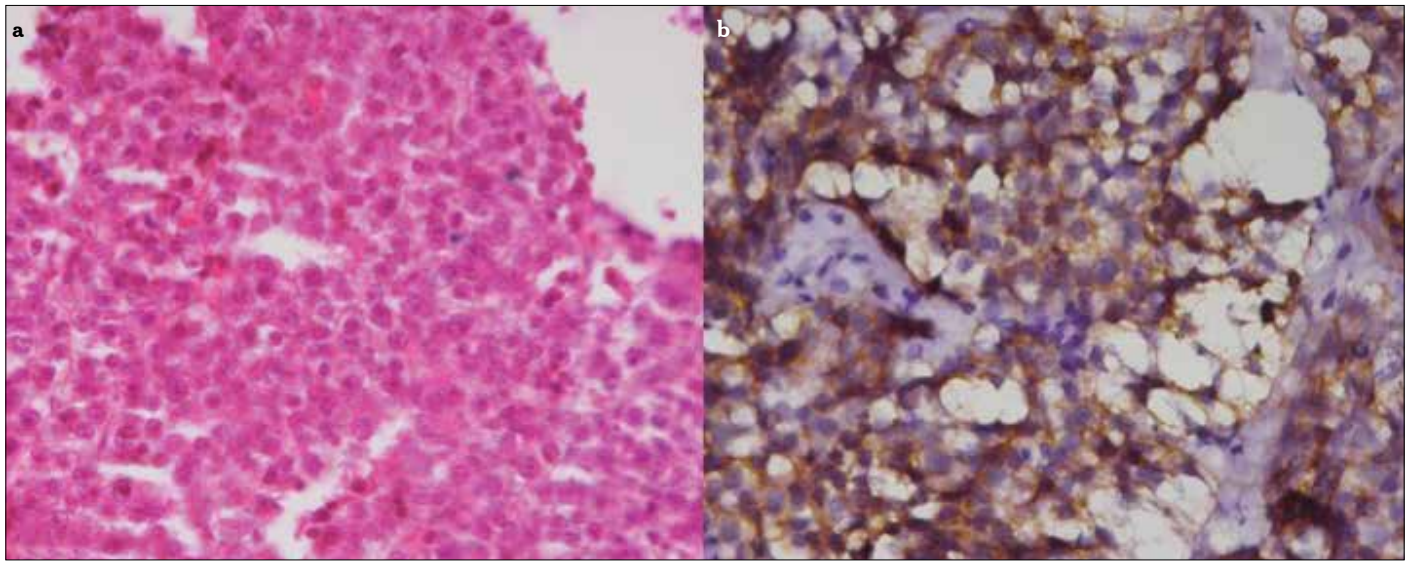

Figure 3. a, b. Microphotograph showing dysgerminoma cells (a) which were immunopositive for placental alkaline phosphatase (PLAP), (b)

Table 1. Summary of patients with Swyer syndrome (non-familial)

Sr. no.	1	2	3	4	5	6
Clinical Findings						
Age (years)	22	19	24	18	8	17
Chief Complaint	Primary amenorrhea	Primary amenorrhea	Primary amenorrhea with abdominal pain and infertility	Primary amenorrhea with poor secondary sexual characters	Lump abdomen (presented at 14 years again with primary amenorrhea and poor secondary sexual characters	Primary amenorrhea with poor secondary sexual characters
Height (cm)	158	165	154	155	NA	138
Weight (kg)	53	60	50	54	NA	38
Breast (Tanner)	2	3	3	1	Not developed	1
Axillary/Pubic Hair	Sparse	Sparse	Sparse	Sparse	Not developed	absent
Abdominal Examination	Soft	Soft	5×4 cm palpable mass in the lower abdomen	Soft	6×6 mass felt per abdomen	Soft
Vaginal Examination	Vagina well canalized	Vagina well canalized	Uterus anteverted Normal sized 7×5 cm, right adnexal mass, 5×5 cm	Vagina well canalized	Vagina well canalized	Vagina well canalized
Rectal Examination	Uterus not felt	Uterus not felt	No nodularity, rectal mucosa free	Uterus not felt	No nodularity, rectal mucosa free	Uterus not felt

NA: not available

amenorrhea and infertility for a duration of 5 years. Karyotype was consistent with 46 XY (pure). Behtash et al. (3) reported the development of dysgerminoma in three phenotypic female patients with 46 XY pure gonadal dysgenesis. All patients presented first with abdominopelvic mass. The youngest case of gonadoblastoma has been reported in a 9-month-old girl with ambiguous genitalia (4). Maleki et al. (5) reported a case of gonadoblastoma and dysgerminoma diagnosed on touch preparation in a dysgenetic gonad of a 16-year-old patient with Swyer syndrome.

Table 2. Investigations and management summary (non–familial)

Sr. no.	1	2	3	4	5	6
LH (mIU/L)	90.19	86.6	26.1	30.2		28.3
FSH (mIU/L)	14.4	22.4	107.1	99.7	Raised	52.87
CA125 (IU/L)			159.5			
AFP (IU/L)			2.2.4			
HCG (mIU/L)			5.88			
LDH (U/L)			1673		Raised	
Imaging	Uterus small bilateral ovaries not seen	Uterus small bilateral ovaries not seen	Uterus small. 14.5×6.4×10.3 cm solid, multilobulated mass, ascites present. Bilateral ovaries not seen separately	Uterus 4.7×6.4×10.3 cm. Left ovary not seen. 1.2×0.6 cm linear structure in the right broad ligament	Bilateral solid ovarian masses with a rudimentary uterus	Uterus small bilateral ovaries not seen
Karyotype	46 XY	46 XY	46 XY	46 XY	Culture failed twice	45 X (40%)/ 46 XY (60%)
Surgery	Laparoscopic gonadectomy	Laparoscopic gonadectomy	Exploratory laparotomy with ascitic fluid cytology with total abdominal hysterectomy with infracolic omentectomy	Laparoscopic right gonadectomy	Exploratory laparotomy with bilateral gonadectomy	Laparoscopic right gonadectomy
Histopathology	Streak gonads	Streak gonads	Dysgerminoma	Dysgerminoma	Dysgerminoma	Streak gonads

LH: luteinizing hormone; FSH: follicle-stimulating hormone; CA 125: carcinoembryonic antigen; AFP: alpha fetoprotein; HCG: human chorionic gonadotropin; LDH: lactate dehydrogenase

Table 3. Summary of the clinical findings of familial Swyer syndrome siblings

Sr. no.	1	2
Clinical findings		
Age (years)	16	20
Chief complaint	Primary amenorrhea	Primary amenorrhea with poor secondary sexual characters
Height (cm)	155	163
Weight (Kg)	37	42
Breast (Tanner)	2	1
Axillary/pubic hair	Sparse	Sparse
Abdominal examination	Soft	Soft
Vaginal examination	Vagina well canalized	Vagina well canalized
Rectal examination	Uterus not felt	Fixed mass felt anteriorly

Because of the dysgenetic gonads, the risk of gonadoblastoma and dysgerminoma has been estimated to be between 15% and 35%, and it is advisable to perform bilateral gonadectomy as soon as the diagnosis is made. This is in contrast with the management in another XY disorder such as AIS or true hermaphroditism that have a lower malignant potential.

Swyer syndrome is characterized by the presence of female internal organs. In most cases, the uterus is small and non-functional. Similar to our series of eight patients, seven had small dimensional uteri, but one of them had a uterine length of 4 cm. Apart from the imaging modalities, direct visualization under laparoscopy also aids in assessing the uterine size and its appropriateness for future reproductive function. In the case series of 29 patients (1), 8 patients had an ultrasound (US) assessment of uterine length, which was found to have a median length of 62 mm (48–82 mm). Three out of

Table 4. Investigations and management summary of familial Swyer syndrome siblings

Sr. no.	1	2
LH (mIU/L)	29.84	22
FSH (mIU/L)	102.3	89
CA 125 (IU/L)		7.9
AFP (IU/L) HCG (mIU/L)		1.08
LDH (U/L)		1.66
Imaging	Uterus not seen , bilateral streak gonads	Small uterus, 7×5 cm right adnexal solid cystic mass
Karyotype	46 XY	46 XY
Surgery	Laparoscopic gonadectomy	Exploratory laparotomy and peritoneal wash cytology with bilateral gonadectomy with bilateral salpingectomy and infracolic omentectomy
Histopathology	Streak gonads	Left streak gonad, right dysgerminoma

LH: luteinizing hormone; FSH: follicle-stimulating hormone; CA 125: carcinoembryonic antigen; AFP: alpha fetoprotein; HCG: human chorionic gonadotropin; LDH: lactate dehydrogenase

these had achieved successful pregnancies following ovum donation.

Swyer syndrome can occur in families, as found in the two siblings in our series. Generally, the diagnosis of Swyer syndrome in one member prompts a screening of other siblings for the presence of primary amenorrhea with or without poor development of secondary sexual characteristics. In our series, one of the siblings first underwent a laparotomy because she presented with an ovarian mass. However, on pedigree analysis, one of her siblings was also diagnosed with Swyer syndrome, and she underwent a laparoscopic gonadectomy. In a report by Kempe et al. (6), an asymptomatic woman (age, 38 years) with a family history of ovarian malignancies was referred for pre-symptomatic genetic testing of mutations in the BRCA genes. The family history revealed three affected paternal aunts. Two of them developed ovarian malignancies at 13 and 15 years of age and died at 19 and 20 years of age. This disease was diagnosed in the third aunt (82 years old) at the age of 35 years. Bagci et al. (7) have reported two siblings with Swyer syndrome along with the same history in their mother's maternal aunt who was subsequently diagnosed to have XY cell lines. Anecdotal case reports of families with XY Gonadal Dysgenesis (GD) have been described (8). The modes of transmission described in these families include autosomal recessive, autosomal dominant with a variable penetrance, and X-linked pattern of inheritance. This case series highlights the role of laparoscopy in the diagnosis and management of Swyer syndrome. Early diagnosis, a minimally invasive approach to gonadectomy, followed by HRT and family screening are the cornerstones of the management of this rare disorder. Menstrual function and pregnancy can be achieved in a select group of patients.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patients who participated in this case.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - N.M., V.D., D.D., A.S.; Design - A.S., V.D., S.A.; Supervision - N.M., V.D.; Materials - A.S., D.G., S.A.; Data Collection and/or Processing - A.S., S.A., D.G.; Analysis and/or Interpretation - A.S., V.D., S.A.; Literature Search - A.S., S.A., D.G.; Writing - A.S., S.A., V.D.; Critical Reviews - N.M., V.D., D.D.*

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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Egg recovery completed with a "manually-created" negative pressure is still an option in cases of emergency or "low-cost" in vitro fertilization?

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Abstract

This is a report of a case of an egg recovery procedure completed with manual suction instead of the automated negative pressure suction. A 35–year-old woman who was undergoing in vitro fertilization (IVF) treatment had to undergo oocyte recovery using manual suction through a syringe instead of the automated negative pressure suction systems because of the failure of both the initial and replacement systems. The treatment cycle ended in a positive pregnancy test and a clinical pregnancy with acceptable oocyte fertilization rates and no complications during procedure. The case presented may be an implication for an alternative implementation in cases of emergency, particularly when low-cost IVF is a target. (J Turk Ger Gynecol Assoc 2015; 16: 257-8)

Keywords: IVF, oocyte retrieval, manual, suction, cost

Received: 18 April, 2015 **Accepted:** 13 July, 2015 **Available Online Date:** 02 November, 2015

Introduction

Oocyte recoveries take place with the use of sophisticated and very efficient equipment worldwide. However, sometimes during emergency conditions, such as failure of equipment of any kind, physicians are required to immediately decide regarding the future of the complete in vitro fertilization (IVF) cycle. We present a case of an oocyte recovery that was successfully completed with the use of a manually created negative pressure for oocyte aspiration because of simultaneous failure of both the main and replacement systems. It is important, primarily for less experienced operators, to know that this is an option in case of emergency conditions, particularly when IVF costs may be an issue.

Case Presentation

A 35-year-old woman, p 0, was admitted to our department with a diagnosis of unexplained primary female, male, and couple infertility with a duration of 24 months. Nothing of clinical importance could be found in the couple's medical history, except for a slightly reduced motility in the male semen analysis.

We used a fixed antagonist protocol with 225 international units of follicle-stimulating hormone/day. The last pelvic ultrasound scan performed revealed the existence of 12 good-sized follicles (>14 mm); hence, human chorionic gonadotropin triggering was decided for the following day.

On the day of oocyte recovery, we discovered that both the suction apparatus and its substitute were not working; moreover, the sooner we would have been able to have a new apparatus would have been 5–7 h later. After having an emergency meeting, we decided to go ahead with the application of a "manual" suction and a single lumen follicle aspiration needle (instead of the double lumen that we use). We applied a 20-mL syringe, and a second doctor aspirated the follicles by creating a negative pressure using the syringe. We managed to aspirate eight oocytes from 10 suitable follicles; however, a previous ultrasonographic finding of the high left ovary was confirmed, making the procedure even more difficult.

IVF was implemented and among the eight eggs, three managed to get fertilized. A single embryo transfer of a 7c2 quality embryo took place three days later, and the other two embryos were discarded because they were not of freezing quality. The urine pregnancy test performed 15 days later proved to be positive; the patient is currently in the second trimester of an ongoing, uncomplicated pregnancy.

Discussion

The case report presented is important for two reasons: firstly, because it demonstrates a feasible and easy solution for similar instances and secondly, it may be a very cheap solution in cases where low-cost IVF is an important issue. To the best of our knowledge, this is the only case described, particularly after the implementation of the automated suction systems for oocyte recovery; a systematic review of the literature conducted revealed no other similar cases (keywords used were: "manual," "suction," "IVF," "oocyte," "aspiration," and "recovery").

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DOI:10.5152/jtgga.2015.15069

In the past (early 1980s), follicle aspiration with manual pressure was the common procedure; nevertheless, it has been reported that follicular aspiration using a syringe suction system may damage the zona pellucida (1). This was not the case in our patient despite the lower fertilization rate that we had achieved (3/8 eggs were fertilized); however, the fact that only one good quality embryo was obtained may present a complication for the procedure.

A disadvantage of the method may be the fact that in cases of application of manual suction, we cannot apply flushing of the aspirated follicles, which is a technique used for oocyte aspiration in our center. Nevertheless, one may say that this is not an obligatory applied technique, and recent research suggests that follicles should not be flushed in cases of normal responders, as in our case (2).

In contrast, taking into consideration the fact that in many IVF centers worldwide cost may be an important issue, using a syringe to manually create the negative pressure, at least in cases of emergency, may be an attractive alternative (3). Considering that the cost of oocyte retrieval is estimated to be at approximately 220 Euro (3, 4), safeguarding the amount required for a pump replacement, may contribute to wider provision of IVF services under the provision of proving that manual suction leads to uncomplicated oocyte retrieval.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patient who participated in this case.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - T.K.; Design - T.K.; Supervision - A.M.; Resource - T.K., A.M.; Materials - T.K., A.M.; Data Collection and/or Processing - T.K.; Analysis and/or Interpretation T.K., A.M.; Literature Search - T.K.; Writing - T.K.; Critical Reviews - A.M.*

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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Torsion of an iatrogenic parasitic fibroid related to power morcellation for specimen retrieval

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Abstract

Parasitic fibroids are generally diagnosed incidentally at the time of surgery performed for symptomatic uterine fibroids. Torsion of a parasitic fibroid causing severe acute onset pain is extremely rare. We report a torsed parasitic fibroid in a patient who underwent hysterectomy using power morcellation for specimen retrieval. A 40-year-old patient with a history of laparoscopic supracervical hysterectomy 8 years prior presented with severe abdominal pain. She was diagnosed with degenerating parasitic fibroids on magnetic resonance imaging and was managed conservatively. Surgery was performed 3 days later for persistent pain, and the parasitic fibroid was found to have undergone torsion. Torsed ischemic fibroids can undergo necrosis and gangrene and can potentially cause life-threatening coagulopathy and peritonitis. Awareness of this potential complication will reduce errors in diagnosis and facilitate timely management.(J Turk Ger Gynecol Assoc 2015; 16: 259-62)

Keywords: Parasitic fibroid, torsion, morcellation

Received: 06 May, 2015 **Accepted:** 13 September, 2015 **Available Online Date:** 02 November, 2015

Introduction

When a fibroid becomes partially or completely separated from the uterus and receives its main blood supply from another source, it is called a parasitic fibroid. This was first described by Kelly and Cullen in 1909. Nezhat et al. (1) described three broad categories of parasitic fibroids: (a) spontaneously developing parasitic fibroids resulting from pedunculated fibroids that have lost their connection to the uterus, (b) fibroids associated with previous uterine surgeries such as hysterectomy and myomectomy, particularly if morcellation of the uterus or fibroids was performed, (c) fibroids associated with the restriction of blood supply to the uterus (e.g., gonadotropin-releasing hormone agonists, uterine artery embolization), which may cause a pedunculated subserosal fibroid to lose its uterine blood supply and become parasitic.

Parasitic fibroids are generally diagnosed incidentally at the time of surgery for symptomatic uterine fibroids. Torsion of a parasitic fibroid causing acute onset pain is extremely rare. We report a patient with a torsed parasitic fibroid that occurred 8 years after a hysterectomy was performed using a power morcellator.

Case Presentation

A 40-year-old female presented to the emergency department of our hospital complaining of severe acute onset lower abdominal pain. She had a history of laparoscopic supracervical hysterectomy performed 8 years prior for fibroid uterus at our hospital. On reviewing her previous records, it was found that power morcellation had been used, and the pathology report showed benign fibroids. She had a low grade fever and neutrophilic leukocytosis. On examination, she had a tender mass in the pelvis.

A computed tomography (CT) scan performed in the emergency department showed a 6×4.6×6-cm solid left adnexal mass and a 6×10 cm pelvic mass. The differential diagnosis on CT scan included left adnexal mass, pelvic abscess, and fibroids. Broad-spectrum antibiotics were administered, and magnetic resonance imaging (MRI) was performed that showed a normal cervix and bilateral normal ovaries. There was a lobulated mass above the cervix measuring 9.6×6.4×7.4 cm. A second smaller mass measuring 7.3×5.3×7.6 cm was found immediately cephalad and to the left of the first mass. A third mass measuring 2.7×2.3 cm (smallest mass) was found in the right lower pelvis (Figure 1, 2). After intravenous contrast, there was only mild posterior enhancement of the largest mass, while the other two masses showed good enhancement (Figure 3). The radiologist made a diagnosis of parasitic fibroids with degeneration of the largest one based on the MRI findings.

The patient was managed conservatively using nonsteroidal anti-inflammatory drugs (NSAIDS) for pain relief. However, she continued to spike low-grade fevers, and her pain did not subside. There was also concern about malignant transformation in the fibroid. Therefore, a decision was made to perform an exploratory laparotomy and excision of pelvic masses. A small amount of blood-tinged free fluid in the pelvis was observed intraoperatively, which was sent for cytopathological analysis. There were mild adhesions between the bladder and the sigmoid colon, which were easily sepa-

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DOI:10.5152/jtgga.2015.15093

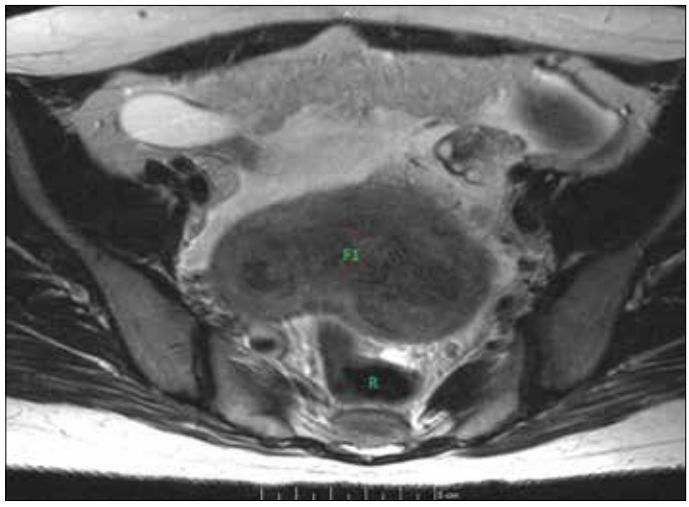

Figure 1. MRI showing a lobulated pedunculated fibroid 9×6×7 cm (F1) arising from the rectum (R)

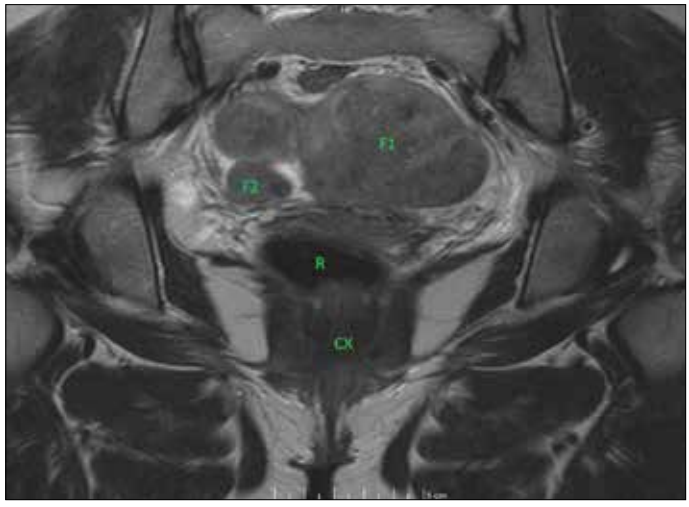

Figure 2. MRI showing a smaller fibroid 2.7×2×3 cm (F2) above the cervix (CX) in addition to the larger fibroid (F1)

rated. After adhesiolysis, two pedunculated parasitic fibroids were identified, arising from the anterior wall of the rectum. The largest fibroid was found to be degenerated and had undergone torsion with two complete turns on its pedicle. A third parasitic fibroid was found arising from the left pelvic side wall with a thin pedicle. Both the ovaries and cervix were normal and separate from the masses. All fibroids were removed and sent for histopathological examination. The patient's postoperative recovery was uneventful, and she was discharged on the second postoperative day. The ascitic fluid cytology showed mesothelial cells, neutrophils, and lymphocytes. The pathology revealed benign fibroids with necrosis and hemorrhagic degeneration in the largest fibroid (Figure 4). The patient agreed to the publication of this report and informed consent was obtained.

Discussion

Parasitic fibroids are rare, with only a few case reports and case series reported in the literature. In the past 10 years, there

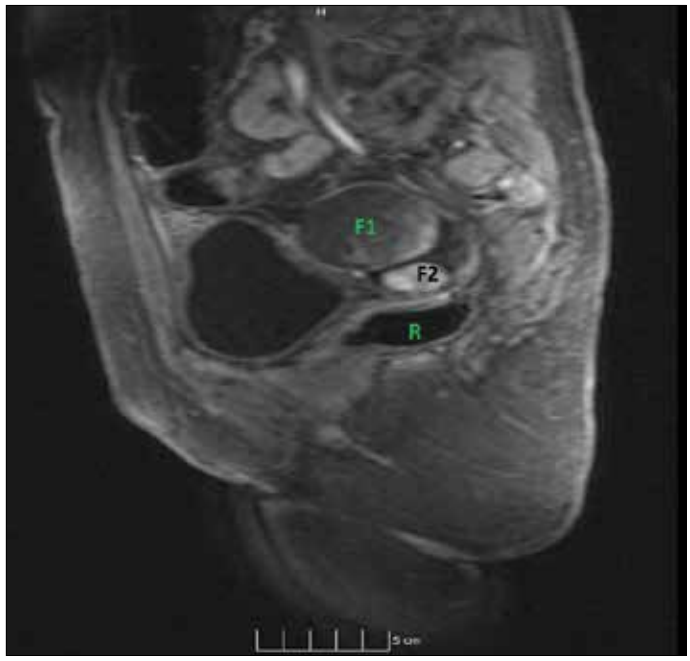

Figure 3. MRI with IV contrast (Panel C) showing an enhancement of the smaller fibroid (F2) but no enhancement of the larger fibroid (F1), suggestive of degenerative changes in the larger fibroid (F1)

has been an increase in the reports of parasitic fibroids attributable to previous surgery (iatrogenic parasitic fibroids). In a recent review of parasitic myomas after laparoscopic surgery, 48 of 53 patients were reported between 2007 and 2014 (2). Morcellation increases the possibility of leaving behind small fragments of fibroids that proceed to implant in the peritoneal cavity and form parasitic fibroids. These lesions are mostly located in the pelvis because of the gravitational movement of retained fragments. Parasitic fibroids are uncommon, whereas the loss of tissue fragments within the abdominal cavity during morcellation is presumably extremely common. Hence, it seems reasonable to hypothesize that other factors such as the exposure of fragments to steroid hormones and growth factors may contribute to the occurrence of this complication (3).

In a case series, Kho et al. (4) reported that 6 out of 12 patients with parasitic fibroids underwent laparoscopic surgery in the past that involved morcellation. Miyake et al. (5) used molecular genetic analysis to show that the parasitic fibroids resected at subsequent surgery originated from the fibroids that were morcellated during the initial surgery. The incidence of parasitic fibroids after laparoscopic surgery with morcellation is probably around 0.1%–1.0% (6). Parasitic fibroids are generally diagnosed incidentally at surgery for symptomatic uterine fibroids. However, there are case reports in which parasitic fibroids have been symptomatic (7-10). Torsion of a parasitic fibroid causing severe pain of acute onset is extremely rare, but there have been a few reports in the past (Table 1).

Two of these reports are from the 1990s, and both of these patients did not undergo previous surgeries; they had spontaneously formed parasitic fibroids that underwent torsion (7, 8). The other two patients had previous myomectomy: one by laparoscopy (9) and the other by laparotomy (10). All of these

patients still had their uteri in situ at the time of presentation as opposed to our patient who had a supracervical hysterectomy in the past. Our patient also had power morcellation during the initial surgery. All patients that presented acutely, including our patient, had leukocytosis and/or neutrophilia, and two out of five patients also had fever.

On preoperative imaging, the fibroids were visualized in four out of the five patients. However, the diagnosis of parasitic fibroid was not made preoperatively in any other cases of torsion, except our patient. This is consistent with the fact that most parasitic fibroids are diagnosed incidentally at the time of surgery. The imaging modality was sonogram in three patients, while the fourth patient underwent a CT scan. Our patient underwent a CT scan and an MRI. The parasitic fibroid was thought to represent a torsed ovarian mass in one patient, a torsed pedunculated fibroid in the second, and an incidental finding in the third. The fourth patient had a sonogram, and the parasitic fibroid was not seen (Table 1). In our patient, the CT scan was not conclusive, and the diagnosis of parasitic fibroid was made on MRI. The sites of origin of the torsed fibroid in the previously reported patients were the omentum, mesentery, and broad ligament, whereas it arose from the rectum in our patient. The histopathology report in all these patients showed some evidence of infarct, necrosis, or degeneration. The ascitic fluid cytology in our patient showed neutrophils and lymphocytes that are indicative of an inflammatory response to the ischemic and necrotic fibroids.

Table 1. Torsed parasitic fibroids, comparison and review of reported cases

Author	Brieger et al. (7), 1995	Yeh et al. (8), 1999	Tan et al. (9), 2012	Park et al. (10), 2013	Our patient
Age (years)	41	33	44	45	40
Previous surgery	None	None	Laparoscopic myomectomy 2 years ago. Morcellator use not specified	Abdominal myomectomy 10 years ago	Laparoscopic supracervical hysterectomy with power morcellation 8 years ago
Presenting symptom	Lower abdominal pain starting 36 hrs before presentation	Lower abdominal pain for 5 days before presentation	Acute right lower abdominal pain	Pain in the right lower abdomen for 2 weeks before presentation	Severe pain in the lower abdomen for 6 h before presentation
Clinical findings	Neutrophilia	Leukocytosis	Fever, Neutrophilic leukocytosis	None	Fever, Neutrophilic leukocytosis
Imaging	Ultrasound: Mass in pouch of douglas seen separately from a normal sized uterus	Ultrasound: mass seen in the upper pelvis and lower abdomen separate from the uterus. 2 cm subserosal uterine fibroid. Small amount of free fluid	CT scan: two masses seen separate from the uterus in the lower abdomen and pelvis. Small amount of free fluid. Appendix mildly dilated	Ultrasound: enlarged uterus with multiple intramural fibroids. The parasitic fibroid was not seen	MRI: three parasitic fibroids, one of them degenerating. Small amount of free fluid
Pre-operative diagnosis	Torsion of ovarian cyst	Torsion of pedunculated fibroid	Acute appendicitis	Fibroid uterus with pain	Degenerated parasitic fibroid
Route of surgery for the parasitic fibroid	Laparoscopy, specimen removal by colpotomy	Not specified	Laparotomy	Laparoscopy Power morcellation	Laparotomy
No. of parasitic fibroids	1	1	3	1	3
Origin of torsed fibroid	Omentum	Broad ligament	Omentum	Mesentery	Rectum
Adhesions (intraoperative finding)	None	None	None	Severe adhesions of torsed fibroid to the omentum and bowel	Severe adhesions of torsed fibroid to the bladder and sigmoid colon
Pathology	Leiomyoma with necrosis and dystrophic calcification	Leiomyoma with hemorrhagic infarction	Leiomyoma with areas of edema, hemorrhage, and infarction	Leiomyoma with infarction	Leiomyoma with hemorrhagic infarction

CT: computerized tomography, MRI: magnetic resonance imaging

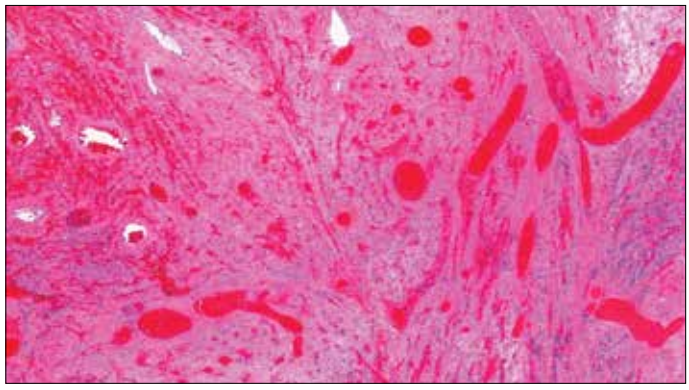

Figure 4. Histopathological examination (Hematoxylin and eosin staining, 40x) revealed leiomyoma with hemorrhagic degeneration in the larger one that was found to be torsed intraoperatively

Our patient is the first reported case of post hysterectomy torsed iatrogenic parasitic fibroid with a documented use of a power morcellator at initial surgery. The site of origin of the fibroid, the rectum, is also unusual, and the other authors reported parasitic fibroids arising from the omentum and mesentery, which are more likely to undergo torsion. Early diagnosis of torsion is important because ischemia or necrosis can cause consumptive coagulopathy, gangrene, and peritonitis, which can be life threatening.

There have been reports of the spread of endometriosis, adenomyomas, cervical tissue, endometrial cancer, and uterine sarcoma in the abdominal cavity after morcellation. The major concern is the possible spread of occult malignancy, and the United States Food and Drug Administration has released a safety communication discouraging the use of power morcellators. However, parasitic fibroids have been reported after abdominal hysterectomy and abdominal myomectomy (4). Hence, restricting the use of power morcellation will not eliminate iatrogenic parasitic fibroids.

To conclude, iatrogenic parasitic fibroids are rare complications after previous uterine surgery, and an acute presentation due to torsion is even rarer. Most of these patients present with severe pain and peritoneal signs. Low-grade fever and leukocytosis may be present. MRI is the best tool to evaluate these lesions. Awareness of this potential complication will reduce the delay in diagnosis and facilitate appropriate management.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patient who participated in this case.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - D.M.N., E.E., S.S; Design - D.M.N., E.E., S.S.; Supervision - E.E., S.S.; Resource - D.M.N.; Materials - D.M.N.; Data Collection and/or Processing - D.M.N.; Analysis and/or Interpretation - D.M.N., E.E., S.S.; Literature Search - D.M.N., E.E., S.S.; Writing - D.M.N., E.E., S.S.; Critical Reviews - E.E., S.S.*

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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What is your diagnosis?

A 40-year-old lady with previous two deliveries by lower segment caesarian section presented with a long history of lower abdominal heaviness and menorrhagia and a mass coming out of vagina recently. There were associated problems in voiding urine.
On local examination, a large, elongated, soft, reddish mass, measuring 14×3.8×4 cm, was protruding out of the vagina. A firm whitish mass with a well-demarcated rim was observed at the end of the large reddish mass, measuring 5×5 cm. The entire mass could be incompletely reduced inside the vagina. On genital examination, the cervical rim could not be posteriorly felt. There was ulceration with discharge of pus from the top of the mass, which was sent for culture and sensitivity tests. The culture was positive for Pseudomonas aeruginosa, which was sensitive to levofloxacin. Prolapsed part was reposited back and was restrained using a glycerine acriflavine-soaked vaginal tampon. The patient was put on oral antibiotics with a dressing of the local wound. Healing was achieved in 10 days. An ultrasonography of the pelvis was ordered. Ultrasonography revealed an upside-down uterine fundus, filling in between the cervix making mirror image of normal uterine contour. Outer serosal surfaces were coming in contact together making typical pseudo stripe sign. The uterine fundus was observed between the vaginal walls giving a typical target sign. There was a heterogeneous mass lesion observed at the uterine fundus that probably arised from the submucosa and exhibited heterogeneous internal vascularity. The uterine artery pedicle and ovaries of both the sides were pulled along with the uterine fundus; however, there was no involvement of the urinary bladder (Figure 1 a-d).

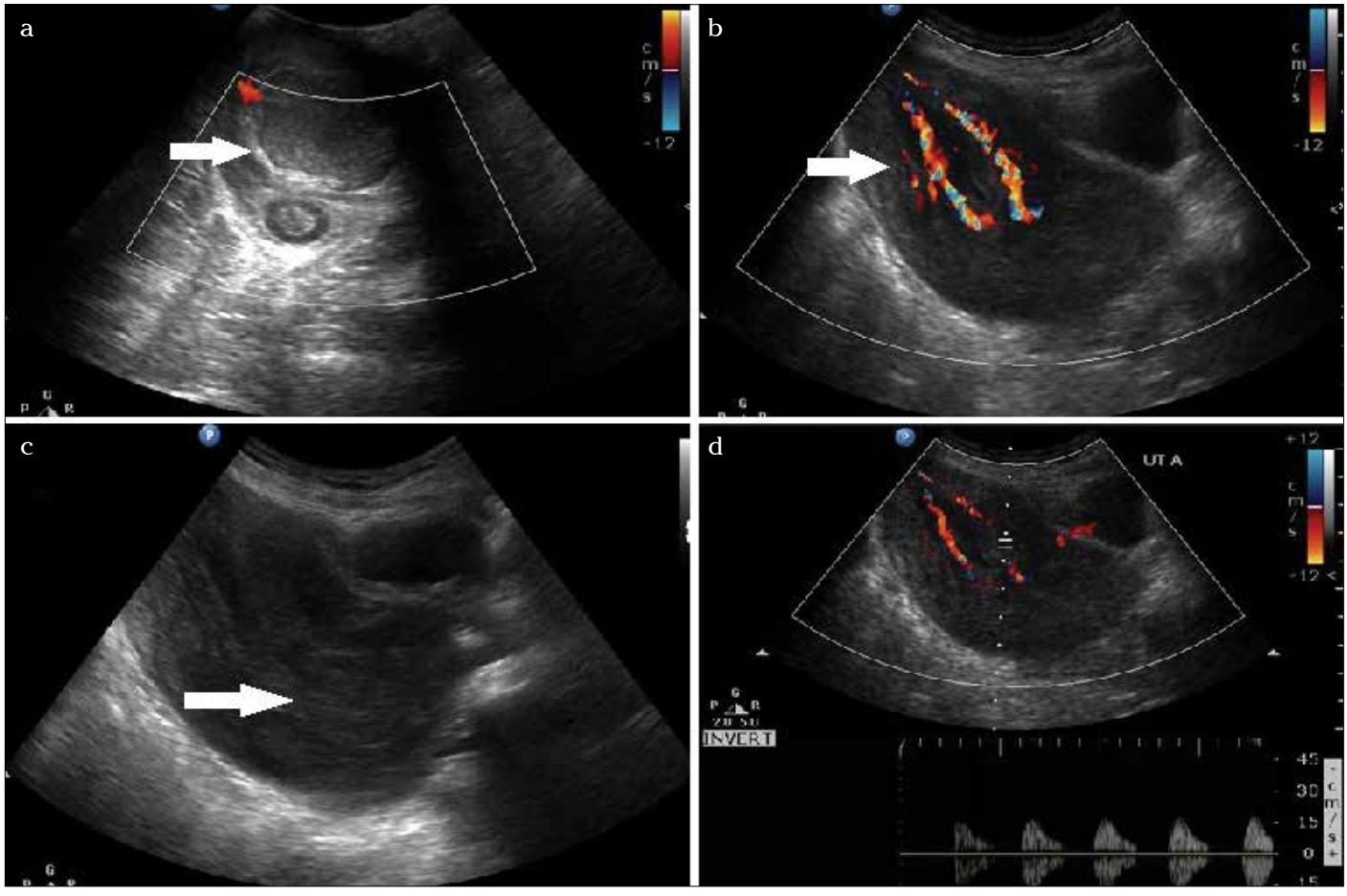

Figure 1. a-d. Pelvic ultrasound image (coronal view) shows target sign (solid arrow), i.e., hyperechoic fundus with mass surrounded by hypoechoic layer of fluid inside the vagina (a), pelvic color mode ultrasound image (sagittal view) shows inverted fundus with pulled pedicles (solid arrow) (b), pelvic ultrasound image (sagittal view) shows pseudo stripe sign (solid arrow), i.e., outer serosal surfaces coming into contact mimicking normal endometrial stripe (c), pelvic spectral Doppler mode ultrasound image (sagittal view) shows a normal uterine artery waveform in pulled uterine vascular pedicle (d)

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DOI: 10.5152/jtgga.2015.0188

Answer

The chronic non-puerperal uterine inversion is an extremely uncommon entity, generally caused by submucosal fibroid, particularly located in the fundus (1). Chronic non-puerperal uterine inversion is generally observed after the age of 40 years, and in addition to large submucous fibroids, rare malignancies, such as rhabdomyosarcoma, endometrial carcinoma, or endometrial polyp, can precede etiologic agents (2). The proposed pathogenesis is thinned out uterine wall in middle-aged patients, giant fundal mass, and gravity with expulsive efforts by uterus (3). By definition, uterine inversion means a descent of the uterine fundus to or through the cervix so that the uterine cavity is anatomically turned inside out (4).

Uterine inversion can be divided into acute and chronic on the basis of the onset and course of the disease. Acute inversion is characterized by pain and hemorrhage, whereas chronic inversion is associated with pelvic discomfort, vaginal discharge, irregular vaginal bleeding, and anemia (5).

Uterine inversion is suspected when gynecological examination detects a protruding mass in the vagina or vulva, and the uterine fundus cannot be palpated by bimanual examination. A constricting ring around the cervix is a convincing clinical finding. The most promising features are inability to palpate the fundus and non-visualization of the cervix separately (5). Acute inversion of the uterus is almost always postpartum and is common, whereas chronic inversion of the uterus is extremely rare. Chronic uterine inversion can be either incomplete or complete. In incomplete type, the fundus everts out of the cervix but stays inside the vagina, whereas in complete type, the fundus, including the cervix, protrudes out of the introitus (6). A high index of suspicion is required as chronic uterine inversion can be mistaken for uterine prolapse or any mass of the vagina or cervix. Correct diagnosis can only be made intra-operatively in many cases. This causes certain surgical difficulties and increased complication rates (7).

Ultrasonography generally is the primary diagnostic modality of choice (7). Classical findings described are the mirror image sign, pseudo stripe sign, and target sign. All these features are present in our case. Mirror-image sign depicts the reverse relationship of the fundus and cervix as the fundus lies lower than the cervix. The pseudo stripe sign is described as a hyperechoic stripe formed because of the apposition of serosal surfaces mimicking the normal endometrial stripe. The target sign is an illustration of the uterine fundus lying in the vagina and outlined by hypoechoic line of fluid collection between the two (8).

Characteristic magnetic resonance imaging (MRI) findings are U-shaped alignment of the uterine cavity in sagittal plane and bull's eye appearance of the fundus inside the vagina on T2-weighted imaging, which is a derivative of the target sign found on ultrasonography. MRI also shows the content of the inversion, such as the ovaries or bladder. MRI is the best modality for the characterization of the mass that is responsible for the inversion, i.e., to differentiate between benign and malignant masses (9).

In this case, MRI revealed an U-shaped configuration of the uterine cavity with complete inversion of the fundus. The uterine artery pedicle and ovaries were also pulled along with the uterine fundus. There was a well-defined heterogeneous mass at the uterine fundus revealing a heterogeneous intense enhancement that was suggestive of a fibroid. The U-shaped configuration was previously described in the sagittal plane

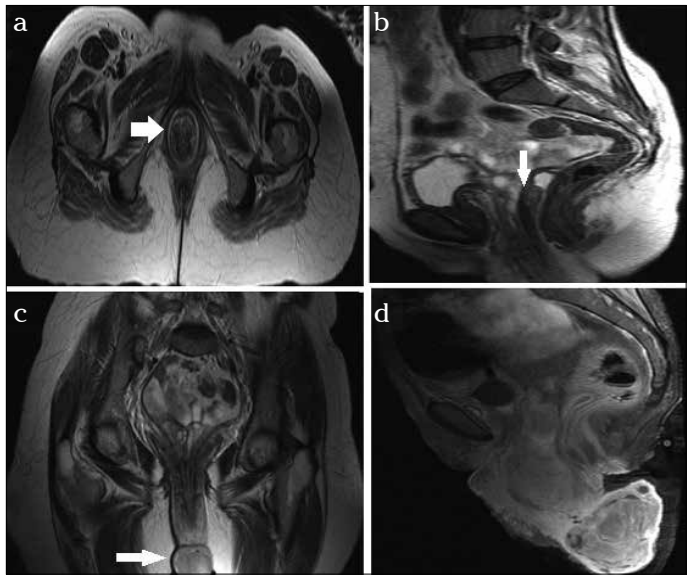

Figure 2. a-d. Axial T2-weighted MRI shows Bull's eye sign (solid arrow), i.e., hypointense fundus outlined by hyperintense layer of fluid within the vagina (a), sagittal T2-weighted MRI shows inverted U-shaped uterine cavity (solid arrow), a mirror image of a normal anatomical configuration (b), coronal T2-weighted MRI shows inverted uterus with a well-defined mass lesion at the top of the uterine fundus (solid arrow) (c), sagittal post contrast T1-weighted MRI shows intense homogeneous enhancement of both the mass and normal uterus (d)

Figure 3. a-d. Clinical pre-operative photograph of the patient, showing exposed red fleshy endometrium with a whitish fleshy mass (solid arrow) at top of it (a), intra-operative photograph shows undergoing excision of the fibroid and vaginal myomectomy (b), intra-operative photograph shows undergoing Kustner's procedure, incision at the posterior cervical lip, and identifying edges of the cervix (c), intra-operative photograph shows repositioning of the uterus for vaginal hysterectomy (d)

and can be observed in the coronal plane, similar to this case (Figure 2 a-d).

Huntington and Haultain procedures are the commonly used abdominal approaches, and Kustner and Spinelli procedures are the commonly used vaginal approaches for the correction of chronic inversion (10).

As the fibroid was present at the fundus; therefore, vaginal myomectomy was first performed and then the uterus was reposited so that safe hysterectomy could be performed. Kustner's procedure was performed as the patient had history of two deliveries by lower segment caesarian section, and there are less chances of injury to the bowel and bladder in this procedure (Figure 3 a-d). The patient had a normal course in the ward and was discharged after 5 days.

The following conclusions can be drawn from this case. This is a very rare entity with typical imaging features; however, many radiologists are unaware of this entity because of its rarity. This differential it must be kept in mind in middle-aged patients presenting with a mass coming out of the vagina. As the method of treatment solely depends on the perfect radiological diagnosis, precise diagnosis must be delivered keeping in mind the above-mentioned imaging features.

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Acknowledgements for the Year 2015

(Reviewers contributed at the review process in 2015)

On behalf of the office staff and the Editorial Board of the Journal of *The Turkish German Gynecological Association*, we would like to thank to all of our reviewers of the past year for their outstanding contributions. Their thorough reviews and expertise enable our journal to improve its scientific quality. We certainly look forward to their ongoing support, suggestions and recommendations as to how to continue to advance the overall quality of the Journal of *The Turkish German Gynecological Association*.

Prominent Reviewers in 2015

We would like to acknowledge, with special thanks and appreciation, the following reviewers for their remarkable contributions to peer review process of the Journal of *The Turkish German Gynecological Association* in 2015. We extend our sincere gratitude for their timely, scholarly and critically evaluations.

Mehmet Sühha Bostancı
Murat Seval
Mustafa Kara
Özge Yıldırım
Özlem Pata
Şebnem Özyer
Tuncay Yüce
Yavuz Emre Şükür
Yılmaz Güzel
Yusuf Üstün

Reviewers in 2015

We are also grateful for the help of the reviewers who devoted time and effort to the Journal of *The Turkish German Gynecological Association* in 2015.

Abdo Ahmed
Abdul H. Sultan
Abimbola O. Famuyide
Ahmar Shah
Ahmed Badawy
Ahmet Akın Sivaslıoğlu
Ahmet Başaran
Ahmet Karataş
Alan Andrea
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Ashraf Ganie
Ashraf Moini
Atalay Ekin
Ates Karateke
Atilla Karaalp
Aykan Yücel
Ayla Aktulay
Aysel Derbent
Aysun Altınok Karabulut
Ayşe Güler Okyay
Ayşe Parlakgümüş
Ayşe Uğur İşleyen
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Batuhan Özmen
Bengt Sorbe
Benny Almog
Berfu Demir
Berna Haliloğlu
Berna Seçkin
Bilge Burçak Annagur
Boris Tutschek
Bruno Borghese
Bruno van Herendael
Bülent Baysal
Bülent Berker
Bülent O. Yıldız
Can Tekin İskender
Carl Della Badia
Carla Tatone
Carol Bower
Cecily M. Begley
Cem Atabekoğlu
Cem Çelik
Cem Ficicioğlu
Cemal Güvercin
Cesare Battaglia
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Eyal Sheiner
Ezzat Khodashenas
Fabricio Da Silva Costa
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Justine Schober
Jyoti Modi
Jyotsna Rani
Kaei Nasu
Kallol Kumar Roy
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Ma Stephanie Fay S. Cagayan
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Marcela G. del Carmen
Marcus Q. Bernardini
María José Aguilar Cordero
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Martin D. Keltz
Mary F. Thompson
Maryam Ayazi Roozbahani
Masood Jawaid
Mazdak Momeni
Mehmet Anıl Onan
Mehmet Aslan
Mehmet Asoğlu
Mehmet Bülbül
Mehmet Doğu
Mehmet Harma
Mehmet Kağıtçı
Mehmet Murat Naki
Mehmet Sakıncı
Mehmet Sıddık Evsen
Mehmet Sühha Bostancı
Mehmet Vural
Mehrdad Mahdian
Mekin Sezik

Melike Doğanay
Mert Turgal
Mesut Özkaya
Michael A. Thomas
Michael Bohlmann
Michael von Wolff
Michalis Varras
Mihaela Grigore
Milena Kralickova
Mine Esin Ocaktan
Minfang Tao
Ming-Ping Wu
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Mohamed A. Elshaikh
Mohamed A. Mohamed
Mohamed Abdel-Fattah
Mohamed EL-Gharib
Mohammad Amin Bahrami
Mohammad Ghasemi-Rad
Mohammad Hossein Ahmadi
Mojgan Barati
Mona M. Aboulghar
Muazez Cevik
Murat Api
Murat Bakacak
Murat Dede
Murat Seval
Mustafa Güven
Mustafa Kemal Demirağ
Müge Harma
Nadine C. Kassis
Naohiro Kajiwara
Neha Gami
Nevin Sağsöz
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Nicole Julia Roberts
Nikolaos Vitoratos
Nikolaos Vrachnis
Nita Khurana
Niyazi Cenk Sayın
Niyazi Tuğ
Olusegun O. Badejoko
Osman Balcı
Oya Demirci
Özge Karadağ Caman
Özlem Moraloğlu
Özlem Seçilmiş Kerimoğlu
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Salih Taşkın
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Şadıman Kıykaç Altınbaş
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Tahir Atik
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Takashi Takeda
Takashi Watanabe
Tamaki Matsumoto
Taner Usta
Tansel Haliç
Tarek Shokeir
Tatjana Croenlein
Tatjana Pekmezovic
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Teera Wacharaprechanont
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Yiğit Çakıroğlu
Young Sik Choi
Yusuf Üstün
Zahra Maleki
Zarko Alfirevic
Zehra Gölbaşı
Zhenyu Zhang
Zhong-Yun Wang

JTGGA CME/CPD CREDITING

Questions on the article titled "*Abdominal anatomy in the context of port placement and trocars*" within the scope of CME/CPD

1. Which of the following statements with respect to adenomyosis uteri are not true?
   a) Entry access in laparoscopic hysterectomy of adenomyosis needs to be at Palmer's point
   b) Vaginal access and vaginal hysterectomy can be feasible
   c) The diagnosis can be made via ultrasound or magnetic resonance imaging (MRI)
   d) It tends to occur in women over 35 years of age
   e) Because the condition commonly occurs in association with endometriosis, surgical treatment can be very challenging even though the uterus itself can be of normal size

2. Which of the following are the shortest diameters of the pelvic cavity?
   a) Transverse
   b) Interspinous
   c) Oblique
   d) Anteroposterior
   e) Interspinous and oblique are of the same lengths

3. Which of the following statements with respect to blood supply are not correct?
   a) The superficial epigastric artery arises from the femoral artery
   b) The circumflex iliac superficial artery originates from the femoral artery
   c) The inferior epigastric artery originates from the femoral artery
   d) The superficial vessels of the abdominal wall can be visualized via diaphanoscopy
   e) Injury of the inferior epigastric artery can be threatening

4. Which of the following statements with respect to the abdominal wall are not correct?
   a) The plica umbilicalis mediana contains the urachus, and injury causes fistula in most of the cases
   b) The plica umbilicalis medialis contains the obliterated umbilical artery, and injury can cause bleeding because the umbilical artery is not obliterated in all of the cases
   c) The plica umbilicalis lateralis contains the inferior epigastric vessels, and the pulsation of the artery can be identified
   d) The working trocars are generally placed lateral to the plica umbilicalis lateralis
   e) Following the plica umbilicalis medialis leads to the internal iliac artery

5. Which statement with respect to single-site surgery is correct?
   a) Laparoscopic hysterectomy can be performed via single-site surgery
   b) The risk for hernia in single-site surgery is lower than in conventional laparoscopy
   c) Single-site surgery has a shorter learning curve than conventional laparoscopy
   d) There are no disposable trocars available for single-site surgery
   e) Flexible optical telescopes are disadvantageous because presentation and orientation is more difficult

6. Which statement with respect to the following instruments is not correct?
   a) The Veress needle is inserted at a variable angle towards the pelvis to avoid injuring the aorta and iliac bifurcation. The angle of insertion is dependent on the grade of obesity
   b) The endotip allows the entry under side and simultaneously prevents umbilical hernia
   c) Uterine manipulators are often used for deep infiltrating endometriosis
   d) Uterine manipulators are sometimes used for myomectomy
   e) Uterine manipulators are only helpful for unexperienced surgeons

JTGGA CME/CPD CREDITING

Answer form for the article titled *"Abdominal anatomy in the context of port placement and trocars"* within the scope of CME/CPD

1st Question				
A	B	C	D	E

2nd Question				
A	B	C	D	E

3rd Question				
A	B	C	D	E

4th Question				
A	B	C	D	E

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CONGRESS CALENDAR

INTERNATIONAL MEETINGS

02 - 05 March, 2016 — **The 17th World Congress of Gynecological Endocrinology**
Firenze, Italy
http://isge2016.isgesociety.com/

19 - 21 May, 2016 — **The 24th European Congress of Obstetrics and Gynaecology (EBCOG)**
Torino, Italy
http://www.ebcog2016.org/

NATIONAL MEETINGS

25 - 26 February, 2016 — **The 2nd National Postpartum Care Congress**
Antalya, Turkey
http://www.dogumsonubakimkongresi.org/

03 - 06 March, 2016 — **Palandöken Gynaecology Congress**
Erzurum, Turkey
http://palandokenkadindogum.com/

06 - 09 March, 2016 — **6th Minimally Invasive Gynaecology Symposium and 2nd Fertility Academy**
Bursa, Uludağ

11 - 15 May, 2016 — **XI Turkish German Gynecologic Congress**
Antalya, Turkey
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