POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

Edited since 1922

no **2**/vol **90**/**2019**

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

IF: **0.621,** MNiSW: **15**

ORIGINAL PAPERS

Hormonal contraception in patients with epilepsy
Magdalena Bosak, Katarzyna Cyranka, Agnieszka Slowik
61

Sensitivity and specificity of HR HPV E6/E7 mRNA test in detecting cervical squamous intraepithelial lesion and cervical cancer
Dominik Pruski, Sonja Millert-Kalinska, Anna Lewek, Witold Kedzia
66

Influence of Human Papilloma Virus (HPV) infection on early pregnancy
Lukasz Bober, Grzegorz Guzowski, Hanna Moczulska, Piotr Sieroszewski
72

MTHFR genetic polymorphism and the risk of intrauterine fetal death in Polish women
Hubert Wolski, Grazyna Kurzawinska, Krzysztof Drews, Magdalena Barlik, Przemyslaw Kadziolka, Zbyszko Malewski, Paula Mikolajska-Ptas, Michal Bylewski, Agnieszka Seremak-Mrozikiewicz
76

Additional secure circular suture during sphincteroplasty — preliminary results on the efficacy of fecal incontinence surgery in urogynecological patients
Aneta Adamiak-Godlewska, Katarzyna Skorupska, Katarzyna Romanek-Piva, Jacek Pilat, Tomasz Rechberger
82

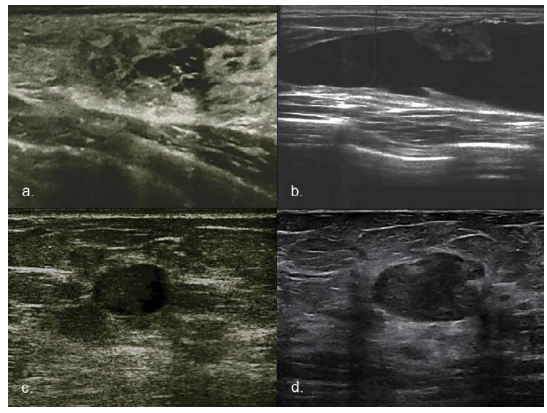
Expression of Cripto-1 in the placenta and its role in placenta accreta and placenta previa
Lingling Jiang, Xiaoya Wu, Jianying Yan, Rongxin Chen, Qing Han, Qinjian Zhang
86

Follow-up of children with antenatally diagnosed idiopathic polyhydramnios
Magdalena Adamczyk, Jakub Kornacki, Przemyslaw Wirstlein, Malgorzata Szczepanska, Ewa Wender-Ozegowska
93

REVIEW PAPERS

Intraductal papilloma of the breast — management
Diana Hodorowicz-Zaniewska, Joanna Szpor, Pawel Basta
100

Food and Drug Administration — approved molecular methods for detecting human papillomavirus infection
Katarzyna Sitarz, Slawa Szostek
104



GINEKOLOGIA POLSKA 2019, VOL 90, NO 2, PAGES 61–114

ISSN 0017–0011

VIA MEDICA

POLISH GYNECOLOGY

GINEKOLOGIA POLSKA

ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS ISSN 0017–0011

EDITOR-IN-CHIEF
Ewa Nowak-Markwitz (Poznań, Poland)

VICE EDITOR-IN-CHIEF
Tomasz Rechberger (Lublin, Poland)

SECTION EDITORS
Gynecology
Artur Czekierdowski (Lublin, Poland)
Basic science section
Agnieszka Seremak-Mrozikiewicz (Poznań, Poland)
Perinatology
Agnieszka Zawiejska (Poznań, Poland)

MANAGING EDITOR
Lucyna Januszek-Michalecka (Poznań, Poland)

PUBLISHER EDITOR
Karolina Klimek (Gdańsk, Poland)

EDITORIAL ADVISORY BOARD
Grzegorz H. Bręborowicz (Poznań, Poland)
Gian Carlo di Renzo (Perugia, Italy)
Krzysztof Drews (Poznań, Poland)
Dan Farine (Ontario, Canada)
Moshe Hod (Tel-Aviv, Israel)
Grzegorz Jakiel (Warszawa, Poland)
Jacques Jani (Brussels, Belgium)
Agata Karowicz-Bilińska (Łódź, Poland)
Jan Kotarski (Lublin, Poland)
Przemysław Oszukowski (Łódź, Poland)
Tomasz Paszkowski (Lublin, Poland)
Ritsuko K. Pooh (Osaka, Japan)
Krzysztof Preis (Gdańsk, Poland)
Joseph G. Schenker (Jerusalem, Israel)
Jim G. Thornton (Nottingham, United Kingdom)
Mirosław Wielgoś (Warszawa, Poland)
Sławomir Wołczyński (Białystok, Poland)

Ginekologia Polska is published monthly, twelve volumes a year, by VM Media sp. z o.o. VM Group sp.k., Świętokrzyska St. 73, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94, fax: (+48 58) 320 94 60, e-mail: redakcja@viamedica.pl, marketing@viamedica.pl, http://www.viamedica.pl

Editorial office address: Gynecologic Oncology Department, Poznan University of Medical Sciences, Polna 33, 60–535 Poznań, Poland, e-mail: ginpol@viamedica.pl

Indexed in: CrossRef, DOAJ, Index Copernicus, Ministry of Science and Higher Education (15), POL-Index, Polish Medical Bibliography, PubMed, Science Citation Index Expanded (0.621), Scimago Journal Rank, Scopus, Ulrich's Periodicals Directory

Advertising. For details on media opportunities within this journal please contact the advertising sales department, Świętokrzyska St. 73, 80–180 Gdańsk, Poland, phone: (+48 58) 320 94 94, e-mail: marketing@viamedica.pl

The Editors accept no responsibility for the advertisement contents.

Manuscripts should be submitted using online submission system only.

All rights reserved, including translation into foreign languages. No part of this periodical, either text or illustration, may be used in any form whatsoever. It is particularly forbidden for any part of this material to be copied or translated into a mechanical or electronic language and also to be recorded in whatever form, stored in any kind of retrieval system or transmitted, whether in an electronic or mechanical form or with the aid of photocopying, microfilm, recording, scanning or in any other form, without the prior written permission of the publisher. The rights of the publisher are protected by national copyright laws and by international conventions, and their violation will be punishable by penal sanctions.

Editorial policies and author guidelines are published on journal website: www.journals.viamedica.pl/ginekologia_polska

Legal note: www.journals.viamedica.pl/ginekologia_polska/about/legalNote

VIA MEDICA © Via Medica 2019

18-0256.002.001

POLISH GYNECOLOGY
GINEKOLOGIA POLSKA
ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGÓW I POŁOŻNIKÓW
THE OFFICIAL JOURNAL OF THE POLISH SOCIETY OF GYNECOLOGISTS AND OBSTETRICIANS

CONTENTS

ORIGINAL PAPERS
GYNECOLOGY

Hormonal contraception in patients with epilepsy
Magdalena Bosak, Katarzyna Cyranka, Agnieszka Slowik 61

Sensitivity and specificity of HR HPV E6/E7 mRNA test in detecting cervical squamous intraepithelial lesion and cervical cancer
Dominik Pruski, Sonja Millert-Kalinska, Anna Lewek, Witold Kedzia 66

Influence of Human Papilloma Virus (HPV) infection on early pregnancy
Lukasz Bober, Grzegorz Guzowski, Hanna Moczulska, Piotr Sieroszewski 72

MTHFR genetic polymorphism and the risk of intrauterine fetal death in Polish women
Hubert Wolski, Grazyna Kurzawinska, Krzysztof Drews, Magdalena Barlik, Przemyslaw Kadziolka, Zbyszko Malewski, Paula Mikolajska-Ptas, Michal Bylewski, Agnieszka Seremak-Mrozikiewicz 76

Additional secure circular suture during sphincteroplasty — preliminary results on the efficacy of fecal incontinence surgery in urogynecological patients
Aneta Adamiak-Godlewska, Katarzyna Skorupska, Katarzyna Romanek-Piva, Jacek Pilat, Tomasz Rechberger 82

ORIGINAL PAPERS
OBSTETRICS

Expression of Cripto-1 in the placenta and its role in placenta accreta and placenta previa
Lingling Jiang, Xiaoya Wu, Jianying Yan, Rongxin Chen, Qing Han, Qinjian Zhang 86

Follow-up of children with antenatally diagnosed idiopathic polyhydramnios
Magdalena Adamczyk, Jakub Kornacki, Przemyslaw Wirstlein, Malgorzata Szczepanska, Ewa Wender-Ozegowska 93

REVIEW PAPER
GYNECOLOGY

Intraductal papilloma of the breast — management
Diana Hodorowicz-Zaniewska, Joanna Szpor, Pawel Basta 100

Food and Drug Administration — approved molecular methods for detecting human papillomavirus infection
Katarzyna Sitarz, Slawa Szostek 104

VIA MEDICA
© Via Medica 2019

REVIEW PAPER
OBSTETRICS

The role of lymphocytes in fetal development and recurrent pregnancy loss
Jan Modzelewski, Anna Kajdy, Michal Rabijewski 109

CLINICAL VIGNETTE

Exploring the fetal brain: is MRI always better than ultrasound?
Michal Lipa, Przemyslaw Kosinski, Miroslaw Wielgos 114

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 61–65
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0010

Hormonal contraception in patients with epilepsy

Magdalena Bosak, Katarzyna Cyranka, Agnieszka Slowik

Collegium Medicum, Jagiellonian University of Cracow, Poland

ABSTRACT

Objectives: The aim of the study was to evaluate hormonal contraception use in women with epilepsy and to assess the risk of potential interactions between contraceptives and antiepileptic drugs (AEDs).

Material and methods: Data on hormonal contraception were obtained prospectively in women of childbearing age treated in the university epilepsy clinic.

Results: We evaluated 334 women with epilepsy (mean age 30.2 years). The majority of patients took one AED (193, 58%); the most commonly prescribed AEDs were: valproate, levetiracetam or lamotrigine. Hormonal contraception was used by 19 (5.7%) of all women of childbearing age. Only 7 patients (37%) of all those using hormonal contraception used preparations that did not interact with AEDs; what is more 145 (46%) patients who did not use hormonal contraception were prescribed AEDs with high teratogenic potential (valproate or/and topiramate).

Conclusions: A very small percentage of women with epilepsy of childbearing potential used hormonal contraception. More than a half of that group simultaneously took AEDs that may interact with oral contraceptives. A large proportion of women taking AEDs with high teratogenic potential were not using hormonal contraception. As interaction between OC and AEDs are common, nonhormonal, highly effective methods, such as IUDs, may be ideal for women with epilepsy. The results of the study indicate the need for closer cooperation between neurologist and gynecologist caring for women with epilepsy.

Key words: hormonal contraception; epilepsy; antiepileptic drugs; interaction

Ginekologia Polska 2019; 90, 2: 61–65

INTRODUCTION

With an estimated point prevalence of 6.4 per 1,000 persons, epilepsy is one of the most frequent chronic neurological disorders [1]. Both epileptic seizures and their pharmacotherapy may negatively affect reproductive health-related issues, especially in women with epilepsy (WWE). Antiepileptic drugs (AEDs) must be used in WWE for many years, and sometimes throughout life, also during reproductive age. One of the most important side effects of pharmacotherapy of WWE is the teratogenic potential of AEDs. The results of prospective observational registers of pregnancies in WWE indicate an increased risk of birth defects in children exposed to AEDs in utero. The latest report of the largest pregnancy and epilepsy register EURAP International showed 4.9% risk of major congenital malformation in offspring of women taking AEDs during pregnancy [2]. Another aspect of the treatment of epilepsy in women are the bidirectional pharmacokinetic interactions between oral contraceptives (OC) and AEDs , which may lead to a reduction in the effectiveness of contraception and/or AEDs [3]. Ethinylestradiol (EE) metabolism may be accelerated by carbamazepine, oxcarbazepine, phenytoin, phenobarbital and high doses of topiramate (> 200 mg/d), progestin by carbamazepine, oxcarbazepine, lamotrigine, phenytoin and phenobarbital. Other AEDs interacting with OC are not available in Poland. Lamotrigine concentration is reduced by EE and increased seizure frequency has been reported. Interaction of EE with AEDs are well known, however possible interactions of progestin with AEDs are much less studied. It has been proved that enzyme-inducing AEDs interact with oral levonorgestrel, oral norethindrone and the subdermal etonogestrel implant [3]. According to recent data more than half of pregnancies in women with epilepsy are unplanned; significant percentage of patients do not use highly effective methods of contraception or use hormonal contraceptives combined with enzyme-inducing AEDs, which can lead to unintended pregnancies [4–6].

Corresponding author:
Magdalena Bosak
Collegium Medicum, Jagiellonian University of Cracow, Poland
e-mail: magdalena.bosak@uj.edu.pl

Objectives

The aim of the study was to evaluate hormonal contraception use in women with epilepsy and to assess the risk of potential interactions between contraceptives and antiepileptic drugs.

MATERIAL AND METHODS

Study population

The study included consecutive WWE of reproductive age (16–49 years) treated at the university epilepsy clinic between 01.08. 2017 and 31.08.2018. Participation in the study was offered to patients diagnosed with epilepsy as defined by the International League Against Epilepsy (ILAE) of 2014 [7]. Patients who did not agreed to participate, pregnant and breastfeeding patients, patients with primary amenorrhea and patients with concomitant psychogenic non-epileptic seizures were excluded from the study.

The study protocol was approved by the University Ethical Committee and all subjects gave their written consent to participate in the study.

Methods

Demographic and epilepsy data were collected using a structured questionnaire and included: age, sex, age at the onset of epilepsy, type and frequency of seizures, AEDs treatment. The type of epilepsy was diagnosed on the basis of the interview, neurological examination, neuroimaging and electroencephalogram. Epilepsy type has been classified according to the new ILAE position paper on classification of epilepsies [8]. Data on hormonal contraception used by patients were obtained prospectively from patients during two subsequent visits to the clinic. The potential for interaction between AEDs and hormonal contraceptives has been assessed on the basis of Reimers et al. [3]. Enzyme-inducing AEDs used by studied women included carbamazepine, oxcarbazepine and topiramate > 200 mg daily.

RESULTS

Sample characteristics

Among 405 female patients who were seen in the epilepsy clinic within the period of the study, 334 fertile women met the inclusion criteria and entered the study. The average age of the analyzed patients was 30.2 (± 7.73). 193 (57.8%) patients were on monotherapy, polytherapy was used in 141 (42.2%) of the studied women. The most commonly used AEDs included valproate, levetiracetam and lamotrigine. At the time of the assessment 127 (36.2%) of the patients were in remission. Counseling regarding the effective methods of contraception and the possible teratogenic effects of the medication on the fetus were documented in a written form in patients' health records in all WWE taking valproate or/and topiramate and in 89% (297) of the whole group. Table 1 presents demographics, the characteristics of epilepsy and its treatment in the studied group.

Table 1. Clinical characteristics of the study patients

Variable	N = 334
Age [years]	30.2 (16–49)
Age at onset of epilepsy	15.0 (1–43)
Type of epilepsy	
• focal	227 (70.0%)
• genetic (idiopathic) generalized	96 (28.7%)
• combined focal & generalized or unknown	11 (3.3%)
Number of AEDs used	
• 1	193 (57.8%)
• 2	104 (31.2%)
• 3	32 (9.6%)
• 4	5 (1.4%)
Seizure frequency	
• more than 1 per month	121 (36.2%)
• less than 1 per month, more than 1 per year	86 (25.8%)
• less than 1 per year	127 (38.0%)
The most commonly used AEDs (in mono-or polytherapy)	
Valproate	135
• levetiracetam	121
• lamotrigine	98
• carbamazepine	56
• topiramate	39
Place of residence	
• village or town < 20 000	198 (59.3%)
• large town 20 000–100 000	80 (24%)
• city 100 000–1000 000	56 (16.7%)
Education	
• none	68 (20.3%)
• primary school	18 (5.4%)
• vocational/secondary school	201 (60.2%)
• university degree	47 (14.1%)

Hormonal contraception

Of the 334 WWE participating in the study, 19 (5.7%) patients in an average age of 27.6 (20–43) years reported the current use of hormonal contraception (18- combined hormonal contraceptive, 1 progestin-only pill). No women used hormonal patch, vaginal ring, implanted progestin or depomedroxyprogesterone. The precise characteristics of AEDs used by these patients as well as hormonal contraception are presented in Table 2.

Of the patients on OC, the majority (12; 63%) had a potential for drug-drug interactions. Only 7 women (37%) of all those on hormonal contraception used preparations that did not interact with AEDs. Additionally 7 patients used sex hormones for other indications (menstrual regulation or hormone replacement therapy): 2 patients — dydrogesterone, 2 — progesterone, 1 — estradiol, 1 — estradiol / norethisterone, 1 — estradiol / norgestrel. All patients from this group were on non-enzyme-inducing AEDs (LEV, VPA, VGB).

Table 2. Hormonal contraceptives, AEDs and interaction risk in the studied patients

AEDs	Estrogen	Progestogen	Interaction risk[a]
OXC	estradiol	nomegestrol	1
CBZ	ethinylestradiol	norgestimate	1
LTG	ethinylestradiol	gestodene	2
LEV	ethinylestradiol	gestodene	3
LEV OXC	ethinylestradiol	gestodene	1
OXC	ethinylestradiol	gestodene	1
LEV	ethinylestradiol	norgestimate	3
VPA	ethinylestradiol	norgestimate	3
LEV LTG	ethinylestradiol	drospirenone	2
VPA LTG LEV	ethinylestradiol	norgestimate	2
VPA LEV	ethinylestradiol	norgestimate	3
VPA	ethinylestradiol	norgestimate	3
VPA LTG	ethinylestradiol	gestodene	2
LTG	ethinylestradiol	dienogest	2
VPA	ethinylestradiol	gestodene	3
LTG	ethinylestradiol	norgestimate	2
LTG	ethinylestradiol	gestodene	2
LTG VGB	ethinylestradiol	dienogest	2
LEV		desogestrel	3

[a]1 — reduced efficacy of OC; 2 — decreased concentration of AED; 3 — without clinically significant interactions; abbreviations: CBZ — carbamazepine; LTG — lamotrigine; LEV — levetiracetam; OXC — oxcarbazepine; VPA — valproate; VGB — vigabatrin

AEDs with teratogenic potential

Nearly half of the patients (145; 46%) who did not use hormonal contraceptives were on AEDs with known teratogenic potential (128 VPA, 17 TPM). In 68 patients of the initial cohort the future pregnancy was extremely unlikely due to concurrent severe disabilities (severe mental retardation, being in a nursing home, significant paresis). They were excluded from the analysis. The remaining group consisted of 266 women, of whom 19 (7.1%) used OC. Out of 247 WWE not using hormonal contraception, 84 (37%) were on AEDs with the highest risk of teratogenicity: VPA or TPM.

DISCUSSION

Our work showed a very low percentage of WWE using hormonal contraceptives (5.7%). In a study of patients with epilepsy in the US, as many as 46.6% used hormonal contraception [5]. The results of study by Polish authors, focused on general population also showed a significantly higher percentage of OC usage in the Polish general population (31.2%) [9]. There may be several reasons for such a low percentage of OC usage in our cohort. With regard to religion, the vast majority of Polish population (88%) is Roman catholic and may accept only methods of natural family planning [10]. Only 14% of patients had a university degree and most of them (60%) lived in villages or small towns, these factor my negatively affect knowledge and availability of the effective contraceptive methods. Several other reasons may play a role in not using contraception by WWE: concerns about its efficacy and interactions with AEDs, sides effects of hormonal OC, menstrual problems and increased seizure frequency [11].

Every woman of childbearing age treated in our epilepsy clinic receives counseling on a contraception plan and on the teratogenicity of AEDs. Despite this, the proportion of patients using hormonal contraceptives is very low. In the case of using drugs with high risk of teratogenicity (VPA, TMP), the patient is counselled on effective methods of contraception during each subsequent visit, and a plan for changing the pharmacotherapy of epilepsy is also presented. Unfortunately, most patients, especially those who are in remission, do not agree to change therapy.

Furthermore, 60% of the patients taking oral contraceptives were on AEDs which could have significant pharmacokinetic interactions with hormonal preparations. These were: induction of hepatic metabolism of OC by CBZ or OXC, and thus the possibility of reducing the contraceptive effectiveness. The second, more frequent mechanism of interaction, involved the stimulation of UDP-glucuronyl

transferase by the estrogen component of hormonal contraception, thereby reduction of the concentration and efficacy of lamotrigine. Our results are in line with the study of Bhakta et al. [4] and indicate that knowledge gaps exist in terms of the potential teratogenic effects of AEDs and pharmacokinetic interactions between AEDs and OC.

Out of 247 childbearing age women who could become pregnant and did not use hormonal contraception, 84 (37%) were on AEDs with the highest risk of teratogenicity: VPA or TPM. According to the recently published European Medicine Agency recommendation valproate must not be used in women able to have children unless the terms of a special pregnancy prevention programs are followed [12].

Appropriate counseling on the forms of hormonal contraception, suited for WWE expectations and needs is crucial for the selection of an optimal birth control method [11, 13]. Polish Society of Epileptology and Polish Gynecological Society have developed guidelines regarding management and care of WWE of childbearing potential, which underline the importance of counseling regarding contraceptive or pregnancy planning and the choice of AEDs [14].

Combined OC and progestin-only pill efficacy may be reduced by enzyme-inducing AEDs. Other hormonal contraceptive methods, such as medroxyprogesterone acetate depot injection, or implantable hormonal contraceptive may have some interaction with AEDs. Nonhormonal, highly effective methods, such as IUDs, may be ideal for women with epilepsy, since the contraceptive mechanism of IUDs is unaffected by changes in hepatic enzyme activity [14–16]. What's more, IUD poses a significantly lower risk for seizure increase in WWE than hormonal contraception [17]. In WWE using a hormonal-IUD, a levonorgestrel level seems to be unaffected by concomitant AEDs therapy [18]. The role of gynecologist in counseling on the forms of hormonal contraception is indispensable for appropriate management of WWE in childbearing age.

Our research has several disadvantages. First of all, it was carried out in a reference outpatient epilepsy clinic in which we treat patients with drug-resistant epilepsy often requiring polytherapy or using drugs with a higher potential for teratogenicity. For this reason, a group of patients may not be representative of the general population of women with epilepsy. The second disadvantage is the lack of a control group. Therefore, it can only be concluded indirectly that the frequency of OC usage in patients with epilepsy is lower than in the Polish population of women in reproductive age. Thirdly, information on the use of other methods of contraception, in particular highly effective methods, such as the intrauterine device (tubal ligation and vasectomy are legally prohibited in Poland), has not been collected. In the studies of Bakhta et al. [4] and Herzog et al. [5], IUD was used by 6.1% and 17% of WWE respectively.

CONCLUSIONS

A very small percentage of WWE in reproductive age used oral hormonal contraception. The ones using contraception frequently applied method that had significant drug–drug interaction which reduced the effectiveness of OC or AED(s). A large proportion of women who were having AEDs with high risk of teratogenicity prescribed were not using hormonal contraception. It is advisable to create gynecological-neurological teams, caring for WWE in reproductive age, and to take care about continuous education of patients regarding effective methods of family planning and about improvement of methods of informing patients about the teratogenicity of AEDs. Nonhormonal, highly effective methods, such as IUDs, may be ideal for women with epilepsy.

Acknowledgements

Magdalena Bosak: Project development, data collection, literature review, writing manuscript; Katarzyna Cyranka: literature review, writing manuscript; Agnieszka Słowik: literature review, writing manuscript, intellectual input.

Conflict of interest

M. Bosak received honoraria for publications from Sanofi; honoraria for lectures, travel expenses and conference fees from Sanofi, Adamed, Teva Pharmaceutical, Neuraxpharm, Glenmark, UCB Pharma.

K. Cyranka reports no conflict of interest.

A. Słowik received honoraria for lectures from Bayer, Boehringer Ingelheim, Novartis, Polpharma, Bristol-Myers Squipp, Novartis, Biogen, Teva Pharmaceutical, Medtronic; for the participation in advisory meetings from Bayer, Boehringer Ingelheim, Novartis.

Funding

This publication was prepared without any external sources of funding.

REFERENCES

1. Jette N, Fiest KM, Sauro KM, et al. Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. Neurology. 2017; 88(3): 296–303, doi: 10.1212/WNL.0000000000003509, indexed in Pubmed: 27986877.
2. EurapInternational http://eurapinternational. http://eurapinternational.org/wp-content/uploads/2018/09/Eurap_Report_May_2018.pdf (2018.09.15).
3. Reimers A, Brodtkorb E, Sabers A. Interactions between hormonal contraception and antiepileptic drugs: Clinical and mechanistic considerations. Seizure. 2015; 28: 66–70, doi: 10.1016/j.seizure.2015.03.006, indexed in Pubmed: 25843765.
4. Bhakta J, Bainbridge J, Borgelt L. Teratogenic medications and concurrent contraceptive use in women of childbearing ability with epilepsy. Epilepsy Behav. 2015; 52(Pt A): 212–217, doi: 10.1016/j.yebeh.2015.08.004, indexed in Pubmed: 26460786.
5. Herzog AG, Mandle HB, Cahill KE, et al. Contraceptive practices of women with epilepsy: Findings of the epilepsy birth control registry.

Epilepsia. 2016; 57(4): 630–637, doi: 10.1111/epi.13320, indexed in Pubmed: 26880331.
6. Herzog A, Mandle H, Cahill K, et al. Predictors of unintended pregnancy in women with epilepsy. Neurology. 2017; 88(8): 728–733, doi: 10.1212/wnl.0000000000003637.
7. Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014; 55(4): 475–482, doi: 10.1111/epi.12550, indexed in Pubmed: 24730690.
8. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017; 58(4): 512–521, doi: 10.1111/epi.13709, indexed in Pubmed: 28276062.
9. Plany prokreacyjne i stosowanie antykoncepcji przez Polaków w wieku reprodukcyjnym. Zdrowie Publiczne i Zarządzanie. 2017; 15(2), doi: 10.4467/20842627oz.17.013.6783.
10. http://stat.gov.pl/obszary-tematyczne/inne-opracowania/wyznania-religijne/wyznania-religijne-w-polsce-20122014,5,1.html (2018.10.31).
11. Mandle HB, Cahill KE, Fowler KM, et al. Reasons for discontinuation of reversible contraceptive methods by women with epilepsy. Epilepsia. 2017; 58(5): 907–914, doi: 10.1111/epi.13734, indexed in Pubmed: 28369748.
12. EuropeanMedicinesAgency. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Valproate_2017_31/European_Commission_final_decision/WC500250216.pdf (2018.09.15).
13. Tomaszewski J, Paszkowski T, Debski R, et al. [The CHOICE study (Contraceptive Health Research Of Informed Choice Experience) – an educational research program for Polish women planning combined hormonal contraceptives use]. Ginekol Pol. 2012; 83(6): 417–423, indexed in Pubmed: 22880460.
14. Jędrzejczak J, Bomba-Opoń D, Jakiel G, et al. Managing epilepsy in women of childbearing age - Polish Society of Epileptology and Polish Gynecological Society Guidelines. Ginekol Pol. 2017; 88(5): 278–284, doi: 10.5603/GP.a2017.0053, indexed in Pubmed: 28580576.
15. Reddy DS. Clinical pharmacokinetic interactions between antiepileptic drugs and hormonal contraceptives. Expert Rev Clin Pharmacol. 2010; 3(2): 183–192, doi: 10.1586/ecp.10.3, indexed in Pubmed: 20369030.
16. Reimers A. Contraception for women with epilepsy: counseling, choices, and concerns. Open Access J Contracept. 2016; 7: 69–76, doi: 10.2147/OAJC.S85541, indexed in Pubmed: 29386938.
17. Herzog AG, Mandle HB, Cahill KE, et al. Differential impact of contraceptive methods on seizures varies by antiepileptic drug category: Findings of the Epilepsy Birth Control Registry. Epilepsy Behav. 2016; 60: 112–117, doi: 10.1016/j.yebeh.2016.04.020, indexed in Pubmed: 27206228.
18. Vieira CS, Pack A, Roberts K, et al. A pilot study of levonorgestrel concentrations and bleeding patterns in women with epilepsy using a levonorgestrel IUD and treated with antiepileptic drugs. Contraception. 2018 [Epub ahead of print], doi: 10.1016/j.contraception.2018.11.018, indexed in Pubmed: 30529085.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 66–71
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0011

Sensitivity and specificity of HR HPV E6/E7 mRNA test in detecting cervical squamous intraepithelial lesion and cervical cancer

Dominik Pruski[1, 2], Sonja Millert-Kalinska[2], Anna Lewek[1], Witold Kedzia[1, 2]

[1]Division of Gynecology, Department of Perinatology and Gynecology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences, Poznan, Poland

[2]Laboratory of Cervical Pathophysiology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences, Poznan, Poland

ABSTRACT

Objectives: The paper assess the relevance of HR HPV E6/E7 mRNA test in women with abnormal Pap results.

Material and methods: Between 2013–2014, 125 women were subjects to the enhanced diagnostics due to abnormal Pap results. According to The Bethesda system, if ASC-US, AGC, LSIL, ASC-H, HSIL or cancer cells were present, the result was abnormal. The patients underwent the enhanced diagnostics which included the following procedures: Pap smear collection for molecular assessment of HR HPV E6/E7 mRNA test, the colposcopic examination and biopsy of clinically suspicious areas.

Results: High-grade squamous intraepithelial lesions constituted the most frequent cervical pathology in women with abnormal Pap test results, as well as with the positive results of HR HPV E6/E7 mRNA test. Test sensitivity in patients with the histopathological diagnosis of high-grade squamous intraepithelial lesion was estimated at 86.1%.

Conclusions: HR HPV E6/E7 mRNA test identifying neoplastic lesions and cervical cancer is characterised by a high relevance which is reflected by means of sensitivity and specificity. In fact, test sensitivity and specificity increased with the age in the group of patients up to 50 years old.

Key words: HPV E6/E7 mRNA; SIL; squamous intraepithelial lesion; HSIL

Ginekologia Polska 2019; 90, 2: 66–71

INTRODUCTION

According to the World Health Organization (WHO), cervical cancer constitutes the 4th most frequent malignant cancer in women worldwide. In 2012, about 530000 new cases were recorded, and nearly 90% of 270000 deaths occurred due to this disease in mid and low socioeconomic status countries. Moreover, high mortality rate may be reduced only if a comprehensive approach is introduced including broadly defined prevention, that is education, effective and efficient screening, as well as early diagnosis and treatment [1].

The introduction of cervical cancer screening has largely decreased both the incidence and the mortality rate of women in Europe over the years, although the success rate is radically different in particular countries [2]. Nowadays, 34000 new cases of cervical cancer are found in Europe every year, with 13000 deaths due to this disease [3]. In Poland, since the 90's the tendency constantly decreases, reflecting the improvement in the epidemiological situation, although further steps need to be taken in order for the method to be fully successful. In Poland in 2014, the diagnosis of cervical cancer was made in 9 women a day, and nearly half of them died of it. [4, 5].

In 2005 a Polish national programme for cervical cancer prevention was implemented which aimed at an early detection of precancerous lesions classified as CIN (Cervical Intraepithelial Neoplasia) 1, CIN 2, CIN 3. According to the current recommendations, CIN 1 is referred to as LG SIL (Low Grade Squamous Intraepithelial Lesion), whereas CIN 2 and CIN 3 are both called HG SIL (High Grade Squamous Intraepithelial Lesion).

The basic factor in cervical cancer development is a persistent infection with HR HPV, where the most cancerogenic

Corresponding author:
Dominik Pruski
Division of Gynecology, Department of Perinatology and Gynecology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences Poznan, Poland
e-mail: dominik.pruski@oxytop.pl

types are HPV 16, 18, 31, 33, 45. Cervical intraepithelial neoplasia lasts ca. 7–10 years, and following another 3–5 years may consequently lead to a pre-invasive and invasive cervical cancer. Moreover, current data indicate the presence of various HPV DNA types in 99.7% cervical cancer biopsies [6].

As early as 2003, the American College of Obstetricians and Gynecologists was the first to include HR HPV DNA test in the screening guidelines. Furthermore, since 2012 more and more recommendations have indicated and proved the HPV DNA test superiority over conventional cytology test in female patients aged 30–65 [7].

There is evidence suggesting that co-testing, i.e. combining Pap test with HR HPV DNA test, contributes to a decrease in the incidence of invasive cancer, as well as generates lower costs in comparison to the annual Pap test performed for 30 years [8, 9].

The current European guidelines recommend HR HPV DNA test as a screening method in women 35–60 years of age [10].

Numerous research indicate a higher diagnostic value of the HR HPV DNA test in comparison with the Pap test. In fact, on the basis of the analysis including over 10000 women in Canada, HR HPV DNA test sensitivity for HG SIL lesions was estimated at 94.6%, as compared to cytological test sensitivity which was estimated at 55.4% [11].

A perfect screening method should comprise a nearly 100% sensitivity and specificity, as well as a high positive predictive value which in practice, however, is extremely difficult to obtain.

Incorporating tests detecting HR HPV E6/E7 mRNA test constitutes one of the most recent discoveries, and allows for the identification of patients with permanent viral infection, where the process of DNA incorporation in the epithelial cells genetic material has already been initiated. In fact, the neoplastic transformation process starts once HPV DNA integrates with the proper epithelial cell genome. Moreover, it is possible when HPV DNA circular form is damaged and chromatin displacement occurs within the chromosomal DNA of host's cells. Oncoprotein E6 and E7 expression in epithelial cells infected with HR HPV types is associated with an increase in proliferation and abnormal differentiation of these cells, and may lead to the development of neoplastic and malignant lesions [12–14]. HR HPV E6 protein contributes to the degradation of p53 protein which protects the genome, an thus may inactivate the genetic mechanisms controlling the cellular cycle and apoptosis. In fact, the function of p53 in the cellular cycle is based on the movement control from G1 phase to the S phase of the cellular cycle by means of inducing expression of p16, p21 and p27 cyclin inhibitors. Due to this mechanism it is possible to stop the cellular cycle in G1/S phase [12].

According to the sources, the described diagnostic procedure is characterised by a high sensitivity and specificity equal to 98% and 85% respectively.

The indisputable advantage of the abovementioned diagnostic method is the objectivity and repeatability, although the screening test of a given patient would not have to be performed as frequently as a conventional cytology. The clinical observations show that the progression risk increases when one of the highly oncogenic types: 16, 18, 31, 33, 45 is responsible for the persistent infection, and its mRNA presence constitutes an even poorer prognostic factor. In fact, it indicates an ongoing carcinogenesis on the molecular level and additionally, in 98% of cases, it entails the continuation and progression of the disease [15]. Further observations may be vital in the future, and may result in the introduction of new guidelines in patients diagnosed with LG SIL who may undergo a spontaneous regression in certain cases. Moreover, observation of regression in women with a negative HR HPV E6/E7 mRNA test could prevent them from additional stress and the necessity of performing unnecessary invasive procedures.

Objectives

The aim of the paper is to assess the relevance of HR HPV E6/E7 mRNA test in women, in female patient population with abnormal Pap test.

MATERIAL AND METHODS

Between 2013–2014 in the Laboratory of Pathophysiology of Uterine Cervix at Poznań University of Medical Sciences, 125 women were subjects to the enhanced diagnostics due to abnormal cytology results. According to The Bethesda system, if ASC-US, AGC, LSIL, ASC-H, HSIL or cancer cells were present, the result was abnormal. All women who participated in the study were adults, not pregnant and not breast-feeding. The study was approved by the Bioethics Committee of the University No 548/18. The paper constitutes a retrospective analysis.

Firstly, all patients were subjects to a detailed medical interview which included the oncological past, earlier cytology and histopathological tests results, if they had been performed, family history, obstetric history, the age of the first menstruation and the date of the last menstrual period. Secondly, the patients underwent the enhanced diagnostics which included the following procedures:

— Pap smear collection for molecular assessment of HR HPV E6/E7 mRNA test;
— The colposcopic examination;
— Biopsy of clinically suspicious areas assessed by a gynaecologist.

Pap smear for molecular assessment — the sample was collected with an endocervical Cyto-Brush, and then it was

cial for the examination to be satisfactory. In all cases, a trial with 3% aqueous solution of acetic acid was performed, as well as the Schiller's test with Lugol's iodine. The colposcopic images were evaluated according to Reid's Colposcopic Index which assesses the colour, lesion borders and surface, blood vessels and iodine test.

Biopsy of the clinically suspicious area visible in colposcopy was performed in each patient classified for the examination. Cervical samples were fixed in buffered 10% formalin solution.

Calculations were performed using the statistical package Statistica (data analysis software system), ver. 13.1 and graphs — using Excel. It was estimated whether increasing age resulted in higher rates of sensitivity, specificity, PPV and NPV by Chi-square test for the trend. Statistical hypotheses were verified at the level of significance of = 0.05.

RESULTS

120 patients participated in the study who were classified into 4 age groups:

— 18–29 years of age → n = 50,
— 30–39 years of age → n = 42,
— 40-49 years of age → n = 15,
— over 50 years of age → n = 13.

The number of participants in particular age groups is shown in Figure 1. In the course of the histopathological analysis of the ectocervix and/or endocervix biopsies, 49.17% of samples were associated with SIL lesions, with the following results:

— 23 patients presented CIN 1 — LG SIL,
— 20 patients showed CIN 2 — HG SIL,
— 13 patients had CIN 3 — HG SIL,
— 2 patients presented squamous cell cancer,
— 1 patient showed adenocarcinoma,
— 61 patients had no SIL.

The incidence of individual histopathological diagnoses with reference to particular age groups is shown in Figure 2.

The average age of patients was 28, with 19 years of age as the youngest, median: 28, and 66 years of age as the oldest.

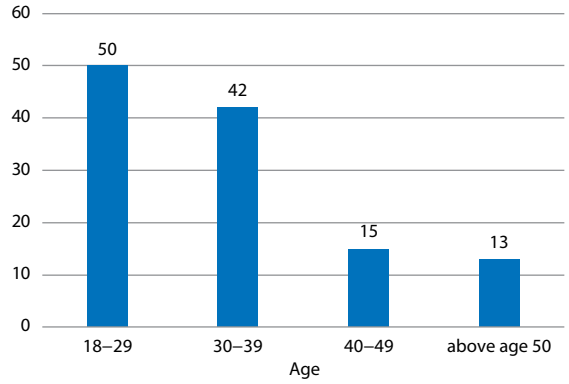


Figure 1. Number of patients

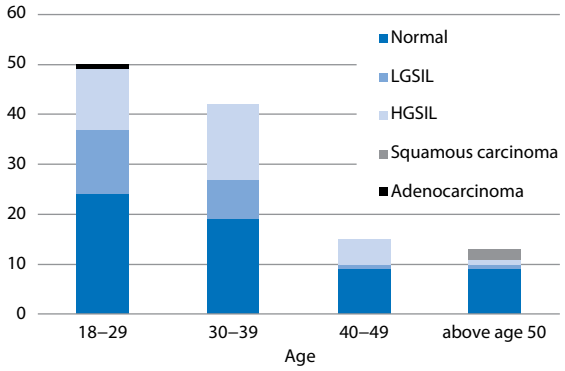


Figure 2. Histopathological diagnose

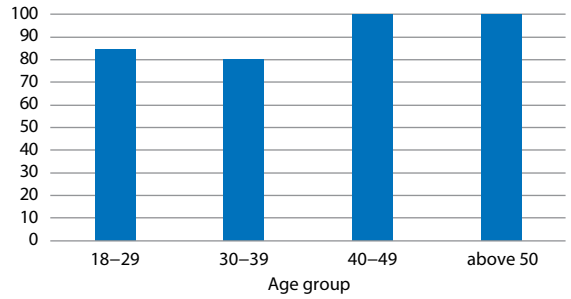


Figure 3. mRNA test sensitivity

Results of molecular HR HPV E6/E7 mRNA test

Test sensitivity in patients with the histopathological diagnosis of low-grade squamous intraepithelial lesion was estimated at 82.6%.

Test sensitivity in patients with the histopathological diagnosis of high-grade squamous intraepithelial lesion was estimated at 86.1% which is shown in Figure 3 with reference to particular age groups.

Test sensitivity of patients with the histopathological diagnosis of both high- and low-grade squamous intraepi-

preserved in PreservCyt® (Hologic Corp.) and SurePath® (BD Diagnostics-TriPath) reserved for the biological material. NucliSENS EasyQ® HPV v1.1 test by bioMérieux was employed for the detection and nucleic acid amplification in real-time, allowing for qualitative identification of E6/E7 messenger RNA (mRNA) for five cancerogenic HPV virus types: 16, 18, 31, 33, 45 in epithelial cells.

Colposcopic examination — the examination was performed in the Laboratory of Pathophysiology of Uterine Cervix by means of the stereoscopic colposcope Olympus OSC-500. In fact, the visualization of the affected area is cru-

Test specificity in patients with the histopathological diagnosis of both high- and low-grade squamous intraepithelial lesion was estimated at 54.1%, which is shown in Figure 5 with reference to particular age groups.

The sensitivity value of the HR HPV E6/E7 mRNA test increases with the patients' age up to 50 years of age, and then decreases.

Sensitivity of detecting squamous intraepithelial lesions by means of this test was the highest in the age group of 40–49 years and above 50 years of age.

Among Pap-test diagnoses listed below: ASC-H, LSIL, HSIL and cervical squamous cell carcinoma, a correlation was found between the diagnosis of pathology and the presence of HR HPV mRNA test. Only in the case of ASC-US diagnosis, in most cases the presence of HR HPV E6/E7 mRNA was not confirmed. Among the Pap-tests in which no pathology was found (NILM), in most cases the presence of HR HPV mRNAs was not confirmed. The results are presented in Figure 6.

The histopathological diagnoses were also taken into account — in the case of confirmed pathology, i.e. LGSIL, HGSIL and cervical squamous cell carcinoma , the presence of HR HPV E6/E7 mRNA was confirmed in the majority of cases. On the other hand, tests for the presence of HR HPV E6/E7 mRNA are still not proper to detect glandular dysplasia (Adenocarcinoma). The results are presented in Figure 7.

There were statistically significant differences in the presence of HR HPV E6/E7mRNA and the occurrence of pathology found in cervical biopsy ($p = 0.00001$). The dependencies in all age groups were also tracked. Only in the group of the youngest patients no statistically significant differences were found ($p > 0.05$). In contrast, in the other age groups, statistically significant differences were found-in the group of women aged 30–39 ($p = 0.01491$) and in the group of women over 50 ($p = 0.01086$). The strongest relationship was observed in the group of patients aged 40–49 ($p = 0.00082$).

DISCUSSION

According to the paper by Sørbye et al. published in 2014, diagnostic tests detecting HR HPV E6/E7 mRNA are characterized by a higher specificity than tests identifying HR HPV DNA. Comparative studies were conducted in Norway in a group of over 300 patients with abnormal Pap test, diagnosed with ASC-US or LSIL according to TBS. Positive predictive value for HSIL histopathological diagnosis in terms of HR HPV DNA molecular test was 21.5%, whereas for HR HPV E6/E7 mRNA test it was 34.6%. What is more, HR HPV DNA test was characterised by a higher sensitivity than the HR HPV E6/E7 mRNA test and detected more cases of histopathologically confirmed high-grade squamous intraepithelial lesion of uterine cervix [16].

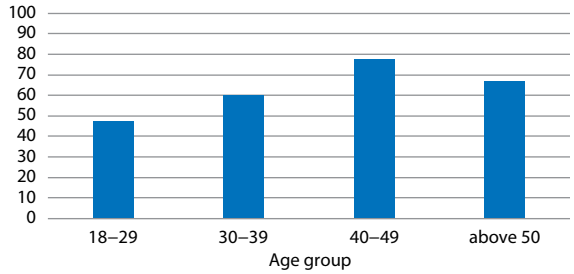


Figure 4. mRNA test sensitivity for LGSIL nd HGSIL

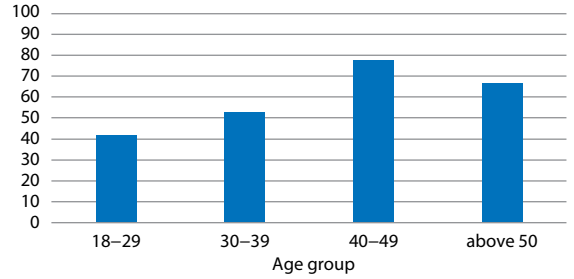


Figure 5. mRNA test specifity

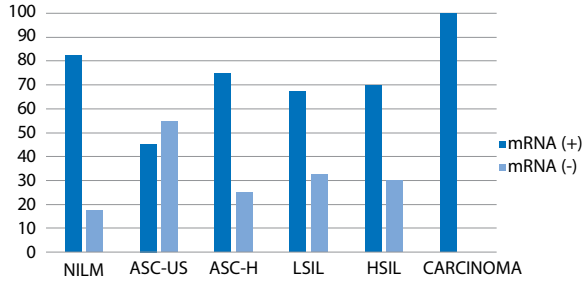


Figure 6. The incidence of mRNA positive and negative results according to PAP tests

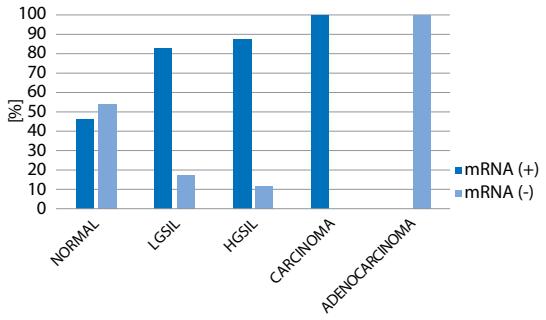


Figure 7. The incidence of mRNA positive and negative results according to histopathological diagnoses

thelial lesion a was estimated at 84.7% with reference to particular age groups is presented in Figure 4.

Yao Yl et al. in 2017 confirmed the relevance of HR HPV E6/E7 mRNA test in monitoring HR HPV positive patients. In the abovementioned paper no statistically relevant difference was shown between the sensitivity and specificity of the Pap test and HR HPV E6/E7 mRNA test in detecting HSIL lesions among HPV positive patients. Moreover, the sensitivity and specificity of the abovementioned test was estimated at 89.52% and 48.96% respectively in the diagnosis of high-grade squamous intraepithelial lesion of uterine cervix. Additionally, the percentage of positive HR HPV E6/E7 mRNA test results was significantly higher in the histopathological HSIL diagnoses than LSIL [17].

According to a 2013 analysis by Perez Castro et al., HR HPV DNA tests are characterized by a high sensitivity, but a relatively low specificity in identifying uterine cervix oncological pathologies. Due to this fact, new and more precise enhanced diagnostic methods are anticipated which could be employed in patients with abnormal cytology results, namely ASCUS or LSIL. It is vital to notice that test detecting HR HPV E6/E7 mRNA test may significantly increase the molecular tests specificity in identifying HSIL lesions, while retaining high sensitivity and negative predictive value. In the already mentioned paper by Perez Castro et al., the HR HPV E6/E7 mRNA test sensitivity for low-grade lesions, i.e. LSIL, was estimated at 81.3%, whereas for high-grade lesions, that is HSIL, at 84.1%. Additionally, positive predictive value (PPV) was estimated at 97.4% for HSIL lesions. In the summary, the authors confirm the relevance of HR HPV E6/E7 mRNA test in the diagnosis of HR HPV DNA positive population [18].

Fontecha et al. in their paper confirmed the high specificity of HR HPV E6/E7 mRNA test in HPV positive patient population, where progression of squamous intraepithelial lesions occurred in a 2-year observation period. In this paper, the molecular test was characterised by 100% sensitivity in HSIL lesions detection [19].

Combining the aforementioned methods, i.e. PAP test and molecular diagnostics detecting HR HPV E6/E7 mRNA, may significantly contribute to the earlier and more precise detection of cervical neoplasia pathology in high-risk patients groups [20]. Furthermore, the aforesaid management algorithm may also considerably influence the number of surgical procedures which is particularly crucial in pregnant patients. In addition, the future identification of patients with the HSIL and cervical cancer risk development on the basis of a negative molecular test result will allow for a decrease in the numbers of invasive cervical biopsy procedures. What is more, the conducted analysis substantiates the diagnostic value of molecular tests enabling the detection of uterine cervix precancerous and cancerous lesions in pregnant patients.

Verification diagnostics of abnormal cytology results in pregnant patients constitutes a difficult task, lacking particular algorithms and guidelines. Furthermore, colposcopic examination in pregnancy is extremely difficult to interpret, and thus involves human error risk due to the examination high subjectivity level. A gynaecologist has to frequently consider the validity of a comprehensive surgical procedure, that is a cervical biopsy, and the risk of complications in normally developing pregnancy in patients with questionable cytology results, according to The Bethesda System. In fact, ASCUS and LSIL cytological diagnosis constitutes the most frequent abnormal result in pregnant patients [21].

In the 2017 paper, Cobas and Aptima tests were compared. The analysis included over 1800 patients with the histopathological HSIL diagnosis. Both tests were characterized by high sensitivity. However, the Aptima test possessed a statistically higher specificity in detection of high-grade lesions, i.e. HSIL, in comparison to the Cobas test which was estimated at 41% and 13% respectively. Positive predictive value of the Aptima and Cobas tests amounted to 41% and 13% respectively, whereas test accuracy was equal to 50% and 25% respectively. High specificity of the Aptima test, combined with its sensitivity, significantly influences cost reduction of verification diagnostics in abnormal cytology results and positive results of HR HPV DNA tests. It is crucial to bear in mind the fact that the Aptima test detects 14 types of HR HPV E6/E7 mRNA [22].

In the paper by Duvlis et al., 413 patients were analysed with both normal and abnormal cytology results. In all patients, the DNA and mRNA tests detecting HR HPV virus types were conducted. The test identifying E6/E7 mRNA transcripts of HPV 16, 18, 31, 33 and 45 was characterized by 50% specificity and 62% positive predictive value in the HSIL detection. In comparison, the specificity of HR HPV DNA test was equal to 18%. What is more, the authors emphasise the fact that the introduction of modern molecular diagnostics may significantly decrease the number of surgical procedures, and thus lower the costs associated with colposcopic examinations and cervical biopsies [23].

In 2017 Granados et al. confirmed the relevance of HR HPV E6/E7 mRNA test in patients under 35 years of age in detection of HSIL lesions. The Aptima test was characterised by a slightly higher sensitivity comparing to a liquid-based cytology in the diagnosis of CIN 2+ in 5000 patients aged 25–65. Furthermore, Aptima test presented 100% sensitivity in HSIL lesion detection. On the other hand, the sensitivity of cytological examination in the group of patients with the positive Aptima HPV molecular test result was estimated at 60.6% [24].

Cadagrande et al. in a 2016 paper confirmed high specificity and negative predictive value of HR HPV

E6/E7 mRNA test in patients with LSIL lesions, or without cervical pathologies. In addition, in patients diagnosed with ASCUS and LSIL, HR HPV E6/E7 mRNA positive test was more frequent. Moreover, in all subjects with negative molecular test result, i.e. with no expression of the genetic material, the Pap test result was also within the normal range — NILM (negative for intraepithelial lesion and malignancy) [25].

CONCLUSIONS

High-grade squamous intraepithelial lesion constituted the most frequent lesion in women with abnormal cytological test results, as well as with the positive results of HR HPV E6/E7 mRNA test.

Furthermore, HR HPV E6/E7 mRNA test identifying neoplastic lesions and cervical cancer is characterised by a high relevance which is reflected by means of sensitivity and specificity. In fact, test sensitivity and specificity increased with the age in the group of patients up to 50 years old.

REFERENCES

1. WHO/ICO Information centre on HPV and cervical cancer (HPV Information Centre). Human papillomavirus and related cancers in Europe. Summary report 2010. Barcelona, Spain: WHO/ ICO HPV Information Centre. 2010.
2. Elfström KM, Arnheim-Dahlström L, von Karsa L, et al. Cervical cancer screening in Europe: Quality assurance and organisation of programmes. Eur J Cancer. 2015; 51(8): 950–968, doi: 10.1016/j.ejca.2015.03.008, indexed in Pubmed: 25817010.
3. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013; 49(6): 1374–1403, doi: 10.1016/j.ejca.2012.12.027, indexed in Pubmed: 23485231.
4. Wojciechowska Urszula, Didkowska Joanna. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii - Instytut im. Marii Skłodowskiej-Curie. http://onkologia.org.pl/raporty/ (27/11/2017).
5. Spaczyński M, Nowak-Markwitz E, Karowicz-Bilińska A, et al. Diagnostyka nowotworów narządów płciowych, Praktyczna ginekologia onkologiczna. Wielkopolskie Towarzystwo Onkologii Ginekologicznej, Poznań. 2012: 23–29.
6. Kędzia W, Karowicz-Bilińska A, Spaczyński M. Nowotwory szyjki macicy, Praktyczna ginekologia onkologiczna. Wielkopolskie Towarzystwo Onkologii Ginekologicznej, Poznań, 2012. 2012: 91–110.
7. Saslow D, Solomon D, Lawson H, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA: A Cancer Journal for Clinicians. 2012; 62(3): 147–172, doi: 10.3322/caac.21139.
8. Saslow D, Runowicz CD, Solomon D, et al. American Cancer Society Guideline for the Early Detection of Cervical Neoplasia and Cancer. CA: A Cancer Journal for Clinicians. 2002; 52(6): 342–362, doi: 10.3322/canjclin.52.6.342.
9. Goldie S, Kim J, Wright T. Cost-Effectiveness of Human Papillomavirus DNA Testing for Cervical Cancer Screening in Women Aged 30 Years or More. Obstetrics & Gynecology. 2004; 103(4): 619–631, doi: 10.1097/01.aog.0000120143.50098.c7.
10. Karsa Lv, Arbyn M, Vuyst HDe, et al. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. Papillomavirus Research. 2015; 1: 22–31, doi: 10.1016/j.pvr.2015.06.006.
11. Mayrand MH, et al. et al.. HPV testing vs Papanicolaou screening tests for cervical cancer. NE J Med. 2007; 357: 1579–1588.
12. Gatenby RA, Vincent TL. An evolutionary model of carcinogenesis. Cancer Res. 2003; 63(19): 6212–6220, indexed in Pubmed: 14559806.
13. Doorbar J. The papillomavirus life cycle. J Clin Virol. 2005; 32 Suppl 1: S7–15, doi: 10.1016/j.jcv.2004.12.006, indexed in Pubmed: 15753007.
14. Miller CS. Pleiotropic mechanisms of virus survival and persistence. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005; 100(2 Suppl): S27–S36, doi: 10.1016/j.tripleo.2005.03.017, indexed in Pubmed: 16037790.
15. Cox JT. Management of women with cervical cancer precursor lesions. Obstet Gynecol Clin North Am. 2002; 29(4): 787–816, indexed in Pubmed: 12509096.
16. Sørbye SW, Fismen S, Gutteberg TJ, et al. HPV mRNA is more specific than HPV DNA in triage of women with minor cervical lesions. PLoS One. 2014; 9(11): e112934, doi: 10.1371/journal.pone.0112934, indexed in Pubmed: 25405981.
17. Yao YL, Tian QF, Cheng B, et al. Human papillomavirus (HPV) E6/E7 mRNA detection in cervical exfoliated cells: a potential triage for HPV-positive women. J Zhejiang Univ Sci B. 2017; 18(3): 256–262, doi: 10.1631/jzus.B1600288, indexed in Pubmed: 28271661.
18. Perez Castro S, Iñarrea Fernández A, Lamas González MJ, et al. Human papillomavirus (HPV) E6/E7 mRNA as a triage test after detection of HPV 16 and HPV 18 DNA. J Med Virol. 2013; 85(6): 1063–1068, doi: 10.1002/jmv.23544, indexed in Pubmed: 23588733.
19. Fontecha N, Basaras M, Hernáez S, et al. Assessment of human papillomavirus E6/E7 oncogene expression as cervical disease biomarker. BMC Cancer. 2016; 16(1): 852, doi: 10.1186/s12885-016-2885-x, indexed in Pubmed: 27816058.
20. Rokita W, Kedzia W, Pruski D, et al. Comparison of the effectiveness of cytodiagnostics, molecular identification of HPV HR and CINtecPLUS test to identify LG SIL and HG SIL. Ginekol Pol. 2012; 83(12): 894–898, indexed in Pubmed: 23488290.
21. Pruski D, Malkowska-Walczak B, Paluszkiewicz A, et al. The incidence of cervical intraepithelial neoplasia in a population of pregnant women with an abnormal cytology. Ginekol Pol. 2017; 88(1): 20–23, doi: 10.5603/GP.a2017.0004, indexed in Pubmed: 28157250.
22. Ge Y, Christensen P, Luna E, et al. Performance of Aptima and Cobas HPV testing platforms in detecting high-grade cervical dysplasia and cancer. Cancer Cytopathol. 2017; 125(8): 652–657, doi: 10.1002/cncy.21875, indexed in Pubmed: 28574670.
23. Duvlis S, Popovska-Jankovic K, Arsova ZS, et al. HPV E6/E7 mRNA versus HPV DNA biomarker in cervical cancer screening of a group of Macedonian women. J Med Virol. 2015; 87(9): 1578–1586, doi: 10.1002/jmv.24199, indexed in Pubmed: 25880030.
24. Granados R, Tellez-Safina H, Solis I, et al. Cervical cancer screening cotesting with cytology and MRNA HPV E6/E7 yields high rates of CIN2+ lesions in young women. Diagn Cytopathol. 2017; 45(12): 1065–1072, doi: 10.1002/dc.23821, indexed in Pubmed: 28949442.
25. Casagrande DC, Ribalta JCL, Leite KD, et al. Expression of human papillomavirus E6 and E7 oncoprotein mRNA in women with low-grade squamous intraepithelial lesions or less. Genet Mol Res. 2016; 15(1), doi: 10.4238/gmr.15017916, indexed in Pubmed: 27051039.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 72–75
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0012

Influence of Human Papilloma Virus (HPV) infection on early pregnancy

Lukasz Bober, Grzegorz Guzowski, Hanna Moczulska, Piotr Sieroszewski

Medical University of Lodz, Poland

ABSTRACT

Objectives: HPV infection in early pregnancy may be a cause of miscarriage. Pregnancy significantly increases the risk of HPV infection. While ascending intrauterine infection with colonization of the trophoblast is commonly observed, descending hematogenous infection should also be considered.

The aim of the study is to assess the prevalence of HPV infection and its influence on pregnancy.

Material and methods: The study was conducted in the years 2010–2015 on a group of 143 pregnant women. The study group consisted of 84 women with abnormal course of the first trimester of pregnancy. The control group consisted of 59 women with normal pregnancy who delivered healthy neonates. Samples of cervix tissue along with samples of trophoblast or placenta were taken for the study. The presence and genotype of the HPV virus were detected using a BIOTOOL B&M Labs set. Statistical analysis was conducted using R software.

Results: The rate of HPV infection in the entire studied population was 13% (19/143): the virus was confirmed in 18% (15/84) of patients in the study group and in 7% (4/59) of the control group. HR HPV was detected in 13 patients in the study group and three patients in the control group. HR HPV infection was more frequent in patients with an abnormal course of the first trimester of pregnancy ($p = 0.03$). HR HPV trophoblast infection was found only in patients in the study group ($p = 0.02$). In two members of the study group, the HPV virus was found in the trophoblast only.

Conclusions:

1. The obtained results may confirm the presence of adverse effects of HPV infection on early pregnancy.
2. HR HPV trophoblast infection was observed only in women with 1st trimester complications.
3. The presence of HPV only in trophoblast samples in some patients may suggest a descending — hematogenous route of primary infection.

Key words: HPV; infection; early pregnancy; miscarriage

Ginekologia Polska 2019; 90, 2: 72–75

INTRODUCTION

Human Papilloma Virus (HPV) infection is one of the most common sexually transmitted diseases. It is estimated that the probability of infection is twice as high during pregnancy [1–3]. This applies to both activations of viruses that previously remained in a latent phase and new primary infections. Of known HPV types, HR types 16, 18, 31, 33 and 35 are activated most often [4, 5–7].

HPV infection in early pregnancy may be a cause of miscarriage [8, 9]. It has been proved that HPV effectively attacks syncytiotrophoblast cells [10–13]. The route of infection could be vertical ascending; however, the possibility of a descending — hematogenous infection should also be considered.

The influence of HPV infection on early pregnancy is not fully understood. Epidemiological data suggests that the HPV infection rate in European population is 8.1% [14].

Objectives

The aim of the study is to assess the prevalence of HPV infection and its influence on pregnancy.

MATERIAL AND METHODS

The study was conducted on a population of 143 pregnant women in the years 2010–2015. The study group consisted of 84 women with an abnormal course of the first trimester of pregnancy (miscarriages and missed miscarriages), who were referred to the Department of Fetal Medi-

Corresponding author:
Lukasz Bober
Medical University of Lodz, Poland
e-mail: lukaszbober@icloud.com

Table 1. Distribution of positive results in both groups (studied and control group)

	Studied group (84 patients)		Control group (59 patients)	
	Sample	(+) Result	Sample	(+) Result
High-risk HPV type (HR HPV)	trophoblast + cervix	6	placenta + cervix	0
	trophoblast only	2	placenta only	0
	cervix only	5	cervix only	3
Low-risk HPV type (LR HPV)	trophoblast + cervix	2	placenta + cervix	1
	trophoblast only	0	placenta only	0
	cervix only	0	cervix only	0
HPV — total	15 (18%)		4 (7%)	

cine and Gynaecology of the Medical University of Lodz. The control group consisted of 59 pregnant women with a normal course of pregnancy, who gave birth to healthy neonates.

A medical history focused on HPV infection risk factors was taken, and an ultrasound scan performed with pregnancy evaluation.

Samples of the cervix and trophoblast were taken from the patients in the study group, while samples of the cervix and placenta were taken after delivery from patients in the control group. The study was approved by the Bioethics Committee of the Medical University of Lodz.

Diagnosis of HPV infection was based on the detection of viral DNA in cervix and trophoblast/placenta samples by PCR. Immediately after sampling, the tissues were incubated for 12 hours at a temperature of 37°C in a reaction mixture for DNA isolation and purification. DNA concentration was measured using a fluorometer with a sensitivity of 2–1000 ng. PCR reaction was conducted using a BIOTOOLS B&M Labs set, which allows qualitative assessment of the presence of HPV DNA in the sampled material. The test detects 32 genotypes of HPV (6, 11, 13, 16, 18, 30, 31, 32, 33, 34,35, 39, 40, 42, 43, 44, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68 and 69).

Statistical analysis was conducted using R software. Results were analysed using Barnard's test and Fisher's exact test.

RESULTS

The prevalence of HPV infection in the entire studied population was 13% (19/143): 18% (15/84) of patients in the study group and 7% (4/59) in the control group (Tab. 1).

HR HPV was identified in 13 patients in the study group and in three patients in the control group (Tab. 2), and was more commonly observed in trophoblastic tissue in the study group than the control group (Tab. 3).

Table 2. Comparison of infection rate with HR HPV in patients in both groups (Barnard's test; p = 0.03)

	Studied group	Control group
HPV HR +	13	3
HPV HR –	71	56
	p = 0.03	

Table 3. Comparison of infection rate with HR HPV in trophoblast and placenta in patients in both groups (Fisher's exact test; p = 0 .02)

	Studied group	Control group
HPV HR + in trophoblast/placenta	8	0
HPV HR – in trophoblast/placenta	76	59
	p = 0.02	

HR HPV infection was found to be significantly more common in patients with an abnormal course of the first trimester of pregnancy (p = 0.03). This finding confirms previous observations that HPV infection has a negative influence on early pregnancy.

The most important finding is that HR HPV trophoblast infection was observed only in patients in the study group (p = 0.02). Coexisting infection of the cervix and the trophoblast was observed in 8/15 patients, while infection in the trophoblast alone was found in 2/15 patients (Fig. 1).

DISCUSSION

Pregnancy is characterized by an increased risk of infections, including HPV. Elevated progesterone serum concentration is used by the virus to regulate its life cycle and activity as the non-coding LCR segment of the viral genome shows high degree of structural similarity to steroid hormone receptors thus enabling a cross-reaction between the

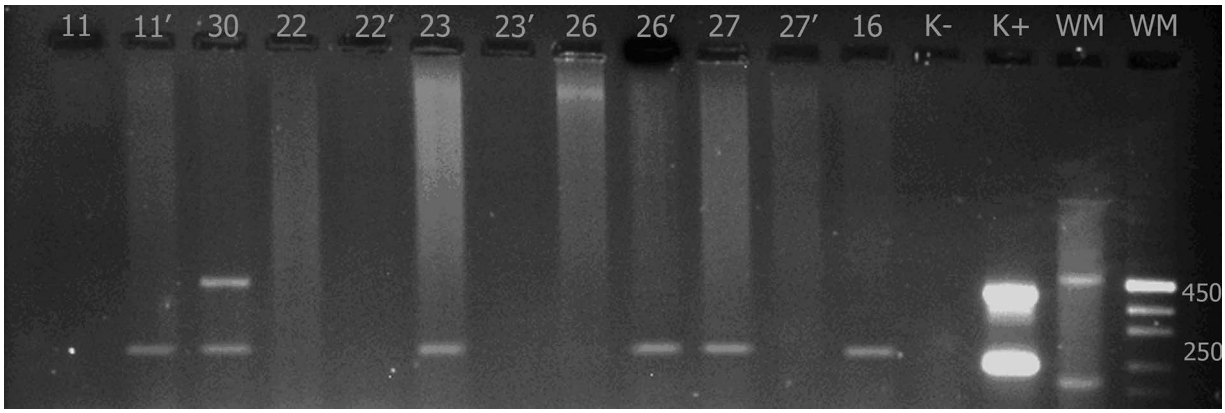


Figure 1. The results of one of the PCR analyses

ligand (i.e. steroid hormone) and glucocorticoid receptor, as well as the analogous LCR sequence. The role of the LCR is to influence the transcription and replication processes by producing signals controlling other viral genes. Furthermore, the immunological response is impaired during pregnancy, which also promotes the development of infections.

In the present study, the mean HPV infection rate among all patients was 13%: 18% in the study group and 7% in the control group. The presence of HR HPV in the trophoblast and placenta was observed significantly more frequently in the group of patients than in the control group. This finding confirms previous reports that HPV infection has a negative influence on early pregnancy [8, 9, 15–17]. An important observation in our study is that HR HPV trophoblast infection was only found in patients in the miscarriage group.

In addition, it is interesting to note that the combined presence of HPV DNA in both trophoblast and cervix was relatively rare, with only nine of 19 women that tested positive (confirmed HPV infection) presenting HPV DNA in both of these tissues. It should be emphasized that HPV was detected in the trophoblast but not the cervix in two cases. This may be accounted for by a descending hematogenous route of primary infection: the virus may choose readily-available, rapidly-dividing trophoblastic cells for infection.

There is clearly a need for further research regarding the relationship between HPV infection and abnormal course of early pregnancy leading to miscarriage or fetal defects.

In 2001, it was discovered that the entire life cycle of HPV virus can occur in trophoblastic cells, not only in keratinocytes [10]. This discovery broadened the perspective on HPV and its biology, and added further support to the proposed association between HPV infection and miscarriage. Later studies have since confirmed this relationship between HPV trophoblast infection and spontaneous miscarriage [13]. It has been established that HPV infection rate is three times

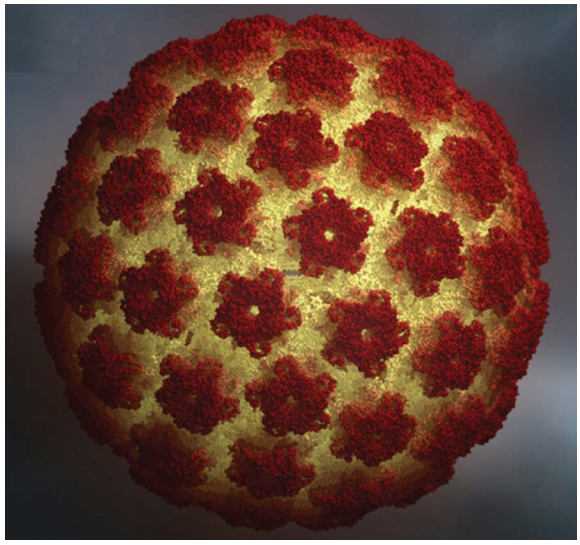


Figure 2. Human Papilloma Virus

higher in tissues from patients after miscarriage compared to those who had undergone induced or surgical abortion [8]. In other studies, the presence of HPV DNA was confirmed in 30% of tissue samples taken from patients after spontaneous miscarriage, while only 17% tested positive on cervical smear [15]. It has since been revealed that asymptomatic HR HPV infection can result in transmission of the virus to the fetus, FGR and preterm labor [1, 3] (Fig. 2).

The HPV infection rate in pregnant women in Poland varies according due to the studied patient group. While Szepietowska reports an infection the rate of 8% in women with third trimester complications [5], a 2007 study found a relatively low rate of 5%; however, this difference may be accounted for by the selection procedure, as only patients with normal cytology were enrolled in the latter study [4]. A study based on global epidemiological data reports HPV

infection rate in Europe in women with normal cytology to be 8% [14].

CONCLUSIONS

1. The obtained results may confirm the presence of adverse effects of HPV infection on early pregnancy.
2. HR HPV trophoblast infection was observed only in women with 1st trimester complications.
3. The presence of HPV only in trophoblast samples detected in some patients may suggest descending — hematogenous route of primary infection.

Acknowledgements

The study was funded by the Medical University of Lodz, Research Task No: 502-03/1-004-02/502-14-092 (Fig. 3).

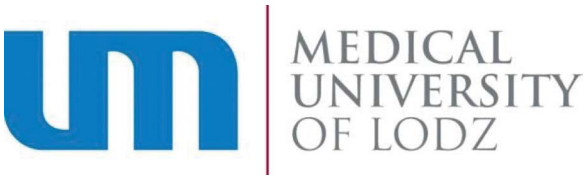


Figure 3. Medical University of Lodz — logo

REFERENCES:

1. Gomez LM, Ma Y, Ho C, et al. Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod. 2008; 23(3): 709–715, doi: 10.1093/humrep/dem404, indexed in Pubmed: 18184644.
2. Hernández-Girón C, Smith JS, Lorincz A, et al. The prevalence of high-risk HPV infection in pregnant women from Morelos, México. Salud Publica Mex. 2005; 47(6): 423–429, indexed in Pubmed: 16983987.
3. Karowicz-Bilińska A. The latent infection of human papilloma virus in pregnat woman and colonization of placenta-preliminary report. Ginekol Pol. 2007; 78(12): 966–970, indexed in Pubmed: 18411921.
4. Nowak Z, Karowicz-Bilińska A. Human papilloma virus infection in pregnant women with normal pap-smears, HPV oncogenity and risk factors. Ginekol Pol. 2007; 78(9): 678–684, indexed in Pubmed: 18159820.
5. Szepietowska M, Słodziński H, Polz-Dacewicz M, et al. Evaluation of frequency human papillomavirus infections during pregnancy. Ginekol Pol. 2002; 73(8): 662–665, indexed in Pubmed: 12369291.
6. Anderson JR. Cancer-associated human papillomavirus types are selectively increased in the cervix of women in the first trimester of pregnancy. J Womens Health. 1997; 6(4): 487–488, indexed in Pubmed: 9279838.
7. Chang-Claude J, Schneider A, Smith E, et al. Longitudinal study of the effects of pregnancy and other factors on detection of HPV. Gynecol Oncol. 1996; 60(3): 355–362, doi: 10.1006/gyno.1996.0055, indexed in Pubmed: 8774639.
8. Hermonat PL, Han L, Wendel PJ, et al. Human papillomavirus is more prevalent in first trimester spontaneously aborted products of conception compared to elective specimens. Virus Genes. 1997; 14(1): 13–17, indexed in Pubmed: 9208451.
9. Hermonat PL, Kechelava S, Lowery CL, et al. Trophoblasts are the preferential target for human papilloma virus infection in spontaneously aborted products of conception. Hum Pathol. 1998; 29(2): 170–174, indexed in Pubmed: 9490277.
10. Liu Y, You H, Chiriva-Internati M, et al. Display of complete life cycle of human papillomavirus type 16 in cultured placental trophoblasts. Virology. 2001; 290(1): 99–105, doi: 10.1006/viro.2001.1135, indexed in Pubmed: 11887784.
11. You H, Liu Y, Agrawal N, et al. Infection, replication, and cytopathology of human papillomavirus type 31 in trophoblasts. Virology. 2003; 316(2): 281–289, indexed in Pubmed: 14644610.
12. Liu Y, You H, Hermonat PL. Studying the HPV life cycle in 3A trophoblasts and resulting pathophysiology. Methods Mol Med. 2005; 119: 237–245, doi: 10.1385/1-59259-982-6:237, indexed in Pubmed: 16350406.
13. You H, Liu Y, Agrawal N, et al. Multiple human papillomavirus types replicate in 3A trophoblasts. Placenta. 2008; 29(1): 30–38, doi: 10.1016/j.placenta.2007.08.005, indexed in Pubmed: 17905430.
14. de Sanjosé S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007; 7(7): 453–459, doi: 10.1016/S1473-3099(07)70158-5, indexed in Pubmed: 17597569.
15. Manavi M, Czerwenka KF, Schurz B, et al. Latent cervical virus infection as a possible cause of early abortion. Gynakol Rundsch. 1992; 32(2): 84–87.
16. Rabreau M, Saurel J. Presence of human papilloma viruses in the deciduous membranes of early abortion products. Presse Med. 1997; 26(36): 1724, indexed in Pubmed: 9452737.
17. Genest DR, Sun D, Crum CP. Human papillomavirus in spontaneous abortion. Hum Pathol. 1999; 30(1): 109–111, indexed in Pubmed: 9923938.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 76–81
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0013

MTHFR genetic polymorphism and the risk of intrauterine fetal death in Polish women

Hubert Wolski[1, 2], Grazyna Kurzawinska[2, 3], Krzysztof Drews[2, 3], Magdalena Barlik[2, 3], Przemyslaw Kadziolka[4], Zbyszko Malewski[2], Paula Mikolajska-Ptas[1], Michal Bylewski[1], Agnieszka Seremak-Mrozikiewicz[2, 3]

[1]Division of Gynecology and Obstetrics, Podhale Multidisciplinary Hospital, Nowy Targ, Poland
[2]Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poland
[3]Laboratory of Molecular Biology in Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poland
[4]Department of Maternal and Child Health, Poznan University of Medical Sciences, Poland

ABSTRACT

Objectives: To evaluate the role of MTHFR genetic variants in the etiology of intrauterine fetal death in the second part of pregnancy at women from Polish population.

Material and methods: A case-control study was performed on a 76 women with a positive history of at least one intrauterine fetal death after 22 gestational week and 400 healthy controls. The MTHFR genotyping for polymorphic sites 667C > T, 1298A > C, 1793G > A was determined by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) method.

Results: For 1298A > C polymorphism, no statistically significant higher frequency of AA vs. AC+CC genotype was observed in the IUFD group 67.1 % vs. 55.2% in the control group (OR = 0.61, p = 0.05, p_{corr} = 0.15). We observed overrepresentation of three-locus haplotype CCG (p = 0.20; p_{corr} = 0.56) and two-locus haplotype CC (p = 0.17; p_{corr} = 0.48) in the IUFD group compared to controls.

Conclusions: There was no observed relationships in genotype frequency of MTHFR 677C > T and 1793G > A variants, however 1298A > C showed a slightly higher but statistically insignificant prevalence in IUFD compared to the controls in Polish population. Further studies on a larger population are needed.

Key words: intrauterine fetal death; MTHFR; genetic polymorphism

Ginekologia Polska 2019; 90, 2: 76–81

INTRODUCTION

Intrauterine fetal death (IUFD) is a very traumatic event for the expectant parents. The reason is often unclear, which poses the challenge of identifying it. Generally, the cause of intrauterine fetal death may be qualified as maternal, fetal or placental. Fetal reasons concern mainly multiple pregnancy, intrauterine growth restriction, fetal defects, genetic disorders and fetal hydrops of various etiology. Placental causes include umbilical disorders, preterm placental abruption, preterm premature rupture of membranes, feto-maternal hemorrhage or placental insufficiency. Finally, among some most important maternal reasons of intrauterine fetal death are post-term pregnancy (> 42 gestational week), improperly controlled diabetes and other chronic diseases, such as systemic lupus erythematosus, antiphospholipid syndrome, infections, hypertension, preeclampsia/eclampsia. This group also concerns inherited and acquired thrombophilia as well as disturbances of folate and choline cycle [1–5]. Unfortunately, the reason of intrauterine fetal death remains unknown in 25–60% cases.

Hiperhomocysteinemia in pregnant patients, apart from increasing significantly the risk of recurrent miscarriage, may also result in development of preeclampsia, fetal hypotrophy, preterm placental abruption, preterm delivery, neural tube defects, cleft palate and intrauterine fetal death.

Corresponding author:
Grazyna Kurzawinska
Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poland; Laboratory of Molecular Biology in Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poland
e-mail: gene@gpsk.am.poznan.pl

5,10-Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism that carries out the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, thus generating the active form of folate required for remethylation of homocysteine to methionine. *MTHFR* gene is highly polymorphic and most of the described genetic variants are functional [6–8]. The most commonly described nonsynonymous single-nucleotide polymorphism (SNP) variants are: alanine-to-valine substitution at codon 222 (*677C > T*, rs1801133), glutamate-to-alanine substitution at codon 429 (*1298A > C*, rs1801131) and Arg594Gln (*1793G > A*, rs2274976).

Aim of the study

The aim of the study was to investigate the association between the three MTHFR SNPs and the IUFD in Polish women, as well as to estimate the effect of haplotypes formed by SNPs localized in the same gene.

MATERIAL AND METHODS

The patients were recruited in the Department of Perinatology and Women's Diseases of Poznan University of Medical Sciences in years 2009–2015. The Bioethical Committee of Poznan University of Medical Sciences approved the study. Written informed consent was obtained from all the participants.

A total of 476 women were enrolled into the case-control study: 76 patients with at least one intrauterine fetal death after 22nd gestational week and 400 healthy controls (Tab 1). The inclusion criteria to the study group were as follows: Polish citizenship, Caucasian race, positive history of intrauterine fetal death after 22nd gestational week, unknown reason of intrauterine fetal death, no chronic diseases at patient. The following data was analyzed: age, parity, gestation age at the time of IUFD, obstetrical and general medical history, accompanying obstetrical complications. Women with known reason of IUFD (eg. hypertension, preeclampsia/eclampsia, placental abruption, infectious diseases, anemia, fetal defects) were excluded from the study group. Women with antiphospholipid syndrome, anatomical, hormonal, autoimmune, infectious disorders at the moment of joining the study group and thrombotic events or chronic diseases in medical history, also did not qualify for the study.

The control group comprised of healthy women with at least two pregnancies ended with a delivery of healthy newborn at term and no history of pregnancy complications, miscarriage, intrauterine fetal death or preeclampsia. All women from the study and control groups were taking folic acid 400 µg per day according to worldwide recommendations as to folate supplementation during pregnancy.

Genomic DNA was extracted from blood cells using QIAamp DNA Blood Mini Kit (Qiagen, Germany). The blood samples (about 5 mL) were taken from elbow vein to the Monovete tube at the opportunity of standard lab tests. Three missense single nucleotide change of the *MTHFR* gene were taken under investigation: *677C > T* (rs1801133), *1298A > C* (rs1801131) and *1793G > A* (rs2274976). Genotyping was performed using a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method previously published by Frost et al. (1995), Hanson et al. (2001) and Rady et al. (2002), respectively [6, 8, 9]. The starters used, restriction enzymes and fragment length after hydrolysis are shown in Table 2.

Statistical analysis

All statistical analyses was performed using R statistical system (version 3.5.0, *http://cran.r-project.org*). Continuous variables are presented as mean ± SD and were analyzed by independent ttest. Genotyping success rate yielded 100% for all the investigated SNPs. Distributions of genotypes were checked with a Hardy-Weinberg equilibrium test.

Two-sided p-values < 0.05 were considered statistically significant. Comparison of genotype frequency differences between groups was performed by unconditional logistic

Table 1. Description of studied polymorphisms

SNP	Sequence of primers	PCR product (bp)	Restriction enzyme	Products
677C > T (rs1801133)	5′TGA AGG AGA AGG TGT CTG CGG GA 3′ 5′ AGG ACG GTG CGG TGA GAG TG 3′	198	HinfI (Eurx)	*CC* – 198 bp *CT* – 198, 175, 23 bp *TT* – 175, 23 bp
1298A > C (rs1801131)	5′ CTT CTA CCT GAA GAG CAA GTC-3′ 5′ CAT GTC CAC AGC ATG GAG-3′	256	MboII (Eurx)	*AA* – 176, 30, 28, 22 bp *AC* – 204, 30, 28, 22 bp *CC* – 204, 30, 22 bp
1793G > A (rs2274976)	5′ CTC TGT GTG TGT GTG CAT GTG TGC G 3′ 5′ GGG ACA GGA GTG GCT CCA ACG CAG G 3′	310	MbiI (Thermo Scientific)	*GG* – 233, 77 bp *GA* – 310, 233, 77 bp *AA* – 310 bp

Table 2. Demographic and clinical characteristics of participants

Parameter		IUFD (n = 76)	Control (n = 400)	p
Age (years)	mean ± SD median min–max	30.46 ± 4.35 31 20–42	30.05 ± 3.51 30 22–44	0.07
Systolic pressure (mm Hg)	mean ± SD median min–max	105.80 ± 11.55 102.5 90–140	107.85 ± 9.82 110 80–130	0.11
Diastolic pressure (mm Hg)	mean ± SD median min–max	66.61 ± 10.49 60 55–110	68.16 ± 8.70 70 50–95	0.17
Height (cm)	mean ± SD median min–max	165.84 ± 8.58 167.0 150–183	166.43 ± 5.41 166.5 150–180	0.60
Weight (kg)	mean ± SD median min–max	62.01 ± 9.91 61 44–99	60.25 ± 9.64 58 45–110	0.15
BMI (kg/m^2)	mean ± SD median min–max	22.58 ± 3.59 21.67 18.03–38.67	21.74 ± 3.21 20.90 16.53–38.57	0.04
IUFD	one two or more	69 (90.79%) 7 (9.21%)	0 0	–
Gestational week of IUFD	mean ± SD median min–max	30.02 ± 4.92 30 22–40	–	–

regression using the SNPassoc package [10]. The Bonferroni method was used to adjust for multiple comparisons (p = 0.017 for three SNPs).

Distribution of haplotypes in the study group was compared with chi-squared tests in Haploview software version 4.2 *(https://www.broadinstitute.org/haploview/haploview)*. Permutation tests were used to correct multiple testing errors with 1000 simulations.

RESULTS

Clinical data analysis

The clinical characteristics of patients enrolled in this study were summarized in Table 2. The mean age of case and control groups was 30.46 ± 4.35 years, (median 31 years, range: 20–42 years) and 30.05 ± 3.51 years, (median 30 years, range: 22–44 years), respectively (p = 0.07). No statistically significant difference was observed in blood pressure, height or weight between the two groups. The study group had a statistically higher BMI compared to the control group (IUFD: 22.58 ± 3.59 vs. 21.57 ± 3.23 kg/m2, p = 0.04). 69 patients (90.79%) had one IUFD episode while 2 or more such episodes occurred in 7 patients (9.21%). The mean gestational age of IUFD was 30.02 ± 4.95 gestational week.

Genetic analysis

The genotype and haplotype frequencies of the three polymorphisms were in accordance with the Hardy-Weinberg equilibrium in both: the case and the control groups. The distribution of the genotypes and their ORs for association with IUFD risk are shown in Table 3. No significant association was found between the presence of MTHFR rs1801133 or rs2274976 polymorphism and the incidence of IUFD overall. The biggest statistical difference was observed for rs1801131 *A > C* polymorphism. Best-fit models for this SNP were dominant (OR = 0.61, 95% CI = 0.36–1.02; *p* = 0.05; AIC = 418.3) and overdominant (OR = 0.61, 95% CI = 0.37–1.00; *p* = 0.05; AIC = 418.2), also after Bonferroni correction $p_{corr} = 0.15$.

We have not found any difference in frequencies for investigated MTHFR gene polymorphism between patients with one or two and more IUFDs. For *667C > T* polymorphism in the seven women that had two or more IUFD, five had *677CC* genotype, one *677CT* and one *677TT*. In turn, their *1298A > C* genotype was as follows: two with *1298AA* (28.6%), four with *1298AC* (57.1%) and one woman with *1298CC* (14.3%). All seven women with two or more IUFDs had *1793GG* genotypes.

Haplotype analysis

The prevalence of *MTHFR* haplotype frequency in controls and women with intrauterine fetal death is presented in Table 4. Haplotype analysis of three (rs1801133, rs1801131, rs2274976) and two (rs1801133, rs1801131) *MTHFR* loci revealed respectively four and three haplotypes with a fre-

Table 3. Logistic regression analyses of associations between the MTHFR polymorphism and the risk of IUFD

Genotypes	IUFD n (%)	Control n (%)	OR (95% CI)	p	AIC
677C > T (rs1801133)					
CC	42 (55.3)	201 (50.2)	1.00		
CT	24 (31.6)	164 (41.0)	1.43 (0.83–2.46)	0.22	421.0
TT	10 (13.2)	35 (8.8)	0.73 (0.34–1.59)		
Dominant *(CC vs. CT + TT)*	34 (44.7)	199 (49.8)	1.22 (0.75–2.00)	0.42	421.4
Recessive *(CC + CT vs. TT)*	66 (86.8)	365(91.2)	0.63 (0.30–1.34)	0.25	420.7
Overdominant *(CC + TT vs. CT)*	52 (68.4)	236 (59.0)	1.51 (0.89–2.54)	0.12	419.6
log-Additive *(0, 1, 2)*	76 (16.0)	400 (84.0)	1.01 (0.70–1.47)	0.94	422.0
Minor allele frequency	44 (28.9)	234 (29.2)	1.01 (0.69–1.49)	0.94	840.1
1298A > C (rs1801131)					
AA	25 (32.9)	179 (44.8)	1.00		
AC	42 (55.3)	172 (43.0)	0.57 (0.33–0.98)	0.12	419.8
CC	9 (11.8)	49 (12.2)	0.76 (0.33–1.73)		
Dominant *(AA vs. AC + CC)*	51 (67.1)	221 (55.2)	0.61 (0.36–1.02)	0.05	418.3
Recessive *(AA + AC vs. CC)*	67 (88.2)	351 (87.8)	1.04 (0.49–2.22)	0.92	422.0
Overdominant *(AA + CC vs. AC)*	34 (44.7)	228 (57.0)	0.61 (0.37–1.00)	0.05	418.2
log-Additive *(0, 1, 2)*	76 (16.0)	400 (84.0)	0.78 (0.55–1.12)	0.18	420.2
Minor allele frequency	60 (39.5)	270 (33.8)	1.28 (0.9 1.83)	0.18	838.3
1793G > A (rs2274976)					
GG	69 (90.8)	368 (92.0)	1.00		
GA	7 (9.2)	31 (7.8)	0.83 (0.35–1.96)	0.70	423.5
AA	0 (0.0)	1 (0.2)	0.00		
Dominant *(GG vs. GA + AA)*	7 (9.2)	32 (8.0)	0.86 (0.36–2.02)	0.73	421.9
Recessive *(GG + GA vs. AA)*	76 (100.0)	399 (99.8)		1.00	421.7
Overdominant *(GG + AA vs. GA*	69 (90.8)	369 (92.2)	0.83 (0.35–1.96)	0.67	421.9
log-Additive *(0, 1, 2)*	76 (16.0)	400 (84.0)	0.89 (0.39–2.05)	0.70	422.0
Minor allele frequency	7 (4.6)	33 (4.1)	0.89 (0.39–2.05)	0.79	840.0

Table 4. Haplotype analysis of SNPs genotyped in the MTHFR gene

Haplotype			*Frequency (overall)*	*Frequency (case,control)*	χ^2	*p value*	*p value**
rs1801133	**rs1801131**	**rs2274976**					
C	A	G	0.361	0.316, 0.370	1.627	0.2022	0.5840
C	C	G	0.305	0.349, 0.296	1.658	0.1979	0.5640
T	A	G	0.292	0.289, 0.292	0.006	0.9400	1.0000
C	C	A	0.042	0.046, 0.041	0.073	0.7867	0.9910
C	A		0.361	0.316, 0.370	1.627	0.2022	0.5160
C	C		0.347	0.395, 0.338	1.848	0.1741	0.4750
T	A		0.292	0.289, 0.292	0.006	0.940	1.0000

*p value calculated using permutation test and a total of 1000 permutations

quency of more than 1%. Higher occurrence of *CAG* haplotype (containing all non-mutated variants) was observed in the control group (0.37 vs. 0.32 in IUFD group, $p_{corr} = 0.584$). The lowest overall p-values, namely $p = 0.20$ and $p_{corr} = 0.56$, were observed for a three-locus haplotype *CCG* and two-locus — the *MTHFR* haplotype *CC* ($p = 0.17$ and $p_{corr} = 0.48$).

These haplotypes were observed more frequently in the IUFD group than controls (0.35 and 0.40 vs. 0.30 and 0.34 at controls).

DISCUSSION

Folate and choline play a pivotal role in many cellular processes including DNA synthesis, methylation and homocysteine metabolism. Folate and choline as well as many reactions that depend on their level have been shown to be essential for proper intrauterine fetal development. Several pregnancy conditions have been indicated to correlate with lower MTHFR activity, with folate and choline deficiency and with several MTHFR and PEMT genetic polymorphisms [11, 12]. The presence of *MTHFR* gene polymorphism causes mild hypercoagulability while disturbances in coagulation cascade during pregnancy may lead to IUFD. Thus, IUFD may be a result of specifically unfavorable *MTHFR* gene polymorphism, especially when combined with some harmful environmental factors [13–15].

It is worth to underline that as far as we know, our study is first in Poland to investigate the association of SNPs and intrauterine fetal death in the second part of pregnancy.

The present study has not revealed any significant role of *677C > T* or *1793G > A MTHFR* gene polymorphism in the etiology of intrauterine fetal death. Yet, the most important observation concerns the role of *1298A > C* polymorphism, which points to increased risk of obstetrical complication in the population of Polish women (genotype *1298AC*: 55.3 vs. 43.0% in the control group, OR = 0.61, $p = 0.05$. Mutated allele *1298C*: 39.5 vs. 33.8% in the control group, OR = 1.28, $p = 0.18$).

In the study of Nurk et al. correlation between Leiden mutation, *MTHFR* gene polymorphism and some obstetrical complications was analyzed. The research involved 5874 women from Norwegian population. The presence of factor Leiden was correlated with increased rate of preeclampsia (OR = 1.63), small gestational weight (OR = 1.34) and IUFD (OR = 2.20). Variant allele for the *677C > T MTHFR* polymorphism was found to strengthen the association between *FVL* and stillbirth (OR 3.34) [16].

Silver et al. analyzed a large population-based case-control study of stillbirths (488 stillbirths and 1342 live birth mothers and 405 stillbirths and 990 live birth fetuses) testing for factor V Leiden, prothrombin 20210G > A, MTHFR 677C > T and 1298A > C, and plasminogen activating inhibitor (PAI)-1 4G/5G mutations in mother and fetus. Maternal factor V Leiden was weakly associated with stillbirth but most maternal and fetal thrombophilia, including 677C > T and 1298A > C MTHFR polymorphism, were not associated with stillbirth [17].

The aim of the study of Murakami et al. was to assess the influence of *MTHFR* genetic variants on the homocysteine serum concentration during early pregnancy. The study involved 816 women between 6 and 12 gestational week. Homocysteine concentration was significantly higher in women with *677TT* ($p < 0.0001$) genotype. Moreover, women with hyperhomocysteinemia in the further course of pregnancy developed preeclampsia ($p < 0.01$) and IUFD ($p < 0.05$) more frequently [18].

On the other hand, the study of Hefler et al. revealed no correlation between genetic variants resulting in thrombophilia (factor V Leiden, *H1299R* factor V gene, *20210G > A* factor II gene, *V34L* factor XIII, *677C > T* and *1298A>C MTHFR* gene, *455G > A* beta-fibrinogen gene, *4G/5G PAI-1*, *L33P* GPIIa, *C282Y* HFE, *R3500Q* apolipoprotein B and E2/E3/E4 apolipoproteins) and increased risk of IUFD. A total of 94 women with IUFD and 94 healthy women with a positive history of at least one normal pregnancy with live birth at term and negative history of IUFD were enrolled into the study [19].

There are also some reports indicating the role of co-existence of *677C > T* and *1298A > C MTHFR* gene polymorphisms in the etiology of obstetrical complications. A proportion of 46% of 113 Turkish women group with obstetrical complications were carriers of *677CT* and *1298AC MTHFR* heterozygotic genotypes [20]. There are also some suggestions that the presence of two mutated genotypes *677C > T* and *1298A > C* of *MTHFR* gene may lead to fetal demise at early stages of pregnancy [21, 22].

The most essential is that the analysis of MTHFR genetic polymorphism could also identify the risk group of IUFD. In light of previous observations that MTHFR polymorphism predisposes to very mild thrombophilia, LMWH prophylaxis could be considered for women in IUFD risk group. Such recommendation would be in line with the findings of Aracic et al. who showed that LMWH prophylaxis has reduced the incidence of fetal growth restriction (FGR), preterm birth (PTB) and IUFD in women carrying the MTHFR, ACE and PAI-1 genetic variants [23].

CONCLUSIONS

In conclusion, our results showed no significant relationship between MTHFR *677C > T* and *1793G > A* genotypes distribution among patients with IUFD and controls. The *1298A > C* variant showed a slightly higher but statistically insignificant prevalence of IUFD compared to the controls in Polish population.

On the basis of obtained results it might be suggested that there is no influence of investigated polymorphisms on the risk of intrauterine fetal death in Polish population. But of course, further studies on a larger population are needed. To better understand the pathobiology of IUFD, we need to know more about interactions of polymorphic variants with each other and with the environment. It is also worth to underline that probable role of MTHFR gene

polymorphisms in the etiology of intrauterine fetal death may be a result of hyperhomocysteinemia caused by these genetic variants [24–26].

REFERENCES

1. Lamont K, Scott NW, Jones GT, et al. Risk of recurrent stillbirth: systematic review and meta-analysis. BMJ. 2015; 350: h3080, doi: 10.1136/bmj.h3080, indexed in Pubmed: 26109551.
2. Man J, Hutchinson JC, Heazell AE, et al. Stillbirth and intrauterine fetal death: role of routine histopathological placental findings to determine cause of death. Ultrasound Obstet Gynecol. 2016; 48(5): 579–584, doi: 10.1002/uog.16019, indexed in Pubmed: 27781319.
3. Stillbirth Collaborative Research Network Writing Group. Association between stillbirth and risk factors known at pregnancy confirmation. JAMA. 2011; 306(22): 2469–2479, doi: 10.1001/jama.2011.1798, indexed in Pubmed: 22166606.
4. Man J, Hutchinson JC, Ashworth M, et al. Stillbirth and intrauterine fetal death: role of routine histological organ sampling to determine cause of death. Ultrasound Obstet Gynecol. 2016; 48(5): 596–601, doi: 10.1002/uog.16020, indexed in Pubmed: 27781316.
5. Barut MU, Bozkurt M, Kahraman M, et al. Thrombophilia and Recurrent Pregnancy Loss: The Enigma Continues. Med Sci Monit. 2018; 24: 4288–4294, doi: 10.12659/MSM.908832, indexed in Pubmed: 29932168.
6. Kim JiY, Kim JiW, Sung SeRa, et al. Impact of RFC1, MTHFR, and MTHFD1 polymorphism on unexplained pregnancy loss (UPL): comparative analysis of maternal and fetal components using mother-abortus paired samples. Eur J Obstet Gynecol Reprod Biol. 2018; 231: 152–157, doi: 10.1016/j.ejogrb.2018.09.017, indexed in Pubmed: 30388610.
7. van der Put NM, Gabreëls F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998; 62(5): 1044–1051, doi: 10.1086/301825, indexed in Pubmed: 9545395.
8. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10(1): 111–113, doi: 10.1038/ng0595-111, indexed in Pubmed: 7647779.
9. Hanson NQ, Aras O, Yang F, et al. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. Clin Chem. 2001; 47(4): 661–666, indexed in Pubmed: 11274015.
10. González JR, Armengol L, Guinó E et al., (2014). SNPassoc: SNPs-based whole genome association studies. R package version 1.9-2. https://CRAN.R-project.org/package=SNPassoc.
11. Drews K, Różycka A, Barlik M, et al. Polymorphic variants of genes involved in choline pathway and the risk of intrauterine fetal death. Ginekol Pol. 2017; 88(4): 205–211, doi: 10.5603/GP.a2017.0039, indexed in Pubmed: 28509322.
12. Seremak-Mrozikiewicz A, Barlik M, Różycka A, et al. Importance of polymorphic variants of phosphatidylethanolamine N-methyltransferase (PEMT) gene in the etiology of intrauterine fetal death in the Polish population. Eur J Obstet Gynecol Reprod Biol. 2018; 231: 43–47, doi: 10.1016/j.ejogrb.2018.10.021, indexed in Pubmed: 30321787.
13. Turgal M, Gumruk F, Karaagaoglu E, et al. Methylenetetrahydrofolate Reductase Polymorphisms and Pregnancy Outcome. Geburtshilfe Frauenheilkd. 2018; 78(9): 871–878, doi: 10.1055/a-0664-8237, indexed in Pubmed: 30258247.
14. Simonidesova M, Simko J, Holoman K. Defects of genes encoding inhibitors of coagulation and their application in early miscarriage aetiology. Bratisl Lek Listy. 2014; 115(11): 730–735, indexed in Pubmed: 25428544.
15. Torabi R, Zarei S, Zeraati H, et al. Combination of thrombophilic gene polymorphisms as a cause of increased the risk of recurrent pregnancy loss. J Reprod Infertil. 2012; 13(2): 89–94, indexed in Pubmed: 23926530.
16. Nurk E, Tell GS, Refsum H, et al. Factor V Leiden, pregnancy complications and adverse outcomes: the Hordaland Homocysteine Study. QJM. 2006; 99(5): 289–298, doi: 10.1093/qjmed/hcl040, indexed in Pubmed: 16613994.
17. Silver RM, Saade GR, Thorsten V, et al. Factor V Leiden, prothrombin G20210A, and methylene tetrahydrofolate reductase mutations and stillbirth: the Stillbirth Collaborative Research Network. Am J Obstet Gynecol. 2016; 215(4): 468.e1–468.e17, doi: 10.1016/j.ajog.2016.04.026, indexed in Pubmed: 27131585.
18. Murakami S, Matsubara N, Saitoh M, et al. The relation between plasma homocysteine concentration and methylenetetrahydrofolate reductase gene polymorphism in pregnant women. J Obstet Gynaecol Res. 2001; 27(6): 349–352, indexed in Pubmed: 11794822.
19. Hefler L, Jirecek S, Heim K, et al. Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. J Soc Gynecol Investig. 2004; 11(1): 42–44, indexed in Pubmed: 14706682.
20. Aytekin E, Ergun SG, Ergun MA, et al. Evaluation of GenoFlow Thrombophilia Array Test Kit in its detection of mutations in Factor V Leiden (G1691A), prothrombin G20210A, MTHFR C677T and A1298C in blood samples from 113 Turkish female patients. Genet Test Mol Biomarkers. 2014; 18(11): 717–721, doi: 10.1089/gtmb.2014.0143, indexed in Pubmed: 25153695.
21. Le Marchand L, Donlon T, Hankin JH, et al. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control. 2002; 13(3): 239–248, indexed in Pubmed: 12020105.
22. Zetterberg H, Regland B, Palmér M, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. Eur J Hum Genet. 2002; 10(2): 113–118, doi: 10.1038/sj.ejhg.5200767, indexed in Pubmed: 11938441.
23. Aracic N, Roje D, Jakus IA, et al. The Impact of Inherited Thrombophilia Types and Low Molecular Weight Heparin Treatment on Pregnancy Complications in Women with Previous Adverse Outcome. Yonsei Med J. 2016; 57(5): 1230–1235, doi: 10.3349/ymj.2016.57.5.1230, indexed in Pubmed: 27401656.
24. Jin H, Cheng H, Chen W, et al. An evidence-based approach to globally assess the covariate-dependent effect of the MTHFR single nucleotide polymorphism rs1801133 on blood homocysteine: a systematic review and meta-analysis. Am J Clin Nutr. 2018; 107(5): 817–825, doi: 10.1093/ajcn/nqy035, indexed in Pubmed: 29722849.
25. Ibrahim S, Maqbool S, Azam M, et al. CBS mutations and MTFHR SNPs causative of hyperhomocysteinemia in Pakistani children. Mol Biol Rep. 2018; 45(3): 353–360, doi: 10.1007/s11033-018-4169-9, indexed in Pubmed: 29600437.
26. Li A, Shi Y, Xu L, et al. A possible synergistic effect of MTHFR C677T polymorphism on homocysteine level variations increased risk for ischemic stroke. Medicine (Baltimore). 2017; 96(51): e9300, doi: 10.1097/MD.0000000000009300, indexed in Pubmed: 29390494.

VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 82–85
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0014

Additional secure circular suture during sphincteroplasty — preliminary results on the efficacy of fecal incontinence surgery in urogynecological patients

Aneta Adamiak-Godlewska[1], Katarzyna Skorupska[1], Katarzyna Romanek-Piva[1], Jacek Piłat[2], Tomasz Rechberger[1]

[1]II Chair and Department of Gynaecology, Medical University of Lublin, Poland

[2]I Chair and Department of General and Transplant Surgery and Nutritional Treatment, Medical University of Lublin, Poland

ABSTRACT

Objectives: The paper is a ten case series study presenting women with complex pelvic floor disorders involving fecal incontinence (FI) with stress urinary incontinence or pelvic organ prolapse.

Our study aimed at ascertaining whether FI-induced sphincteroplasty with an additional secure circular suture around the external anal sphincter muscle (EAS) may improve long term success rates.

Materials and methods: Twelve patients had scheduled urogynecological surgery and overlapping sphincteroplasty with the placement of an additional circular suture around the EAS. Of these, the status of ten women was established by way of the Cleveland Clinic Fecal Incontinence Score/Wexner Score before and about 70 months after surgery.

Results: Statistical analysis of fecal incontinence score showed that patients were not completely cured from FI, but were significantly better ($p = 0.011$).

Conclusions: A circular secure suture around the external anal sphincter in FI patients may help to improve anal sphincter function.

Key words: fecal incontinence; pelvic organ prolapse; urinary incontinence; sphincteroplasty

Ginekologia Polska 2019; 90, 2: 82–85

INTRODUCTION

Women fecal incontinence (FI) due to obstetric injuries and "end-to-end" or an "overlap" sphincteroplasty the most commonly used surgical techniques to fix the problem. Post-operative complications are generally low, but success declines with post-procedure time. Indeed, only 28% were continent at 40 months in one study [1] and predicted median time to FI relapse postsphincteroplasty is five years [2]. If an end-to-end repair is performed after a significant delay from primary injury, outcomes are poorer than an overlapping repair. Outpatient clinic data reveal FI prevalence of 5.6% in the general population and 15.9% in urogynecological patients [3]. Herein, some patients also needed surgery because of vaginal or uterine prolapse or stress urinary incontinence (SUI).

Objectives

Our study aimed at ascertaining whether FI-induced sphincteroplasty with an additional secure circular suture around the external anal sphincter muscle (EAS) may improve long term success rates.

MATERIALS AND METHODS

The study group consisted of 12 urogynecological patients afflicted with FI because of EAS injury — (Tab. 1). All patients provided informed consent to participate in the study, and the study was approved by the Medical University Ethical Board.

FI severity was evaluated via Cleveland Clinic Fecal Incontinence Score (CCFIS)/Wexner Score pre-/post-surgery. The summary score is derived from 5 parameters, the fre-

Corresponding author:
Aneta Adamiak-Godlewska
II Chair and Department of Gynecology Medical University of Lublin; 8 Jaczewski St., 20–954 Lublin, Poland
tel.: +48 605 851 796; fax: +48 7244849
e-mail: adamiak@yahoo.com

Table 1. Characteristics of the patients. Surgery procedures: 1 — T-sling; 2 — TVM anterior; 3 — TVM posterior; 4 — distal levatorplasty; 5 — sphincteroplasty with circular suture; 6 — total vaginal hysterectomy

No	Initials	Age (years)	BMI (kg/m^2)	No of vaginal deliveries	No of cc	Clinical diagnosis	Surgery	Observation time (months)
1.	SL	54	28.3	3	0	POPQ IIIaIIp, FI	1, 4, 5	79
2.	WA	31	21.1	1	0	3-rd degree obstetric injury 6 mths before, POPQ IIp, FI	4, 5	75
3.	BH	49	34.0	3	0	SUI, FI	1, 4, 5	73
4.	BP	38	25.8	1	1	SUI, FI	1, 4, 5	71
5.	TL	65	32.0	3	0	SUI, POPQ IIp, FI	1, 4, 5	71
6.	ZU	67	38.0	1 (forceps)	2	SUI, FI	1, 4, 5	68
7.	MS	66	25.0	2	0	POPQ IIIaIIp IIIc, SUI, FI	1, 4, 5, 6	68
8.	AN	50	23.8	2	0	SUI, FI	1, 4, 5	63
9.	RS	26	23.3	1	0	3-rd degree obstetric injury 7 mths before, POPQ IIp, FI	4, 5	51
10.	ZJ	76	27.0	2 (forceps)	0	SUI, FI	1, 4, 5	40
11.	BW	56	26.7	3	0	POPQ IVc, SUI, FI	1, 2, 3, 4, 5	28
12.	KS	74	24.2	3	0	POPQ IVc, SUI, FI	1, 2, 3, 4, 5	33

quency of which is ranked on a scale from 0 (= absent) to 4 (daily): incontinence to solid stool, to liquid stool, or to gas, need to wear a pad, and lifestyle changes. A score of 0 means perfect control, a score of 20 complete incontinence [4]. EAS defect was confirmed preoperatively by endoanal ultrasound. 3D volumes were obtained by using a 360° mechanical rotational probe with the automatic 3D acquisition (type 2052, Ultraview-800; BK-Medical), at a frequency of 13 MHz. All women had scheduled urogynecological surgery and overlapping sphincteroplasty [5, 6] with the placement of an additional circular suture around the EAS to secure proper tension-free healing of the muscle – Figures 1, 2, 3. Briefly, a perineal incision was used with inverted-U incision at the outer edge of the external sphincter of up to 180° to allow healthy muscle exposure (Fig. 1). The sphincter muscle was then mobilized from the fatty tissue, and the severed ends were reapproximated *en bloc* with both the internal and the external sphincter by way of placement of, typically, 4–6 slow reabsorbing sutures. In such surgery, the incision should not be extended passed 180° to avoid pudendal nerve injury. In the more common delayed repair, scar tissue which bridges the sphincter's distracted ends is maintained in situ while the sphincter muscle's severed ends are overlapped and held with long-term absorbable suture in a horizontal mattress fashion (Fig. 2). This repair lengthens the perineal body and the perineal incision comes together in a Y-shaped formation so that the incision mid-portion is left open for drainage. Next, the skin beneath the anus is incised minimally and a circular secure suture (similar to Shirodkar cervical cerclage - Ethibond-Excel 5, needle 55) is placed around the EAS (Fig. 3). A distal anterior levatorplasty is also performed to augment its function.

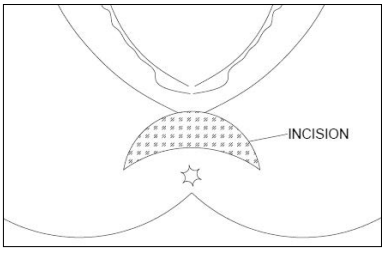


Figure 1. Sphincteroplasty — step 1. A perineal incision was used with inverted-U incision at the outer edge of the external sphincter of up to 180° to allow healthy muscle exposure

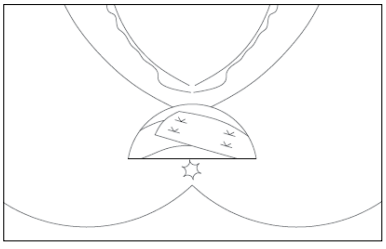


Figure 2. Sphincteroplasty — step 2. Typically, 4–6 slow reabsorbing sutures were placed

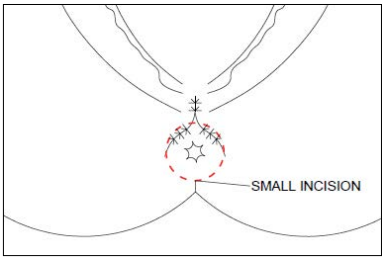


Figure 3. Sphincteroplasty and EAS circular secure suture — step 3. The skin beneath the anus is incised minimally and a circular secure is placed around the EAS

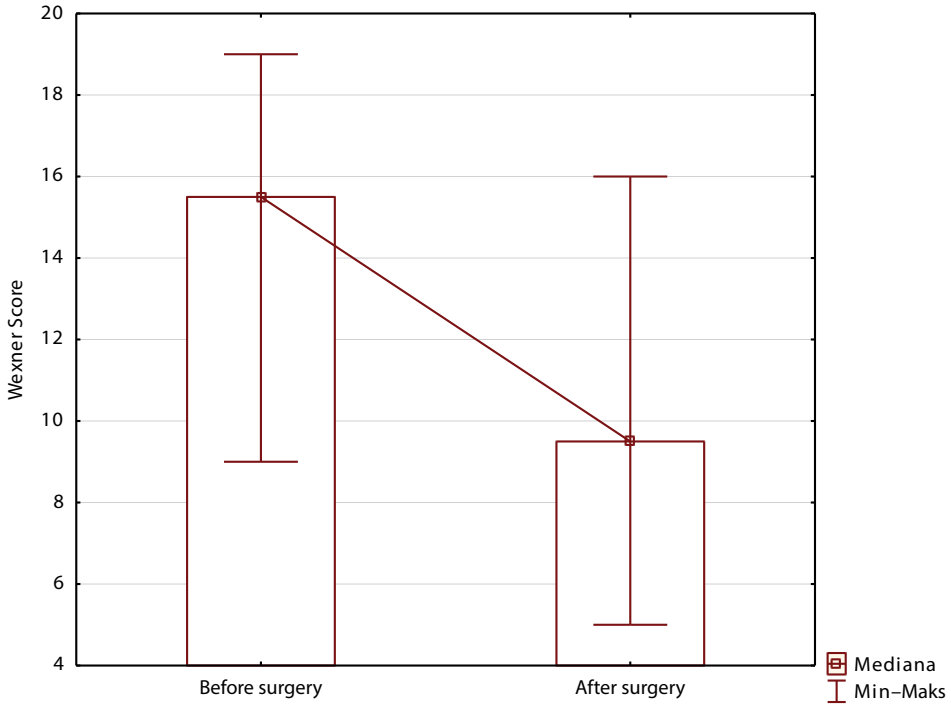


Figure 4. Wexner Score pre-/post-surgery

Post-operation, all patients were on a 5-day liquid diet with stool softeners throughout the postoperative period.

RESULTS

Final analysis of 10 women was performed (1 died in car accident, 1 lost in follow-up). Characteristics of these patients are shown in Table 2. Wexner Score pre-/postsurgery was compared using Statistica v. 12.0 software (StatSoft, Poland) (significance: $p < 0.05$). Wilcoxon signed-rank test was also applied. The patients were not completely cured from FI but were significantly better (p = 0.011) (Fig.4). Circular secure suture around EAS on endoanal ultrasound scan presents (Fig.5).

Table 2. Patient demographics — statistics

Patients (n = 10)	Me (min–max)
Age (years)	59.5 (31–76)
BMI (kg/m2)	26.4 (21.1–38)
Vaginal deliveries	2 (1–3)
Caesarean section	0 (0–2)
Observation time (months)	69.5 (33–79)

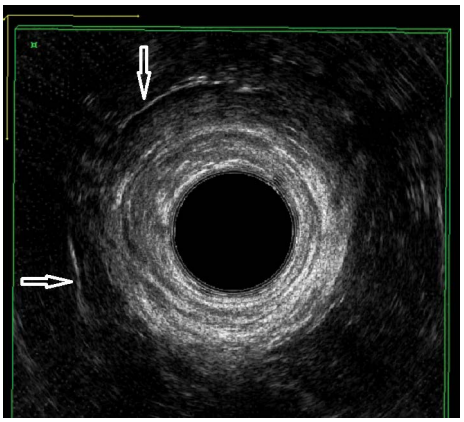


Figure 5. Endoanal ultrasound post-surgery. Arrows indicate the circular suture

DISCUSSION

Fecal incontinence, although less common than POP and SUI, is a very distressing condition also associated with substantial adverse affects the quality of life. The concomitant FI occurrence has been demonstrated in 21% of all patients with UI and/or pelvic organ prolapse [7]. Overall, Jelovsek et al. [8] report that the odds of finding both FI and UI in their cohort of 302 urogynecology patients was 6.3. In a cross-sectional survey of 174 patients with pelvic floor disorders, Bezerra et al. [9] found that patients affected by both FI and UI had significantly worse QoL scores than those with either condition alone. Combined FI and UI is also known to negatively impact patient QoL. The relationship between these three pelvic floor disorders is poorly understood and little investigated. The pivotal clinical questions whether these symptoms shared the common pathological process, risk factors, or often co-exist simply by chance is still unanswered. Therefore the complex

management of patients with such multiple pelvic floor disorders is always challenging and should be performed only in high-volume urogynecological departments.

According to our best knowledge, there are no clinical guidelines on complex surgery in patients affected by FI coexisting with UI and/or pelvic organ prolapse. Therefore we consider our study as preliminary. We decided to check the efficacy of modified by additional circular suture sphincteroplasty hoping that such suture allows proper tension-free healing of disrupted anal sphincter and will secure durability of repair as well. Numerous long-term studies have shown that the clinical efficacy of classical sphincteroplasty markedly decreases over time from 60% to even 0% [10–18]. We introduced an original additional new element to the classical overlapping sphincteroplasty namely circular secure suture around the EAS. By adding this suture we hope to increase the passive tone of the sphincter and actively secure proper tension-free healing of the repaired sphincter muscles.

In a recent Cochrane Review, Omar and Alexander [19] identified 6 trials for medications that enhance the anal sphincter tone (phenylephrine gel or sodium valproate) in patients with structurally intact anal sphincter. More people in these trials achieved full continence or improved incontinence symptoms, hence, EAS tone may help in fecal continence. The problem that arises is the adverse effects of these drugs when administered. These include localized dermatitis, burning sensation or headaches.

Many studies on sphincteroplasty have concluded that advanced age at the time of the surgery was a risk factor for long-term failure [10, 11, 15], but a recent systematic review did not find any consistent factors, including age, that were predictive for failure [17]. In addition, a recent large retrospective review of 321 women did not show any significant difference in long-term severity of FI, quality of life, or postoperative satisfaction between younger versus older women [20].

The problem of fecal incontinence coexisting with other pelvic floor dysfunction shows the need for physicians to cross disciplines or to create centres where urologists, gynecologists and colo-rectal surgeons can interact to manage complex patients [21].

CONCLUSIONS

Circular secure suture around EAS in FI patients may help to improve anal sphincters function after classical overlapping sphincteroplasty however further studies are needed.

REFERENCES

1. Wald A. Clinical practice. Fecal incontinence in adults. N Engl J Med. 2007; 356(16): 1648–1655, doi: 10.1056/NEJMcp067041, indexed in Pubmed: 17442907.
2. McManus BP, Allison S, Hernánchez-Sánchez J. Anterior sphincteroplasty for fecal incontinence: predicting incontinence relapse. Int J Colorectal Dis. 2015; 30(4): 513–520, doi: 10.1007/s00384-015-2162-5, indexed in Pubmed: 25694138.
3. Faltin DL, Sangalli MR, Curtin F, et al. Prevalence of anal incontinence and other anorectal symptoms in women. Int Urogynecol J Pelvic Floor Dysfunct. 2001; 12(2): 117–120; discussion 121, indexed in Pubmed: 11374509.
4. Jorge M, Wexner S. Etiology and management of fecal incontinence. Diseases of the Colon & Rectum. 1993; 36(1): 77–97, doi: 10.1007/bf02050307.
5. Karram MM. Chirurgiczne leczenie nietrzymania stolca. In.: Baggish MS, (eds). Atlas chirurgii ginekologicznej i anatomii miednicy. Wyd polskie. ; 2009: 971–980.
6. Garlandand B, Hull T. Overlapping repair. In: Wexner SD, Fleshman JD (eds). Master Techniques in Surgery. Colon and Rectal Surgery: Anorectal Operations. Wolters Kluwer, Philadelphia. : 2012.
7. Jackson SL, Weber AM, Hull TL, et al. Fecal incontinence in women with urinary incontinence and pelvic organ prolapse. Obstet Gynecol. 1997; 89(3): 423–427, doi: 10.1016/S0029-7844(96)00499-1, indexed in Pubmed: 9052598.
8. Jelovsek JE, Barber MD, Paraiso MF, et al. Functional bowel and anorectal disorders in patients with pelvic organ prolapse and incontinence. Am J Obstet Gynecol. 2005; 193(6): 2105–2111, doi: 10.1016/j.ajog.2005.07.016, indexed in Pubmed: 16325624.
9. Bezerra LR, Vasconcelos Neto JA, Vasconcelos CT, et al. Prevalence of unreported bowel symptoms in women with pelvic floor dysfunction and the impact on their quality of life. Int Urogynecol J. 2014; 25(7): 927–933, doi: 10.1007/s00192-013-2317-2, indexed in Pubmed: 24562788.
10. Oom DMJ, Gosselink MP, Schouten WR. Anterior sphincteroplasty for fecal incontinence: a single center experience in the era of sacral neuromodulation. Dis Colon Rectum. 2009; 52(10): 1681–1687, doi: 10.1007/DCR.0b013e3181b13862, indexed in Pubmed: 19966598.
11. Bravo Gutierrez A, Madoff RD, Lowry AC, et al. Long-term results of anterior sphincteroplasty. Dis Colon Rectum. 2004; 47(5): 727–31; discussion 731, doi: 10.1007/s10350-003-0114-6, indexed in Pubmed: 15037931.
12. Lehto K, Hyöty M, Collin P, et al. Seven-year follow-up after anterior sphincter reconstruction for faecal incontinence. Int J Colorectal Dis. 2013; 28(5): 653–658, doi: 10.1007/s00384-013-1663-3, indexed in Pubmed: 23440365.
13. Halverson AL, Hull TL. Long-term outcome of overlapping anal sphincter repair. Dis Colon Rectum. 2002; 45(3): 345–348, indexed in Pubmed: 12068192.
14. Maslekar S, Gardiner AB, Duthie GS. Anterior anal sphincter repair for fecal incontinence: Good longterm results are possible. J Am Coll Surg. 2007; 204(1): 40–46, doi: 10.1016/j.jamcollsurg.2006.10.008, indexed in Pubmed: 17189111.
15. Zutshi M, Tracey TH, Bast J, et al. Ten-year outcome after anal sphincter repair for fecal incontinence. Dis Colon Rectum. 2009; 52(6): 1089–1094, doi: 10.1007/DCR.0b013e3181a0a79c, indexed in Pubmed: 19581851.
16. Karoui S, Leroi AM, Koning E, et al. Results of sphincteroplasty in 86 patients with anal incontinence. Dis Colon Rectum. 2000; 43(6): 813–820, indexed in Pubmed: 10859083.
17. Glasgow SC, Lowry AC. Long-term outcomes of anal sphincter repair for fecal incontinence: a systematic review. Dis Colon Rectum. 2012; 55(4): 482–490, doi: 10.1097/DCR.0b013e3182468c22, indexed in Pubmed: 22426274.
18. Barisic GI, Krivokapic ZV, Markovic VA, et al. Outcome of overlapping anal sphincter repair after 3 months and after a mean of 80 months. Int J Colorectal Dis. 2006; 21(1): 52–56, doi: 10.1007/s00384-004-0737-7, indexed in Pubmed: 15830204.
19. Omar MI, Alexander CE. Drug treatment for faecal incontinence in adults. Cochrane Database Syst Rev. 2013(6): CD002116, doi: 10.1002/14651858.CD002116.pub2, indexed in Pubmed: 23757096.
20. El-Gazzaz G, Zutshi M, Hannaway C, et al. Overlapping sphincter repair: does age matter? Dis Colon Rectum. 2012; 55(3): 256–261, doi: 10.1097/DCR.0b013e31823deb85, indexed in Pubmed: 22469791.
21. Mannella P, Giannini A, Russo E, et al. Personalizing pelvic floor reconstructive surgery in aging women. Maturitas. 2015; 82(1): 109–115, doi: 10.1016/j.maturitas.2015.06.032, indexed in Pubmed: 26142653.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2019, vol. 90, no. 2, 86–92
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0015

Expression of Cripto-1 in the placenta and its role in placenta accreta and placenta previa

Lingling Jiang[1], Xiaoya Wu[2], Jianying Yan[1*], Rongxin Chen[1], Qing Han[1], Qinjian Zhang[1]

[1]Fujian Provincial Maternity and Children's Hospital, affiliated hospital of Fujian Medical University, Fuzhou, Fujian, China
[2]Fujian Provincial Hospital, affiliated hospital of Fujian Medical University, Fuzhou, Fujian, China
**Co-first author: Jianying Yan*

ABSTRACT

Objectives: This study Aims to explore the role of placental Cripto-1 in the incidence of an adherent placenta.

Material and methods: Ten pregnant women with placenta increta, 20 pregnant women with placenta previa and 30 women with normal pregnant were enrolled in this study. Reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the expression of Cripto-1 in the placenta while as the analysis of placental Cripto-1 was performed by Western blotting

Results: The placenta increta group showed higher levels of Cripto-1 in the center of the increta as compared to the non-implantation area. The level of placental Cripto-1 in the placenta increta was higher than that of the placenta accrete. The expression of placental Cripto-1 in the placenta increta and placenta previa groups was higher than that of control.

Conclusions: Placental Cripto-1 is involved in the regulation of placental tissue invasion. Additionally, excessive placental growth or penetration into the myometrium are likely to be involved in the development of placenta increta.

Key words: Cripto-1; placenta increta; placenta previa; pathogenesis; pregnancy

Ginekologia Polska 2019; 90, 2: 86–92

INTRODUCTION

Placenta increta (PA) refers to the chorionic villi invasion of the myometrium caused by an abnormal placenta. It can be divided into three categories: adhesive placenta, placenta accrete and placenta percreta, according to the depth of the invasion. Placenta increta, also referred to as pathological placenta adhesion [1], is a serious complication in the field of obstetrics. Recent researches on placenta implantation especially those exploring risk factors, diagnosis, treatment methods, and maternal and fetal outcome are lacking. To date, there are few studies on the etiology and pathogenesis of placental implantation. The mechanisms responsible for placenta accreta are not completely understood. Although it is generally accepted that decidual dysplasia, trophoblast cell invasion ability enhancement, and vascular remodeling may be of importance in elucidating the pathophysiology of placenta accreta [2], the mechanisms responsible for its occurrence are still not completely understood. Cripto-1 is a glycosylphosphatidylinositol-anchored small molecular signaling protein and a member of epidermal growth factors EGF-CFC family. In early embryonic development, Cripto-1 plays an important role in germ layer differentiation and later in each organ development. It plays a role in the activation of multiple signaling pathways regulating tumor cell proliferation, differentiation, and migration. Placental trophoblast cells and tumor cells have similar biological characteristics, and Cripto-1 can control their migration and invasion as well. So, we assume that the effect of placental trophoblast cells on cell morphology and their ability to promote placenta implantation might be an important factor in the pathogenesis of placenta increta. Higher expression of Cripto-1 can lead to an increase in cell proliferation, migration and invasion, abnormal placenta angiogenesis, enhancement of placental trophoblast cell invasive ability, and placental invasion. The present study examined the levels of Cripto-1 in pregnant women with placenta accreta.

MATERIAL AND METHODS

Enrolled subjects were pregnant women with regular antenatal care and hospitalized for cesarean section at

Corresponding author:
Jianying Yan
Fujian Provincial Maternity and Children's Hospital, affiliated hospital of Fujian Medical University, Fuzhou, Fujian, China
e-mail: yanjy2004@163.com

the Obstetric Department of Fujian Provincial Maternity and Children's Hospital from January to December 2015. All subjects were Chinese nationals. Based on clinical manifestations, ultrasonic testing, and pathological examination, 10 cases were identified as placenta accreta (PA) and 20 as placenta previa (PP). An additional thirty pregnant women who received cesarean section due to a scarred uterus, abnormal fetal position, abnormal obstetric canal, and social factors were selected to form the control group (NC). This study referred to the relevant literature [3] for the diagnostic criteria of PP and PA. Late pregnancy is defined as 28 weeks or later. Those with both PP and PA were included in the PA group. No included subject underwent labor and nor had a premature rupture of membranes at the time of cesarean section. None of the women were in active labor, had rupture of fetal membranes, or had clinical signs of infection. Patients with pregnancy complications and surgical complications were excluded from the study. All subjects had a single pregnancy. Informed consents were obtained from each subject, and the protocol for this study was approved by the local Institutional Review Board (Ethics committee of Fujian provincial maternal and child health hospital, 20140928).

Specimen collection

Immediately after cesarean section, the placental tissues were taken aseptically from the maternal surface of the placenta. Specific sites of sample collection in each group are described as follows: samples were collected from an accreta area and a none-accreta area for the PA group whereas for the PP group, placental tissues were collected from a marginal location and a central location. For the normal group, placental tissues were only collected from the central area. Tissue samples were incised at 1.0×1.0×1.0 cm under sterile conditions. Sites with hemorrhage, necrosis, and calcification were avoided. After rinsing with cold saline, placental samples were immediately put into a 1.5 mL EP tube, frozen in liquid nitrogen and subsequently transferred into sterile tubes for storage at -70°C until assayed. Repeated freezing and thawing were avoided.

Main materials

The real-time fluorescence quantification PCR assay was purchased from Applied Biosystems (ABI), while nucleic acid and protein quantitative determination apparatus, and low-temperature high-speed centrifugal machine were from Thermo. The horizontal nucleic acid electrophoresis apparatus was from the Beijing Liuyi Biotechnology Co., Ltd. The gel-imaging scanner was from BIO-Rad, USA. The rabbit anti-human Cripto-1 monoclonal antibody was purchased from Abcam. The rabbit anti-human GAPDH polyclonal antibody, horseradish peroxidase-conjugated anti-rabbit secondary antibody, and high-sensitivity chemiluminescence assay kit were from Kangwei Shiji Biotechnology Company, Beijing. The PCR primers were from Beijing Dingguo Changsheng Biotechnology company and SYBR Green I (10x) was from Genview.

Immunohistochemistry

After dehydration and paraffin embedding, tissues were sliced into 3μm thick sections and adsorbed on adhesion slides for hot repair (88°C for 10 min). The slides were dewaxed, hydrated with graded ethanol and immersed in sodium citrate solution under high-temperature conditions for 1–2 min for antigen repair. After cooling, the slides were washed with Tris-buffered saline (TBS). A 30% hydrogen peroxide solution was used to block endogenous peroxidase for 10 min followed by incubation with sheep serum for 30 min at room temperature and binding with the nonspecific antibody. Monoclonal mouse anti-human antibodies against Cripto-1 (Abcam, USA) were added to the slides at a dilution of 1:100 and incubated at 4°C overnight. Biotinylated rabbit anti-mouse antibody (Abcam, USA) was used at a dilution of 1:100 for 30 min at room temperature. After washing with TBS, the specimens were stained with diaminobenzidine, and hematoxylin and coverslipped for microscopic observation. Phosphate buffer solution instead of the primary antibody was used as a negative control.

Western blot

Tissue samples were first washed with PBS three times then washed with lymphocyte lysis buffer. Proteins were purified with the addition of the extraction buffer. Protein concentration was measured by the BCA assay. According to the results of the protein quantification, the corresponding volume of total protein and 5x protein gel electrophoresis buffer were mixed and the protein denatured for 10 min at 95°C. The gel was pre-electrophoresed for 10 min at a constant 80V until the leading edge of the bromophenol blue reached the separation gel. The voltage was adjusted to provide a constant 120V until the dye reached the bottom of the separation gel. The protein was transferred at a constant 110 V for 1 h. Membranes were blocked for two hours at room temperature in Tris-buffered saline-Tween-20 (TBS-T) containing 10% skimmed milk. Membranes were then incubated overnight with the appropriate primary antibody (a rabbit monoclonal antibody against Cripto-1, *Abcam* Company, UK) diluted in TBS-T with 3% BSA at 4°C. Membranes were then incubated with secondary antibodies (an anti-rabbit IgG antibody, Beijing Dingguo Changsheng Biotechnology Company, China) for one hour at room temperature after washing with TBS-T. Blots were washed three times with TBS-T, and the detection was performed using the BM Chemiluminescence system. After exposure, membranes were stained with Amido Black Staining Solution to calculate variations in protein content among samples. Densitometric analysis of band

Table 1. Comparison of the clinical situations of the three groups of pregnant women

Group	No. of Cases	Age (Y)	Gestational Weeks (Week)	Number of Pregnancies (Times)	Number of Uterine Surgeries (Times)	Hospital Stay (Day)
PA	10	31.7 ± 6.6	35.3 ± 4.0*	2.7 ± 1.8*	0.7 ± 0.7	8.0 ± 4.3*
PP	20	30.3 ± 5.8	36.2 ± 2.5*	1.5 ± 1.5	0.4 ± 0.8	6.5 ± 2.9
CON	30	30.2 ± 3.8	39.2 ± 1.0	1.0 ± 0.8	0.5 ± 0.6	5.4 ± 1.7

* P < 0.05

Table 2. Comparison of the clinical situations of the three groups of pregnant women

Group	Duration of Operation (Hour)	Intraoperative Hemorrhage (ML)	Placental Weight (G)	Weight of Newborn (G)	Hospitalization Costs (in RMB 10,000)
PA	1.6 ± 1.0*	1280.0 ± 1316.9*	511.0 ± 147.8*	2441 ± 913.7*	2.2 ± 1.6*
PP	0.9 ± 0.3	543 ± 179.7	577.8 ± 106.5*	2651.1 ± 644.7*	1.2 ± 0.3
CON	0.9 ± 0.3	383.3 ± 130.9	673.6 ± 147.3	3368.8 ± 528.6	0.9 ± 0.1

* P < 0.05

intensities was made with Basic Quantifier software. β-actin was used as a loading control. We compared the gray values of the Cripto-1 and β-actin protein bands.

RTQ-PCR

Trizol was used to extract total RNA from placental tissues. A spectrophotometer was used to measure the light absorption values (A), and total RNA concentration and purity were calculated from 260 nm and 280 n absorption. RNA integrity was determined by 1% agarose gel electrophoresis. A sample of 2ug of total RNA was reverse transcribed into cDNA. Real-time fluorescence quantification PCR was used for quantitative determination. The full-length sequence of the target gene mRNA was obtained from GenBank. Primer 5.0 was used to design the primer sequence. After Blast analysis, the primer sequence was shown to have the necessary specificity. This study entrusted Beijing Dingguo Changsheng Biotechnology Co., Ltd. to synthesize all the primers. β-actin: The upstream primer is 5′-ATC ATG TTT GAG ACC TTC AAC A-3′, while the downstream primer is 5′-CAT CTC TTG CTG AAG ECC A-3′. Cripto-1: The upstream primer is 5′-CAG GGA GAC TGG GTA GGA A-3′, while the downstream primer is 5′- TGT TGG GGA CAT TGA GGT A-3′. The RT-PCR reaction conditions and procedures were performed according to the instructions, and $2^{-\Delta\Delta CT}$ was used to conduct a relative quantitative analysis of the results.

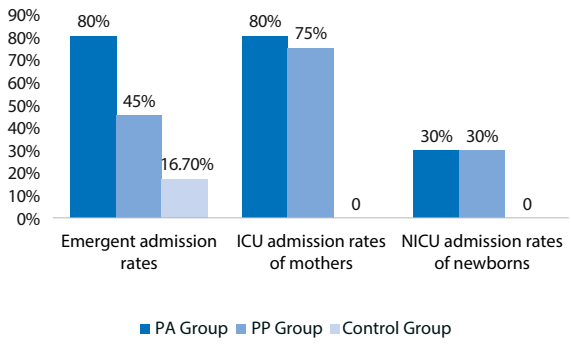


Figure 1. Analysis of hospitalization

Statistical analysis

Statistical analysis was carried out with the Statistical Package for Social Science Software (SPSS19.0). Data are presented as mean ± SD. Differences among groups were compared with one-way ANOVA. Comparison between two groups was performed with t-test or Wilcoxon and Kruskal Wallis tests, while comparison among the three groups was performed with one-way ANOVA. A P-value < 0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics of subjects from the three groups

The differences in age and number of cesarean sections between patients in the groups were not statistically significant. The number of pregnancies, hospital stays, duration of operation, intraoperative hemorrhage, and hospitalization costs of the PA group were significantly higher than those of the Control group, (P < 0.05). Gestational age, the placenta weight, and weight of the infant in both the PA and PP groups were significantly lower than those of the Control group (P < 0.05) (Tab. 1, 2).

The emergency admission rates of the PA group, PP group, and the Control group were 80%, 45%, and 16.7%, respectively. Meanwhile, the ICU admission rates of the mothers were 80% for the PA group, 75% for the PP group and 0% for the Control group. The NICU admission rates of newborns were 30%, 30%, and 0%, respectively. (Fig. 1).

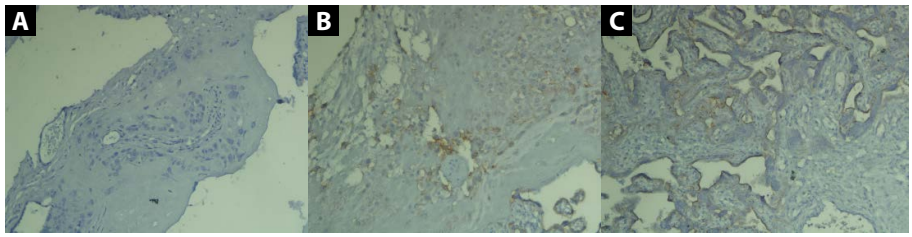


Figure 2. Expression and distribution of Cripto-1 in placental tissue
A — negative control (100x); **B** — showed the expression of Cripto-1 in placenta tissues in group PA (100x); **C** — showed the expression of Cripto-1 in placenta tissues in group PA (100x). It was positive for cytoplasmic brown staining

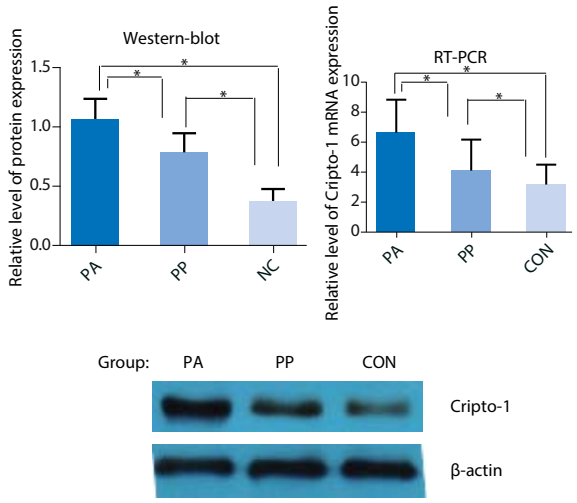


Figure 3. Cripto-1 expression of the pregnant women of the three groups

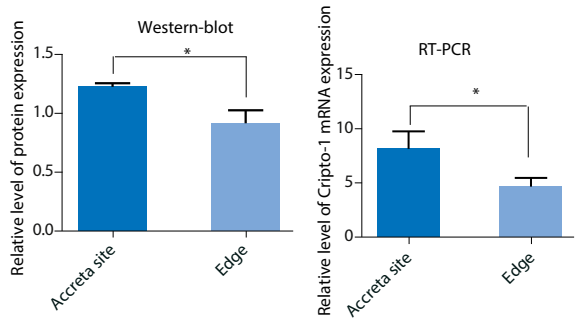


Figure 4. Cripto-1 expression of different sites of PA Group

Cripto-1 expression levels in placental tissues of the three groups

Localization of Cripto-1 in the placenta

Immunohistochemistry indicated the presence of Cripto-1 in placental tissues of subjects from the three groups. The expressions of Cripto-1 in placental tissues of PA and PP groups were higher than that of the control group. Positive staining showed brown yellow granules within the tissue (Fig. 2).

Cripto-1 expression levels in placental tissues of the three groups

The ratios of Cripto-1 protein gray value/β-actin protein gray value of the three groups were: PA group 1.054 ± 0.178, PP group 0.774 ± 0.170, and the Control group 0.369 ± 0.110. The relative content of Cripto-1 protein in the PA and PP groups were significantly higher than that of the Control group ($P < 0.05$). The Cripto-1 protein level in the PA group was much higher than that of PP group ($P < 0.05$). (Fig. 3).

Cripto-1 expression at different sites in the PA group

There was a significant difference in average levels of Cripto-1 protein between samples from the accreta area 1.206 ± 0.038 and the none-accreta area 0.901 ± 0.119 in the PA group. The relative content of Cripto-1 mRNA in the PA group was 7.971 ± 1.751 at the central area and 4.520 ± 0.852 at the none-accreta area. The difference between the two sites was statistically significant ($P < 0.05$). (Fig. 4).

Cripto-1 expression at different sites in the PP group and the Control group

The relative content of Cripto-1 protein in the PP group was 0.739 ± 0.136 at the central area and 0.810 ± 0.196 at the edge. The difference between the two sites was not statistically significant. No significant difference in the relative content of Cripto-1 protein was seen between the central area (0.368 ± 0.112) and the edge (0.370 ± 0.110) in the Control group.

The relative content of Cripto-1 mRNA in the PP group was 3.680 ± 2.227 at the central area and 4.262 ± 2.227 at the edge. The difference between the two sites was not statistically significant. The relative content of Cripto-1 mRNA in the Control group was 3.045 ± 1.447 at the central area and 3.030 ± 1.411 at the edge. The difference between the two sites was not statistically significant. (Fig. 5).

Cripto-1 expression in different types of accreta

In the PA group, the Cripto-1 expression levels were significantly higher in the placenta increta (PI) tissues than in the placenta accreta ($P < 0.05$). (Tab. 3, Fig. 6).

DISCUSSION

Placenta accreta is a serious obstetric complication. The recent years have seen a sharp increase in its incidence with the increase in uterine surgeries like cesarean section and

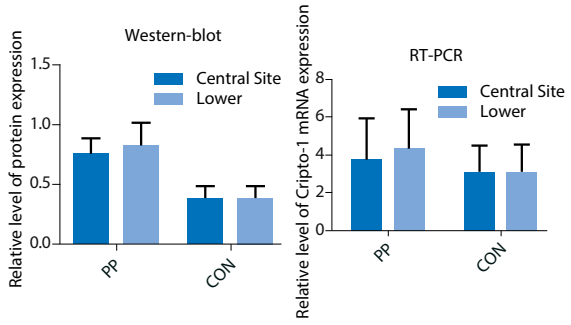


Figure 5. Cripto-1 expression of different sites of PP Group and the Control Group

Table 3. Cripto-1 expression of different accreta types

Group	Western-blot	RT-PCR
Placenta increta (n = 8)	1.071 ± 0.219	6.676 ± 2.224
Placenta accreta (n = 2)	0.984 ± 0.170	4.523 ± 1.250

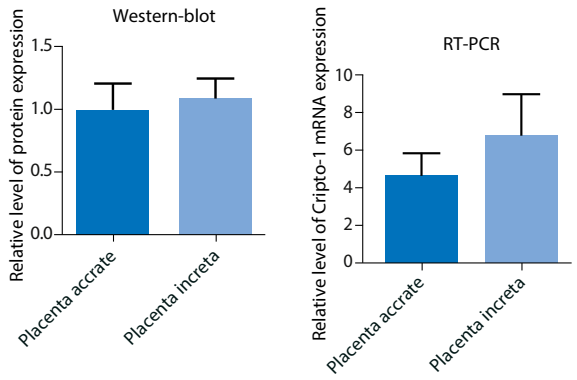


Figure 6. Cripto-1 expression of different accreta

abortion. China reports that the morbidity from placental accreta has reached 0.4% domestically [4]. Its clinical manifestations include spontaneous uterine perforation during pregnancy and no, or partial, separation of the placenta during delivery. These can lead to life-threatening complications such as hemorrhage, shock, secondary infection, or even death during childbirth. It is reported that the rate of hysterectomy caused by PA has reached 77.8% currently making PA a leading cause of hysterectomy due to massive bleeding [5]. The mortality rate of pregnant women due to PA has reached 7%. The clinical data of this study indicate that the gestational age at delivery in the PA group is lower than that of the Control group. Additionally, the length of hospitalization, the duration of operation, the occurrence of intraoperative hemorrhage, hospitalization costs, emergency admission rate, and ICU admission rate are also higher in the PA group. Newborns in the PA group have been shown to have a lower birth weight, a higher NICU admission rate, and premature birth rate. Comparison within the emergency admission cases has shown that planned delivery can reduce the amount of bleeding, the incidence of other complications, and the length of an ICU stay. PA not only represents a serious health threat to mother and child but also increases the costs of medical services and is a great economic burden on families.

At present, the etiology of PA is still not fully understood, and multiple factors are implicated in its pathogenesis. It is of tremendous significance to be able to clarify its pathogenesis, develop early detection means and interventional methods to avoid its complications. Recent research has indicated that decidual maldevelopment, enhanced trophoblast invasion, and abnormal vascular remodeling may be important causes [6].

The results of this study found that, compared with the Control group, the PA group had higher expressions levels of Cripto-1. Moreover, the expression levels varied by anatomic sites. Cripto-1 expression level at the central area is much higher than that in the none-accreta area. This is indicative of enhancement of trophoblast invasion and excessive placental invasion. The PP group also showed higher levels of Cripto-1 expression when compared to the Control group. However, the difference between Cripto-1 expressions in the central site and the edge in the PP group is not statistically significant. Excessive trophoblast invasion was not observed in the PP group even though Cripto-1 expressions varied according to the sampling site. This might be due to lower Cripto-1 expressions in the PP group as compared to the PA group. The cell signal intensity was low and did not reach the threshold of excessive trophoblast invasion in the PP group. There is also the possibility that the up-regulation of Cripto-1 expression is only one of the conditions for the enhancement of trophoblast invasion. There are other vital factors to be explored that might affect excessive trophoblast invasion. Only two cases of placenta accreta were collected in this study. Western-blot results indicated that Cripto-1 expression levels in placental tissues are significantly higher in subjects with placenta increta than those with placenta accreta.

During normal pregnancy, trophoblast cells shift from epithelial phenotype to mesenchymal phenotype via EMT and differentiate into extravillous trophoblast cells with strong invasive ability. The EVTS migrate to the uterine decidua, infiltrating along the maternal spiral artery in a retrograde direction, gradually replacing vascular endothelial cells, and transforming into non-invasive cell phenotypes like endothelial trophoblasts and myometrial multinuclear giant cells via MET. During a normal pregnancy, the invasive ability of trophoblast cells is confined within a certain time and space. Therefore, the invasion is limited in depth.

The Cripto-1 gene was first found and separated by Ciccodicola et al. in the human teratoma NTERA2/D1 cell line cDNA library in 1989 [7]. Cripto-1 is expressed at a higher level during early embryonic development but is weakly expressed or not detected in the placenta in the second and the third trimesters of normal pregnancy. The up-regulation of Cripto-1 may destroy normal EMT and MET regulatory mechanisms of trophoblast cells and activate abnormal signal pathways to strengthen the duration and intensity of trophoblast invasion.

Consistent with other studies, the results of this study indicate that the expression levels of Cripto-1 protein and mRNA in both the PA and PP groups are higher than in the Control group. Bandeira et al. [8] adopted an immunohistochemical technique to determine the Cripto-1 expression in the placenta during the third trimester of pregnancy. They have reported that Cripto-1 expression in the PA group was significantly higher than in the Control group and that the expression level increases along with the depth of accreta. Additionally, they have put forward for the first time the idea that EVT is the main cell expressing Cripto-1 and that With the morphological changes of EVT at the site of accreta, trophoblast cells clustered together clone-like or were dispersedly distributed. They have exhibited a greater cell volume with scattered cells showing migrational characteristics and a star-shaped cytoplasm or the presence of long-axis protrusions. The number of multinucleated giant cells in the myometrium was significantly reduced. Kim et al. [9] found that the thickness of the ETV cell layer in the accreta area was significantly higher than that of a normal placenta. In the third trimester of normal pregnancy, EVT cells showed no proliferative activity and demonstrated a low apoptotic index. Moreover, the differentiation of many multinuclear giant cells from trophoblast cells at their final stage was detected. Trophoblast cells invading the myometrium and expressing high levels of Cripto-1 in EVT is consistent with Cripto-1 promoting the migration and invasion of trophoblast cells.

A high-level of expression of Cripto-1 may mediate several mechanisms thus enhancing the invasive ability of trophoblast cells and participating in the pathogenesis of placenta accreta. The increased expression of Cripto-1 in trophoblast cells may interact with the ability of the TGF-β family to regulate classic EMT transcription factors. The transcription and translation of Snail, Twist, and Slug will promote EMT [10]. In contrast, for PA, the EMT of trophoblast cells does not develop toward tumor formation. There may be a complex regulatory network that limits EMT within a controllable range. The wnt/β-catenin signaling pathway plays a crucial role in promoting the migration and invasion of trophoblast cells. In the Cripto-1 gene promoter area, there is a binding area for T-cell factor/lymphoid enhancer factor (Tcf/Lef) which is regulated by the Wnt/β-catenin signal pathway. Up-regulated Cripto-1 can also serve as a Wnt 11 co-receptor in the cell membrane and, together with Glypican-4 and Frizzled 7, activate β-catenin. The β-catenin in the cytoplasm can enter into the nucleus and, as a coactivator of specific DNA-binding protein transcription in the nucleus, upregulate Cripto-1 expression but also regulate the expression of c-Myc and cyclin D1 (factors related to cell adhesion and cell morphological changes). The migration and invasion of the EVTS are enhanced through a complex cell regulatory network and interaction with expressed factors. Previous studies have found that E-cadherin participates in placenta accreta by regulating trophoblast invasion [11]. Cripto-1 activates the Wnt/β-catenin signal pathway, reduces free β-catenin in the cytoplasm, affects the interaction between β-catenin and E-cadherin as well as the number and stability of cadherin-catenin complexes, a connecting structure of cell adhesion, and increases cell migration and invasion.

Cripto-1 promotes the differentiation of HUVECs into vascular-like structures in vitro and increases the microvessel density of MCF-7 tumors in nude mice [12]. Furthermore, by regulating a specific signaling pathway, it can promote vascular remodeling and neovascularization in PA. Members of the Cripto family can interact with glucose regulatory protein 78, promoting the activation of both TGF-beta and Src/MAPK/PI3K signaling pathways. Its downstream signaling molecules TGF-beta, a bone morphogenetic protein, and Akt are involved in the regulation of natural killer cells and Sertoli cell differentiation [13]. Natural killer cells infiltrate the decidual tissue and promote abnormal vascular remodeling by regulating trophoblast cells which in turn invade the uterus to replace the endothelial cells and the membrane integrity of the uterine spiral artery [14]. MMPs also play a crucial role in vascular remodeling. Cripto-1 promotes MMP2 production via the TGF-beta body/Cripto-1/Smad2 signaling pathway [15]. Cripto-1 participation in placenta increta may be through vascular remodeling.

CONCLUSIONS

In summary, the pathogenesis of placenta accreta may include decidua maldevelopment, enhanced trophoblast invasion, and abnormal vascular remodeling. Upregulation of Cripto-1 expression strengthens trophoblast invasion. Excessive placental invasion may penetrate the myometrium and lead to the development of placenta accreta. Therefore, the role of Cripto-1 in the promotion of vascularization during the development of placenta accreta is worth being further explored.

Acknowledgments

This work was supported by the Key Clinical Specialty Discipline Construction Fujian, P.R.C. (2015 No. 593). And thanks to the people who helped me during specimen collection.

Conflicts of interest

All authors declared no potential conflicts of interest related to the authorship and publication of this article.

REFERENCES

1. Lim BH, Palacios-Jaraquemada JM. The morbidly adherent placenta--a continuing diagnostic and management challenge. BJOG. 2015; 122(12): 1673, doi: 10.1111/1471-0528.13561, indexed in Pubmed: 26498304.
2. Garmi G, Salim R. Epidemiology, etiology, diagnosis, and management of placenta accreta. Obstet Gynecol Int. 2012; 2012: 873929, doi: 10.1155/2012/873929, indexed in Pubmed: 22645616.
3. Leveno KJ, Cunningham FG, Norman FG, et al. Williams Manual of Obstetrics, 24 st edition. McGraw-Hill Education - Europe .
4. Dunjin C, Chunhong S. Placenta Accreta.Chunhong S. ed. Hunan Science and Technology Press, Changsha : 1–6.
5. Sakinci M, Kuru O, Tosun M, et al. Clinical analysis of emergency peripartum hysterectomies in a tertiary center. Clin Exp Obstet Gynecol. 2014; 41(6): 654–658, indexed in Pubmed: 25551957.
6. Parra-Herran C, Djordjevic B. Histopathology of Placenta Creta: Chorionic Villi Intrusion into Myometrial Vascular Spaces and Extravillous Trophoblast Proliferation are Frequent and Specific Findings With Implications for Diagnosis and Pathogenesis. Int J Gynecol Pathol. 2016; 35(6): 497–508, doi: 10.1097/PGP.0000000000000250, indexed in Pubmed: 26630223.
7. Ciccodicola A, Dono R, Obici S, et al. Molecular characterization of a gene of the 'EGF family' expressed in undifferentiated human NTERA2 teratocarcinoma cells. EMBO J. 1989; 8(7): 1987–1991, indexed in Pubmed: 2792079.
8. Bandeira CL, Urban Borbely A, Pulcineli Vieira Francisco R, et al. Tumorigenic factor CRIPTO-1 is immunolocalized in extravillous cytotrophoblast in placenta creta. Biomed Res Int. 2014; 2014: 892856, doi: 10.1155/2014/892856, indexed in Pubmed: 25165718.
9. Kim KR, Jun SY, Kim JY, et al. Implantation site intermediate trophoblasts in placenta cretas. Mod Pathol. 2004; 17(12): 1483–1490, doi: 10.1038/modpathol.3800210, indexed in Pubmed: 15205687.
10. Wendt MK, Allington TM, Schiemann WP. Mechanisms of the epithelial-mesenchymal transition by TGF-beta. Future Oncol. 2009; 5(8): 1145–1168, doi: 10.2217/fon.09.90, indexed in Pubmed: 19852727.
11. Qinjian Z, Jianying Y. Role of E-cadherin in Placenta Accreta [J]. Chinese Journal of Perinatal Medicine. 2015; 4: 306–309.
12. Bianco C, Strizzi L, Ebert A, et al. Role of human cripto-1 in tumor angiogenesis. J Natl Cancer Inst. 2005; 97(2): 132–141, doi: 10.1093/jnci/dji011, indexed in Pubmed: 15657343.
13. Gray PC, Vale W. Cripto/GRP78 modulation of the TGF-β pathway in development and oncogenesis. FEBS Lett. 2012; 586(14): 1836–1845, doi: 10.1016/j.febslet.2012.01.051, indexed in Pubmed: 22306319.
14. Wallace AE, Fraser R, Cartwright JE. Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. Hum Reprod Update. 2012; 18(4): 458–471, doi: 10.1093/humupd/dms015, indexed in Pubmed: 22523109.
15. Lee CC, Jan HJ, Lai JH, et al. Nodal promotes growth and invasion in human gliomas. Oncogene. 2010; 29(21): 3110–3123, doi: 10.1038/onc.2010.55, indexed in Pubmed: 20383200.

VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2019, vol. 90, no. 2, 93–99
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0016

Follow-up of children with antenatally diagnosed idiopathic polyhydramnios

Magdalena Adamczyk, Jakub Kornacki, Przemyslaw Wirstlein, Malgorzata Szczepanska, Ewa Wender-Ozegowska

Division of Reproduction, Department of Gynecology and Obstetrics, Poznan University of Medical Sciences, Poland

ABSTRACT

Objectives: The aim of our work was to assess the development of children with antenatally diagnosed idiopathic polyhydramnios, over 12 months from the end of pregnancy.

Material and methods: The study included 91 healthy pregnant patients with idiopathic polyhydramnios. Diagnostic tests results and perinatal medical history were obtained retrospectively. Parents of children were contacted by phone and by mail. The answers were obtained from 64 (70%) parents. For statistical analysis SigmaStat3.5 software was used.

Results: Ninety six percent of parents declared that in their opinion the development of children was normal. Abnormalities were found in 44% of the children. Thirty percent of neonates demonstrated mild abnormalities which may be due to organic or functional neuromuscular disorders: abnormal muscle tone, speech apparatus and development disorders, swallowing and breathing problems (manifested as vomiting, excessive regurgitation, idiopathic apnoeas).

Isolated small malformations were diagnosed in 12 (19%) children. Two children (3%) with SGA were diagnosed with genetic syndromes. More than one of the abnormalities described above were diagnosed in 14% of children. Gestational age at the time of polyhydramnios diagnosis and its severity were not prognostic factors for abnormalities. Seventy percent of newborns were male.

Conclusions: Despite the subjectively positive assessment of the development of children by the majority of parents, groups of common disorders requiring long-term follow-up have been identified. Functional disorders of the gastrointestinal tract, CNS and the group of neuromuscular disorders may be responsible for idiopathic polyhydramnios. SGA with co-existing idiopathic polyhydramnios is associated with the risk of genetic diseases. The more frequent incidence of idiopathic polyhydramnios in male fetuses requires further research.

Key words: congenital anomalies; development; functional disorders; idiopathic polyhydramnios; neuromuscular disorders

Ginekologia Polska 2019; 90, 2: 93–99

INTRODUCTION

Polyhydramnios is involved in 1–2% of pregnancies [1]. Congenital defects, aneuploidy, maternal diabetes, isoimmunization diseases, intrauterine infections, multiple pregnancies and placental tumours are causes of polyhydramnios. Prenatal diagnostics for fetal defects, chromosomal aberrations, gestational diabetes, immunization, selected viral and parasitic infections is a necessary procedure in the case of abnormal, increased amount of amniotic fluid. About 50–60% of cases of polyhydramnios have the unknown etiology [2]. This condition is called idiopathic polyhydramnios.

Polyhydramnios is associated with the risk of complications and adverse perinatological outcomes, such as: premature births, premature rupture of the membranes, premature abruptio of the placenta, prolapse of the umbilical cord, operative delivery by caesarean section or postpartum atony of the uterus.

In most cases of idiopathic polyhydramnios, the mechanism responsible for the excessive accumulation of amniotic fluid remains unclear. The underlying cause, which is organic, functional or a consequence of the impaired distribution of the amniotic fluid in the amniotic cavity, may be difficult to diagnose using available diagnostic methods. The increased production or reduced absorption of amniotic fluid, as well as a combination of both these causes [3–6] may be responsible for the disturbance of the balance of the amniotic fluid circulation.

We currently have little knowledge about the development of children with the history of idiopathic polyhydramnios during pregnancy. In these cases, prenatal counselling

Corresponding author:
Magdalena Adamczyk, University Hospital of Obstetrics and Gynecology, 33 Polna St., 60–535 Poznan
tel.: +48 61 8419302; fax: +48 61 8419625
e-mail: oddzpiv@gmail.com

is difficult, and prognosis about the health of the child is unpredictable. The lack of reliable data in this regard is due to, among others, the necessity to refer to the results of research from the last dozen or even several dozen years in which methods and diagnostic procedures were less sensitive than those used today. The rapid advancement of technology and the consequent higher quality of ultrasound devices gives us a much greater possibility of detailed imaging diagnostics, while improved neonatological procedures allow for a high standard of postnatal care.

The aim of our work is to assess the development of children within 12 months to 8 years from the end of pregnancy complicated by idiopathic polyhydramnios.

The knowledge about the fate of these children may help to identify new causes of polyhydramnios and give an opportunity to develop new prenatal diagnostic methods and appropriate obstetric and perinatal management.

MATERIAL AND METHODS

The study design was approved by the Ethics Committee of the Karol Marcinkowski University of Medical Sciences in Poznan, Poland (#701/18 on 14-06-2018).

The study included 185 patients with polyhydramnios hospitalized in the second and third trimester of pregnancy at the Gynaecology and Obstetrics Department of the Clinical Hospital in Poznan in the years 2008–2015.

The AFI (Amniotic Fluid Index) > 20 cm was accepted as the criterion for diagnosis of polyhydramnios [7]. Mild polyhydramnios was diagnosed with AFI < 30 cm and severe with AFI ≥ 30 cm.

During the hospitalization at the Department, the patients were subjected to diagnostic procedures to identify possible causes of polyhydramnios. Ultrasound examination with the biometric evaluation and the detailed assessment of fetal anatomy and the severity of polyhydramnios was performed in all patients. Imaging examinations were carried out by the same, qualified team of ultrasound specialists with many years of experience in fetal diagnostics. In justified cases, if fetal anaemia was suspected, the diagnosis was extended to include TORCH tests, serological examinations to identify alloantibodies against red blood cell antigens and the assessment of middle cerebral artery peak systolic velocity (MCA PSV). Screening and diagnostic tests for gestational diabetes were reinterpreted. All patients with a prenatally diagnosed probable cause of polyhydramnios were excluded from further analysis.

Demographic data, information about the obstetric past, current pregnancy history, diagnostic tests results and perinatal medical history were obtained retrospectively on the basis of hospitalization medical records.

Idiopathic polyhydramnios was diagnosed in 91 healthy pregnant patients enrolled in the study.

Parents of children who were qualified to participate in the study were contacted by phone and by mail to get information on the development and health status of children over 1 year of age. The medical history included defects diagnosed after birth, malformations, causes of possible prolonged hospitalization, development of children in infancy and early childhood, including motor and intellectual skills, the necessity of rehabilitation and other specialist medical care, results of diagnostic examinations, operations and hospitalizations.

The answers were obtained from 64 (70%) parents qualified to participate in the study. Eighty percent of them provided the answer by phone, the remaining 13 parents sent a letter response.

The endpoints of the study were the mortality rate, percentage of postnatally diagnosed congenital malformations, genetic syndromes, metabolic and neurological diseases, including cognitive and motor disorders.

Statistical analysis

In order to assess the significance of differences observed between variables in the study groups, *Student's t-test* was used and univariate analysis of variance (ANOVA) with the Holm-Sidak multiple repeat test for variables with normal distribution and their rank versions for variables with non-parametric distribution. In order to evaluate the differences in the distribution of non-quantitative variables, the Fisher-Freeman-Halton exact test, Fisher's exact test for 2×2 tables and Chi-square test were applied. The p value < 0.05 was accepted as statistically significant.

RESULTS

The analysis covered the development and fate of 64 children with the obstetric history of idiopathic polyhydramnios. Seventy five percent of the pregnancies were found with mild polyhydramnios (AFI < 30 cm), in 16 patients AFI exceeded 30 cm and polyhydramnios was classified as severe.

Idiopathic polyhydramnios was most often diagnosed in the second half of the third trimester (approximately in the 33rd week of gestation on average). Nearly 90% of pregnancies ended with birth at term. Seven births took place before the completion of the 37th week of gestation. The percentage of caesarean sections was 51%. The birth weight of 84% of newborns was normal, two children (3%) were diagnosed with small for gestational age (SGA) and macrosomia (> 4000 g) was found in eight newborns. Seventy percent of newborns were male. Perinatological results showing the severity of idiopathic polyhydramnios are presented in Table 1.

The mortality rate was 0%. No abnormalities were found in 56% of the children.

Table 1. Demographic and obstetric characteristics of patients with isolated polyhydramnios in relation to polyhydramnios severity

	AFI < 30 n = 48	AFI ≥ 30 n = 16	p-value
Maternal age (years) Median (range)	31 (17–40)	31 (23–37)	0.608
Gestational age at diagnosis (week) Median (range)	34 (25–39)	33 (24–40)	0.870
Early 20–29 wk	11	4	0.814
Medium 30–34 wk	15	6	
Late +35 wk	22	6	
Gestational age at delivery (week) Median (range)	39 (32–42)	40 (35–42)	0.224
Delivery mode (%)			
Spontaneous vaginal delivery	22 (45.8)	4 (25.0)	0.299
Cesarean section (CC)	22 (45.8)	11 (68.8)	
Vacuum extractor (VE)	4 (8.4)	1 (6.2)	
Newborns birth weight [g] Mean (± SD)	3546 (± 590)	3502 (± 535)	0.793
Macrosomy (> 4000 g), n (%)	6 (12.5)	2 (12.5)	1.000
Sex, n (%)			
Male	35 (72.9)	10 (62.5)	0.530
Female	13 (27.1)	6 (37.5)	

Two children were diagnosed with genetic syndromes — Down syndrome and Rubinstein-Taybi syndrome (RTS), however the diagnosis of RTS was based on the clinical presentation. The low birth weight of children with genetic syndromes was a premise to make the diagnosis of SGA.

Isolated small malformations were diagnosed in 12 children, which constituted over 19% of newborns without genetic syndromes. Congenital anomalies included various systems. Among malformations important in terms of polyhydramnios we can indicate: an unspecified anomaly of the central nervous system, mandibular defect and hypospadias.

Thirty percent of neonates born in a good condition, with the normal Apgar score demonstrated abnormalities which may be due to organic or functional neuromuscular disorders: abnormal muscle tone, speech apparatus and development disorders, swallowing and breathing problems (manifested as vomiting, excessive regurgitation, idiopathic apnoeas). The most numerous group were children requiring rehabilitation due to the abnormal muscle tone (14.5%).

Approximately 5% of children were deficient in growth and body weight adequate to a given calendar age, despite the normal birth weight; two (3%) children required intensive treatment due to haemolytic disease caused by the incompatibility in the AB0 blood group system of the isoimmunization etiology.

More than one of the abnormalities described above were diagnosed in 14% of children. Abnormalities diagnosed in children after childbirth and their frequency, broken down into proposed groups of disorders, are presented in Table 2.

In the group of healthy children and those with abnormalities, including multiple defects, gestational age at the time of polyhydramnios diagnosis and its severity were not prognostic factors. SGA was associated with the risk of numerous genetic abnormalities. The percentage of healthy children with macrosomia was similar to that of children with neuromuscular disorders. The boys made up 56% of healthy children. A disproportion in the sex distribution was clear in the group of children with neuromuscular disorders and multiple abnormalities (82 vs. 18% and 78 vs. 22%). The abnormal muscle tension and the need for rehabilitation were reported only in boys. Perinatological results in the group of healthy children and those with diagnosed abnormalities are presented in Table 3.

DISCUSSION

The causes of polyhydramnios could not be determined in 49% of patients hospitalized in our Department. This percentage is lower than described in the literature (50–70%). No significant congenital defect, which could be diagnosed prenatally, was overlooked in diagnostic imaging. An experienced team of ultrasound specialists and perinatal medicine specialists provides the high quality of prenatal diagnosis, burdened with a low risk of diagnostic failures.

Research on the fate of children with the medical history of idiopathic polyhydramnios, discussed below, used mainly medical databases, including medical opinions and diagnoses based on diagnostic procedures. The medical history taken from children's carers is burdened with the risk of misinterpreting medical information received from the primary care physician and specialists, and often proves the lack of any diagnostics. Undoubtedly, however, the subjective assessment of the child's development made by parents and their expectations are an advantage of the study. On the basis of phone conversations and letter correspondence, 96% of parents declared that in their opinion the development of children was normal. However, a detailed, rigorous analysis of the information allowed to formulate conclusions and hypotheses on the possible causes and consequences of the increased amount of amniotic fluid in pregnancy.

At the beginning, we would like to answer the question: does the normal weight of the child or its disorders, such as SGA or macrosomia accompanying idiopathic polyhydramnios may be a prognostic factor of abnormalities after delivery?

Table 2. Disorders diagnosed after birth in children with idiopathic polyhydramnios

Disorders	n			%	
Genetic diseases	2/64	Down syndrome		3.1	
		Rubinstein–Taybi syndrome			
Malformations	12/62*	Bicuspid aortic valve (BAV)		19.4	
		Atrial septal defect II (ASD II)			
		Central Nervous System (CNS) anomaly			
		Hypospadias			
		Phimosis (No. 2)			
		Duplication of renal pelvis and calyces			
		Laryngomalacia			
		Inguinal hernia			
		Mandibular defect			
		Hip dysplasia			
		Congenital cataract			
Neuromuscular disorders	17/55#	Abnormal muscle tone (rehabilitation)	8	30.9	14.5
		Speech disorders (speech therapist)	3		5.5
		Pathological vomiting / excessive regurgitation	4		7.3
		Idiopathic apnoeas	2		3.6
Deficient in growth and body weight for age (< 3 Cp)	3/62*			4.8	
Newborn hemolytic disease, AB0 main groups	2/62*			3.2	
With > 1 disorders	9/64			14.1	

*Number of children, excluding children with genetic diseases; #Number of children, excluding children with genetic diseases and those born prematurely

The percentage of fetuses with macrosomia in our study was 12.5% (8/64) and was similar to the results obtained by Yefet [8]. Yefet showed a statistically significant relationship between fetal macrosomia and idiopathic polyhydramnios, and compared the outcome to the control group without polyhydramnios (11% vs. 5%). The study conducted by Dorleijn et al. found fetal macrosomia (> 4000 g) coexisting with idiopathic polyhydramnios in 25% of cases and was associated with a good prognosis [9]. Unlike the Dorleijn's results, our analysis revealed that the risk of abnormalities after delivery in children with macrosomia was the same as in children with the normal body weight.

In all cases of SGA, that we have identified, the prognosis was poor and genetic syndromes were diagnosed in children after birth. Like in our study, Kollmann showed that too low foetus weight and polyhydramnios are risk factors for abnormalities [10].

Abnormalities in children with idiopathic polyhydramnios are a group of heterogeneous disorders. These include: genetic syndromes, developmental malformations (facial defects: cleft lip, cleft palate, Pierre-Robin syndrome), metabolic syndromes (Bartter syndrome — tubulopathy disease), neuromuscular disorders (including those genetically determined as for example myotubular *myopathy* linked to chromosome X) and others [9].

The percentage of genetic defects estimated in our study was 3.1%. This result is similar to the outcomes obtained by Yefet et al. (3.7%) and the meta-analysis published in 2015 [8, 11]. On the basis of the analysis of 1729 pregnancies with polyhydramnios of the unknown etiology, Sagi-Dain estimated the risk of chromosomal aberration at 2.8 ± 3.7%. However, he criticized the result obtained, because he believes that the real risk of chromosomal aberrations is lower than estimated. The drawback of this meta-analysis is the lack of assessment of advanced molecular techniques, including microarray methods used in the diagnosis of sub-microscopic chromosome rearrangements as a possible cause of idiopathic polyhydramnios. The genetic syndromes diagnosed after childbirth include not only those conditioned by an abnormal karyotype, but also chromosomal microrearrangements and single gene mutations (Noonan syndrome, Beckwith-Wiedermann syndrome, Shprintzen-Goldberg syndrome) [9].

The average rate of congenital defects in the general population is approximately 5% [12]. The results of our study showed higher than in the population (19.4%) percentage of defects in children with the history of idiopathic polyhydramnios. Similarly, the authors of a large retrospective study found twice the risk of congenital malformations in this group of children compared to the control group [8].

Table 3. Obstetric and neonatal characteristics of healthy children and those with abnormalities in particular groups of disorder

		Healthy	Neuromuscular disorders	Abnormal muscle tone (rehabilitation)	Speech disorders, Vomiting, Apneas	Genetic diseases	Newborn hemolytic disease, AB0	Low body mass and growth for age (< 3 Cp)	With > 1 disorder	p-value
N =		36	17	8	9	2	2	3	9	
Gestational age at diagnosis (week). Median (range)		34 (24–40)	34 (25–38)	34 (28–38)	37 (25–38)	29 (25–33)	35 (33–36)	38 (28–40)	34 (25–38)	0.839
Early 20–29 wk		7	6	3	3	1	0	1	3	
Medium 30–34 wk		12	3	2	1	1	1	0	2	> 0.05
Late +35 wk		17	8	3	6	0	1	2	4	
The severity of polyhydramnios (AFI) [cm]	< 30	25	16	8	9	1	1	8	2	> 0.05
≥ 30	11	1	0	1	1	1	1	1		
Newborns birth weight [g] Mean (± SD)		3670 ± 420*	3720 ± 416	3759 ± 432	3699 ± 402	2068 ± 131*	4170 ± 608	3297 ± 331	3439 ± 852	< 0.001*
SGA n (%)		0#	0	0	0	2 (100%)#	0	0	2 (22%)	< 0.001#
Macrosomy (> 4000 g) n (%)		5 (14%)	2 (12%)	1 (12.5)	1 (10%)	0	1 (50%)	0	1 (11%)	> 0.05
Sex n (%)	Male	20 (56%)	14 (82%)	8 (100%)	7 (70%)	1 (50%)	2 (100%)	7 (78%)	1 (33%)	
	Female	16 (44%)	3 (18%)	0	3 (30%)	1 (50%)	0	2 (22%)	2 (67%)	

*One Way ANOVA with Holm Sidak multiple comparisons method (vs. ctrl). Power = 0.952: Genetic diseases vs Healthy $p < 0.05$; #Fisher-Exact Test for 2×2 contingency tables: Genetic diseases vs Healthy $p < 0.05$; SGA — small for gestational age

They have noticed, however, that the diagnosis of over 60% of defects, which were postnatally diagnosed, was still possible during pregnancy. The analysis of defects, including the division into systems, did not allow the authors to indicate a system which is significantly more often affected than others.

The relationship between idiopathic polyhydramnios and neuromuscular disorders is interesting. Our work indicated that 14.5% of children born at term (8/55) with the normal Apgar score needed rehabilitation after delivery due to the abnormal muscle tone. Yefet also showed more than three times higher risk of neurological problems (especially motor disorders) and delayed development in children with the history of idiopathic polyhydramnios compared to the control group (9.7% vs. 3%) [8].

In 2010, Sekulić set an interesting hypothesis on the possible effect of polyhydramnios on the fetal development and ossification [13]. In this concept, the author refers to the results of studies carried out in professional divers. This hypothesis assumes that, by reducing the apparent fetal body weight (from the normal 60–80% to 10–20% in polyhydramnios) and mechanical stress, the increased amount of amniotic fluid can lead to disturbances of ossification and the abnormal bone development in the prenatal period. According to the authors, these changes can lead to disorders in the skeletal-muscular system in children, muscle tone problems with the delayed motor development as the clinical manifestation.

We can treat neuromuscular disorders as a cause of polyhydramnios in the mechanism of the abnormal amniotic fluid swallowing reflex. In generalized neuromuscular disorders, we observe the consequences of the increased amount of amniotic fluid, as in the hypothesis described above. Therefore, it should be explained whether neuromuscular disorders are the cause or effect of polyhydramnios?

The distribution of newborn sex is particularly noteworthy. A predominance of male is clearly visible in the group of examined children (70%). Our last work on non-idiopathic polyhydramnios revealed that the distribution of sex was similar to the one in population (54% vs. 46%), with a slight male predominance [14]. A similar observation was made by Stanescu et al. [15]. In their work, almost 74% of newborns with idiopathic polyhydramnios were boys. In the commentary to the above publication, Kim indicated the possible association between idiopathic polyhydramnios and nocturnal enuresis [16]. In both cases, these problems more often affect boys. This raises the question of whether it is possible to assess the daily rhythm of urinary output and whether the evaluation of bladder capacity after delivery will help to identify children at risk for nocturnal enuresis in the future?

Noteworthy in our work is that muscular tension disorders, that required rehabilitation after delivery, were found

only in boys. Sex-linked diseases are determined by the presence of alleles located on the X chromosome. Recessively inherited diseases are manifested in men because they have only one X chromosome. 1184 genes have been identified on the X chromosome.

Many genetic neurodegenerative and neuromuscular diseases, including those sex-linked, are triggered by a dynamic mutation related to the expansion of trinucleotide repeats [17]. Myotonic dystrophy is also a neurodegenerative disease. The relationship between myotonic dystrophy and idiopathic polyhydramniosis was well documented [18]. According to Rudnik-Schöneborn et al., myotonic dystrophy is the cause of 9.7% of cases of idiopathic polyhydramnios [19]. Yee C showed that 66% of children with myotonic dystrophy were diagnosed with idiopathic polyhydramnios and proposed the appropriate diagnostic procedures to be introduced in a situation of polyhydramnios coexisting with a positive family history of dystrophy, improper limb position and the reduction of fetal movements visible in the ultrasound image [20]. Children with the medical history of idiopathic polyhydramnios and deficiency in growth and body weight, suffering from idiopathic apnoeas in the long-term follow-up should undergo detailed diagnostic procedures. We failed to determine causes of these symptoms in all analysed cases.

The etiology of apnoea in premature babies is well known, but in full-term children it remains a challenge. Apnoea may be central, associated with depression of the respiratory centre or obstructive. The coexistence of apnoea and gastroesophageal reflux was reported on numerous occasions, however, the last literature review did not ultimately dispel doubts on the presence of this correlation [21]. Apnoea in infants is still one of possible causes of Sudden Infant Death Syndrome (SIDS) which occurs in the mechanism of aspiration of chyme into the lungs. In our study we did not encounter a similar situation, while in the literature the cases of SIDS were described in children with the medical history of idiopathic polyhydramnios [9].

We did not diagnose a congenital infection (from the TORCH group) in any child. Authors of many separate studies on the relationship between intrauterine infections (TORCH, including parvovirosis) and polyhydramnios agree that such correlation is strongly doubtful. Therefore, it seems that in the cases of unexplained polyhydramnios, the diagnosis for TORCH is unjustified [22].

Summing up, polyhydramnios is a symptom of many pathologies. In the absence of other obstetrical disorder or abnormality in the ultrasound image, functional pathologies should be suspected, resulting in the ineffective amniotic fluid swallowing reflex or excessive urine production. A large group of neuromuscular disorders, central nervous system problem or genetic syndromes without anatomical defects are possible causes of idiopathic polyhydramnios. Diagnostic procedures based on CNS functional imaging (functional magnetic resonance imaging fMRI), tests for myopathy (electromyography EMG) and diseases of the peripheral nervous system would allow for an objective assessment of this hypothesis [23].

It is worth noting that, except for children with genetic syndromes, the prognosis in children with idiopathic polyhydramnios in a short several-year observation is good and the accompanying diseases are mild. However, long-term observation is necessary to make a complete assessment.

CONCLUSIONS

1. Despite the subjectively positive assessment of the development of children by the majority of parents, groups of common disorders requiring long-term follow-up have been identified.
2. Functional disorders of the gastrointestinal tract, central nervous system and the group of neuromuscular disorders may be responsible for idiopathic polyhydramnios.
3. Postnatal diagnosis of neuromuscular disorders and long-term observation is necessary for the objective evaluation of their possible connection with idiopathic polyhydramniosis.
4. SGA with co-existing idiopathic polyhydramnios is associated with the risk of genetic diseases.
5. The more frequent incidence of idiopathic polyhydramnios in male fetuses requires further research.

Conflicts of interest

The authors have stated explicitly there are no conflicts of interest in connection with this article.

REFERENCES

1. Volante E, Gramellini D, Moretti S, et al. Alteration of the amniotic fluid and neonatal outcome. Acta Biomed. 2004; 75 Suppl 1: 71–75, indexed in Pubmed: 15301296.
2. Magann EF, Chauhan SP, Doherty DA, et al. A review of idiopathic hydramnios and pregnancy outcomes. Obstet Gynecol Surv. 2007; 62(12): 795–802, doi: 10.1097/01.ogx.0000290349.58707.e0, indexed in Pubmed: 18005456.
3. Lee SM, Jun JK, Lee EJ, et al. Measurement of fetal urine production to differentiate causes of increased amniotic fluid volume. Ultrasound Obstet Gynecol. 2010; 36(2): 191–195, doi: 10.1002/uog.7519, indexed in Pubmed: 20069667.
4. Touboul C, Picone O, Levaillant JM, et al. Clinical application of fetal urine production rate in unexplained polyhydramnios. Ultrasound Obstet Gynecol. 2009; 34(5): 521–525, doi: 10.1002/uog.6440, indexed in Pubmed: 19830790.
5. Brace RA, Anderson DF, Cheung CY. Fetal swallowing as a protective mechanism against oligohydramnios and polyhydramnios in late gestation sheep. Reprod Sci. 2013; 20(3): 326–330, doi: 10.1177/1933719112453510, indexed in Pubmed: 22872543.
6. Zhu X, Jiang S, Hu Y, et al. The expression of aquaporin 8 and aquaporin 9 in fetal membranes and placenta in term pregnancies complicated by idiopathic polyhydramnios. Early Hum Dev. 2010; 86(10): 657–663, doi: 10.1016/j.earlhumdev.2010.07.012, indexed in Pubmed: 20732771.
7. Harlev A, Sheiner E, Friger M, et al. Polyhydramnios and adverse perinatal outcome - what is the actual cutoff? J Matern Fetal Neonatal Med. 2014; 27(12): 1199–1203, doi: 10.3109/14767058.2013.853736, indexed in Pubmed: 24111654.

8. Yefet E, Daniel-Spiegel E. Outcomes From Polyhydramnios With Normal Ultrasound. Pediatrics. 2016; 137(2): e20151948, doi: 10.1542/peds.2015-1948, indexed in Pubmed: 26755694.
9. Dorleijn DMJ, Cohen-Overbeek TE, Groenendaal F, et al. Idiopathic polyhydramnios and postnatal findings. J Matern Fetal Neonatal Med. 2009; 22(4): 315–320, doi: 10.1080/14767050802531870, indexed in Pubmed: 19085623.
10. Kollmann M, Voetsch J, Koidl C, et al. Etiology and perinatal outcome of polyhydramnios. Ultraschall Med. 2014; 35(4): 350–356, doi: 10.1055/s-0034-1366115, indexed in Pubmed: 24729436.
11. Sagi-Dain L, Sagi S. Chromosomal aberrations in idiopathic polyhydramnios: A systematic review and meta-analysis. Eur J Med Genet. 2015; 58(8): 409–415, doi: 10.1016/j.ejmg.2015.06.010, indexed in Pubmed: 26186913.
12. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012; 366(19): 1803–1813, doi: 10.1056/NEJMoa1008095, indexed in Pubmed: 22559061.
13. Sekulić SR, Ilić D, Novakov-Mikić A. Polyhydramnios and bone development: an unexplored relationship. Med Hypotheses. 2010; 75(3): 312–314, doi: 10.1016/j.mehy.2010.03.011, indexed in Pubmed: 20347231.
14. Kornacki J, Adamczyk M, Wirstlein P, et al. Polyhydramnios - frequency of congenital anomalies in relation to the value of the amniotic fluid index. Ginekol Pol. 2017; 88(8): 442–445, doi: 10.5603/GP.a2017.0081, indexed in Pubmed: 28930371.
15. Stanescu AD, Banica R, Olaru G, et al. Idiopathic polyhydramnios and fetal gender. Arch Gynecol Obstet. 2015; 291(5): 987–991, doi: 10.1007/s00404-014-3504-1, indexed in Pubmed: 25288271.
16. Kim TH, Kim JM, Lee HH. Questions about and speculations on the incidence of idiopathic polyhydramnios by fetal gender. Arch Gynecol Obstet. 2015; 291(6): 1195, doi: 10.1007/s00404-015-3688-z, indexed in Pubmed: 25786637.
17. Richards RI, Sutherland GR. Dynamic mutations: a new class of mutations causing human disease. Cell. 1992; 70(5): 709–712, indexed in Pubmed: 1516128.
18. Güler B, Kılıç SH, Kızıltan MY. Variable genetic penetrance of myotonic dystrophy following the diagnosis of idiopathic polyhydramnios. Int J Gynaecol Obstet. 2016; 134(1): 103, doi: 10.1016/j.ijgo.2016.01.004, indexed in Pubmed: 27039051.
19. Rudnik-Schöneborn S, Zerres K. Outcome in pregnancies complicated by myotonic dystrophy: a study of 31 patients and review of the literature. Eur J Obstet Gynecol Reprod Biol. 2004; 114(1): 44–53, doi: 10.1016/j.ejogrb.2003.11.025, indexed in Pubmed: 15099870.
20. Yee C, Choi SJ, Oh SY, et al. Clinical characteristics of pregnancies complicated by congenital myotonic dystrophy. Obstet Gynecol Sci. 2017; 60(4): 323–328, doi: 10.5468/ogs.2017.60.4.323, indexed in Pubmed: 28791262.
21. Smits MJ, van Wijk MP, Langendam MW, et al. Association between gastroesophageal reflux and pathologic apneas in infants: a systematic review. Neurogastroenterol Motil. 2014; 26(11): 1527–1538, doi: 10.1111/nmo.12405, indexed in Pubmed: 25080836.
22. Pasquini L, Seravalli V, Sisti G, et al. Prevalence of a positive TORCH and parvovirus B19 screening in pregnancies complicated by polyhydramnios. Prenat Diagn. 2016; 36(3): 290–293, doi: 10.1002/pd.4769, indexed in Pubmed: 26970557.
23. Jakab A, Pogledic I, Schwartz E, et al. Fetal Cerebral Magnetic Resonance Imaging Beyond Morphology. Semin Ultrasound CT MR. 2015; 36(6): 465–475, doi: 10.1053/j.sult.2015.06.003, indexed in Pubmed: 26614130.

VIA MEDICA

REVIEW PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 100–103
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0017

Intraductal papilloma of the breast — management

Diana Hodorowicz-Zaniewska[1,2], Joanna Szpor[2,3], Pawel Basta[2,4]

[1] 1st Chair of General Surgery, Department of General, Oncological and Gastroenterological Surgery, Jagiellonian University Medical College, Cracow, Poland
[2] University Center for Breast Diseases, University Hospital in Cracow, Poland
[3] Chair of of Pathomorphology, Jagiellonian University Medical College, Cracow, Poland
[4] Chair of Gynaecology and Obstetrics, Department of Gynaecology and Oncology, Jagiellonian University Medical College, Cracow, Poland

ABSTRACT

In light of the growing availability of ultrasound testing and invasive diagnostic methods of the breast in everyday gynecologic practice, lesions of uncertain malignant potential, classified histologically as B3, have become a significant health issue. Intraductal papillomas (IPs) are the most common pathology in that group of lesions. Despite their benign histologic appearance, IPs may accompany malignant growths and the diagnosis made on the basis of biopsy material carries the risk of breast cancer (BC) underestimation. The article presents a review of the available literature on the management of patients diagnosed with intraductal papilloma at a standard core needle biopsy or vacuum-assisted core needle biopsy. The management is not uniform and depends not only on the verification technique or the accompanying pathological growths, but also on the result of clinical-pathological correlations. As it turns out, open surgical biopsy should not necessarily be recommended to every affected woman, and a growing number of sources have recently suggested that a control program would be sufficient in many cases. Thus, it is vital for gynecologists to be able to differentiate between those women who may be included in the annual ultrasound control program and those who require further surgical management.

Key words: intraductal papilloma; B3 breast lesions; core needle biopsy; vacuum-assisted core needle biopsy; underestimation; breast cancer

Ginekologia Polska 2019; 90, 2: 100–103

INTRODUCTION

Ultrasound imaging of the breast is one of the components of complex gynecologic care offered to a patient. In Poland, as in many other European countries, gynecologic care is not limited to secondary prevention of breast cancer, and the number of gynecologists who perform histopathological verification of the focal lesions using different biopsy techniques continues to grow. Thus, it is vital that they are able to interpret the histologic result of a biopsy, conduct clinical-pathological correlations, and identify those patients who require further surgical management. An intraductal papilloma (IP), a benign growth originating from the epithelium of the milk duct, is an example of a problematic histologic diagnosis. Owing to its heterogeneity and the risk for coexisting malignant growths, IP is classified as B3, i.e. a lesion of uncertain malignant potential [1].

Over the last century, the management of patients diagnosed with IPs has undergone a radical change. Initially, clinical suspicion of IP, with an accompanying sanguinous nipple discharge, was a direct indication for mastectomy. In the years to follow, segmental resection of the breast tissue, removal of the papillary tissue or isolated resection of the milk ducts, have been recommended [2]. The above-mentioned radical management was directly responsible why nipple discharge, especially sanguinous, was believed to be indicative of malignant neoplasm of the breast for decades. Nowadays, in the era of advanced diagnostic techniques and minimally invasive procedures, the number of indications for surgical management of IP has notably decreased. Apparently, open surgical biopsy should not necessarily be recommended to all patients with IP and numerous publications suggest that follow-up program would be sufficient in many cases.

Corresponding author:
Diana Hodorowicz-Zaniewska
1st Chair of General Surgery, Department of General, Oncological and Gastroenterological Surgery, Jagiellonian University Medical College, 40 Kopernika St, 31-501 Cracow, Poland; University Center for Breast Diseases, University Hospital in Cracow, 40 Kopernika St, 31-501 Cracow, Poland
e-mail: diana.hodorowicz-zaniewska@uj.edu.pl

EPIDEMIOLOGY

Intraductal papillomas (IPs) constitute approximately 10% of all benign growths within the breast [3]. Their incidence has been estimated at 2–3% among the female population, but the risk increases to 40–70% in case of nipple discharge [4]. Papillomas may develop in women of all ages, most often between 30–77 years of age [5]. Almost 90% of IPs are central, single lesions localized within the large collective ducts, usually developing in the older women and manifesting as nipple discharge (serous, serosanguinous, or sanguinous) [6]. Coexisting atypical growths are rare and IPs do not significantly increase the risk for the development of BC (breast cancer) [7]. Peripheral papillomas are significantly less common; they usually develop in young women and typically have multiple, occasionally bilateral, presentation. They may present as palpable tumors but are most often clinically silent, and are diagnosed accidentally during preventive screening tests [8]. Unlike central papillomas, they usually coexist with atypical growths, e.g. atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), lobular carcinoma *in situ* (LCIS), or even ductal carcinoma *in situ* (DCIS), and notably increase the risk for developing invasive breast cancer [8–10].

ULTRASOUND AND PATHOLOGY DIAGNOSIS

Intraductal papillomas have various imaging presentations, from hyperechogenic growths in the ducts or cysts, to hypoechogenic, well-differentiated hypervascular solid masses [5, 11]. In some cases, IP morphology may resemble that of clustered breast microcysts [12] (Fig. 1).

As far as pathology is concerned, papillary lesions include hyperplastic lesions, presumably benign or malignant tumors. Benign presumed neoplastic papillary lesions include large duct papilloma, peripheral duct papilloma, sclerosing papilloma, nipple adenoma, papilloma with low-grade neoplastic atypia and rare adenomyoepithelioma with papillary morphology [13, 14]. Structurally, they bear resemblance to papillary malignant lesions such as low-grade papillary DCIS, encapsulated papillary carcinoma or solid papillary carcinoma, and the use of immunohistochemistry is required in differential diagnosis [14]. Significant heterogeneity of papillary lesions is the reason why fine needle aspiration biopsy is not applicable in the diagnosis of IPs (high rate of false negative results), and even core needle biopsy presents a challenge for the pathologist [14]. In contrast, a vacuum-assisted core needle biopsy may generate an almost unlimited number of specimens. In terms of tissue volume, vacuum-assisted core needle biopsy is more similar to surgical biopsy than core needle biopsy, and its diagnostic accuracy reaches 98–100% [15]. Nevertheless, material fragmentation makes it impossible to determine the histologic evaluation of resection margins.

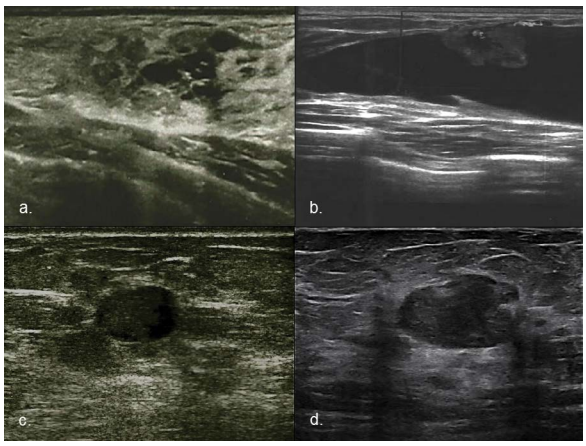
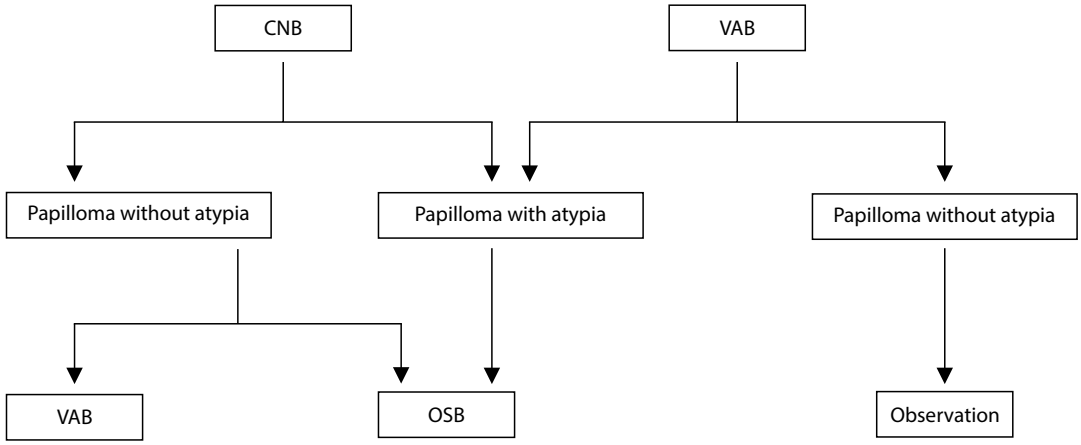


Figure 1. Intraductal breast papilloma images in ultrasound
a —clustered microcysts; **b** — hyperechogenic mural nodule in the major milk duct; **c** — hyperechogenic growth in the cyst; **d** — hypoechogenic solid mass

INTRADUCTAL PAPILLOMA DIAGNOSED AT BIOPSY — THE NEXT STEPS

The diagnosis of intraductal papilloma at biopsy requires careful management. First, sample representativeness needs to be evaluated, followed by the analysis of adequate clinical-pathological correlations, meaning that a reanalysis of the biopsy material needs to be performed to verify whether the result corresponds to the most probable diagnosis made on the basis of the imaging tests. That particular course of action is undertaken due to the significant heterogeneity of the lesions in question. In case of doubt, the biopsy should be repeated, or surgical excision should be performed.

The method of verification is the next parameter to be considered. The literature reports indicate that the diagnosis of intraductal papilloma without atypia at a standard core needle biopsy is associated with a 2.3–16% risk of BC underestimation [16, 17]. Despite the fact that some authors, in case of clinical pathological concordance, advocate in favor of follow-up program [18], most clinicians lean towards radical local excision, either with the use of vacuum-assisted core biopsy or open surgical biopsy [19, 20]. When IP is accompanied by atypical ductal hyperplasia, the risk for BC underestimation increases to 13–92%, in which case surgical excision is common practice [3, 21]. The rate of false negative results for breast cancer at core needle biopsy is distinctly lower and has been estimated at 0%–2.6% for IPs without atypia [22, 23], and at 9–21% for IPs with accompanying atypia [24, 25]. According to the current recommendations, surgical excision is still mandatory in case of atypical lesions, whereas vacuum-assisted core needle biopsy may be considered as a therapeutic option in case of IPs without atypia, on condition that a 5-year follow-up program is implemented [21, 22] (Fig. 2).



CNB–core needle biopsy, VAB–vacuum-assisted biopsy, OSB–open surgical biopsy

Figure 2. Recommendations for intraductal breast papilloma on core biopsy

CONCLUSIONS

According to the literature, after the diagnosis of IP without atypia at a standard core needle biopsy, surgical excision, either using vacuum-assisted core needle biopsy or open surgical biopsy, should be immediately recommended. Both methods have high reliability and although the biopsy method does not allow for histologic evaluation of the resection margins, lower invasiveness of the procedure is an undeniable asset. In case of primary vacuum-assisted core needle biopsy and clinical pathological concordance, the management may be considered as definitive. Still, it is vital to remember about the annual ultrasound follow-up for the affected women, not only due to the risk for recurrence but also for the development of breast cancer. Regardless of the verification method, the diagnosis of intraductal papilloma with atypia at biopsy is always and without question an indication for further surgical management.

REFERENCES:

1. Ellis IO, Humphreys S, Michell M, et al. UK National Coordinating Commmittee for Breast Screening Pathology, European Commission Working Group on Breast Screening Pathology. Best Practice No 179. Guidelines for breast needle core biopsy handling and reporting in breast screening assessment. J Clin Pathol. 2004; 57(9): 897–902, doi: 10.1136/jcp.2003.010983, indexed in Pubmed: 15333647.
2. Dennis MA, Parker S, Kaske TI, et al. Incidental treatment of nipple discharge caused by benign intraductal papilloma through diagnostic Mammotome biopsy. AJR Am J Roentgenol. 2000; 174(5): 1263–1268, doi: 10.2214/ajr.174.5.1741263, indexed in Pubmed: 10789774.
3. Boufelli G, Giannotti MA, Ruiz CA, et al. Papillomas of the breast: factors associated with underestimation. Eur J Cancer Prev. 2018; 27(4): 310–314, doi: 10.1097/CEJ.0000000000000343, indexed in Pubmed: 28296665.
4. Paterok EM, Rosenthal H, Säbel M. Nipple discharge and abnormal galactogram. Results of a long-term study (1964-1990). Eur J Obstet Gynecol Reprod Biol. 1993; 50(3): 227–234, indexed in Pubmed: 8262300.
5. Ganesan S, Karthik G, Joshi M, et al. Ultrasound spectrum in intraductal papillary neoplasms of breast. Br J Radiol. 2006; 79(946): 843–849, doi: 10.1259/bjr/69395941, indexed in Pubmed: 16641415.
6. Al Sarakbi W, Worku D, Escobar PF, et al. Breast papillomas: current management with a focus on a new diagnostic and therapeutic modality. Int Semin Surg Oncol. 2006; 3(1): 1–8, doi: 10.1186/1477-7800-3-1, indexed in Pubmed: 16417642.
7. Jacobs TW, Connolly JL, Schnitt SJ. Nonmalignant lesions in breast core needle biopsies: to excise or not to excise? Am J Surg Pathol. 2002; 26(9): 1095–1110, indexed in Pubmed: 12218567.
8. Maxwell AJ. Ultrasound-guided vacuum-assisted excision of breast papillomas: review of 6-years experience. Clin Radiol. 2009; 64(8): 801–806, doi: 10.1016/j.crad.2009.04.007, indexed in Pubmed: 19589419.
9. Bonaventure T, Cormier B, Lebas P, et al. [Benign papilloma: is US-guided vacuum-assisted breast biopsy an alternative to surgical biopsy?]. J Radiol. 2007; 88(9 Pt 1): 1165–1168, indexed in Pubmed: 17878878.
10. Haagensen CD, Bodain C, Haagensen DE. Breast carcinoma risk and detection. WB Saunders, Philadelphia 1981: 146.
11. Brookes MJ, Bourke AG. Radiological appearances of papillary breast lesions. Clin Radiol. 2008; 63(11): 1265–1273, doi: 10.1016/j.crad.2008.02.012, indexed in Pubmed: 18929044.
12. Athanasiou A, Aubert E, Vincent Salomon A, et al. Complex cystic breast masses in ultrasound examination. Diagn Interv Imaging. 2014; 95(2): 169–179, doi: 10.1016/j.diii.2013.12.008, indexed in Pubmed: 24433921.
13. Hoda S, Kaplan R. World Health Organization (WHO) Classification of Breast Tumours, 4th ed. The American Journal of Surgical Pathology. 2013; 37(2): 309–310, doi: 10.1097/pas.0b013e318273b19b.
14. Agoumi M, Giambattista J, Hayes MM. Practical Considerations in Breast Papillary Lesions: A Review of the Literature. Arch Pathol Lab Med. 2016; 140(8): 770–790, doi: 10.5858/arpa.2015-0525-RA, indexed in Pubmed: 27472236.
15. Nakano S, Sakamoto H, Ohtsuka M, et al. Evaluation and indications of ultrasound-guided vacuum-assisted core needle breast biopsy. Breast Cancer. 2007; 14(3): 292–296, indexed in Pubmed: 17690507.
16. Pareja F, Corben AD, Brennan SB, et al. Breast intraductal papillomas without atypia in radiologic-pathologic concordant core-needle biopsies: Rate of upgrade to carcinoma at excision. Cancer. 2016; 122(18): 2819–2827, doi: 10.1002/cncr.30118, indexed in Pubmed: 27315013.
17. Leithner D, Kaltenbach B, Hödl P, et al. Intraductal Papilloma Without Atypia on Image- Guided Breast Biopsy: Upgrade Rates to Carcinoma at Surgical Excision. Breast Care (Basel). 2018; 13(5): 364–368, doi: 10.1159/000489096, indexed in Pubmed: 30498423.
18. Kiran S, Jeong YJu, Nelson ME, et al. Are we overtreating intraductal papillomas? J Surg Res. 2018; 231: 387–394, doi: 10.1016/j.jss.2018.06.008, indexed in Pubmed: 30278958.
19. Khan S, Diaz A, Archer KJ, et al. Papillary lesions of the breast: To excise or observe? Breast J. 2018; 24(3): 350–355, doi: 10.1111/tbj.12907, indexed in Pubmed: 28845569.
20. Shiino S, Tsuda H, Yoshida M, et al. Intraductal papillomas on core biopsy can be upgraded to malignancy on subsequent excisional biopsy regardless of the presence of atypical features. Pathol Int. 2015; 65(6): 293–300, doi: 10.1111/pin.12285, indexed in Pubmed: 25801805.
21. Am Soc Breast Surg: Consensus guideline on concordance assessment of image-guided breast biopsies and management of borderline or high-risk le-

sions. Nov 2, 2016. https://www.breastsurgeons.org/new_layout/about/statements/PDF_Statements/Concordance_and_High%20RiskLesions.pdf.
22. Rageth CJ, O'Flynn EAm, Comstock C, et al. First International Consensus Conference on lesions of uncertain malignant potential in the breast (B3 lesions). Breast Cancer Res Treat. 2016; 159(2): 203–213, doi: 10.1007/s10549-016-3935-4, indexed in Pubmed: 27522516.
23. Mosier AD, Keylock J, Smith DV. Benign papillomas diagnosed on large-gauge vacuum-assisted core needle biopsy which span <1.5 cm do not need surgical excision. Breast J. 2013; 19(6): 611–617, doi: 10.1111/tbj.12180, indexed in Pubmed: 24102818.
24. Chang JM, Han W, Moon WK, et al. Papillary lesions initially diagnosed at ultrasound-guided vacuum-assisted breast biopsy: rate of malignancy based on subsequent surgical excision. Ann Surg Oncol. 2011; 18(9): 2506–2514, doi: 10.1245/s10434-011-1617-3, indexed in Pubmed: 21369740.
25. Saladin C, Haueisen H, Kampmann G, et al. MIBB Group. Lesions with unclear malignant potential (B3) after minimally invasive breast biopsy: evaluation of vacuum biopsies performed in Switzerland and recommended further management. Acta Radiol. 2016; 57(7): 815–821, doi: 10.1177/0284185115610931, indexed in Pubmed: 26552694.

VIA MEDICA

REVIEW PAPER / GYNECOLOGY

Ginekologia Polska
2019, vol. 90, no. 2, 104–108
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0018

Food and Drug Administration — approved molecular methods for detecting human papillomavirus infection

Katarzyna Sitarz[1, 2], Slawa Szostek[1]

[1]*Department of Virology, Chair of Microbiology, Jagiellonian University Medical College, Cracow, Poland*
[2]*Faculty of Chemistry, Jagiellonian University, Cracow, Poland*

ABSTRACT

In the world, there are many tests that allow the detection of HPV infection. These tests are based on different operating principles and have different levels of sensitivity. The first test to detect HPV infection was approved by the Food and Drug Administration in 2003. Since then, the FDA has approved five more commercial tests for this purpose, the last one in 2018. This paper discusses the principles of molecular tests to detect HPV, which have been approved by the FDA, the main differences between them, as well as their advantages and disadvantages.

Key words: HPV detection; Hybrid Capture; Cervista; COBAS; Aptima; Onclarity

Ginekologia Polska 2019; 90, 2: 104–108

INTRODUCTION

Cervical cancer is currently the fourth most common cancer in terms of both incidence as well as mortality among women in the world [1]. According to estimated data, in 2018 in the world, there were 570,000 new cases of this cancer and 311,000 deaths. However, in 28 countries, it is the most commonly diagnosed cancer among women, and in 42 countries, it is the cancer with the highest mortality rate among women [1]. In Poland, cervical cancer now ranks seventh in terms of cancer incidence and ninth as regards cancer mortality among women [2]. Main known etiological factors of cervical cancer are oncogenic types of human papillomavirus (HPV) [3]. HPV is a sexually transmitted virus. There are both high-oncogenic and low-oncogenic HPV types. The group of high-oncogenic types includes HPV –16, –18, –31, –33, –35, –39, –45, –51, –52, –56, –58 and –59 [4]. However, manufacturers of most commercial tests described here have also included the –66 and –68 types to high–risk (HR) types. In the further part of the publication, this classification will be adopted for simplification, however, these types are classified by the authors of the latest publications as probably (–66) and possibly (–68) carcinogenic [4]. The operation of molecular tests lies primarily in the detection of these types.

The important aspects in preventing the development of cervical cancer involve both cytological examination and the detection of HPV infection [5, 6]. The current guidelines of the Polish Society of Gynecologists and Obstetricians recommend performing an HPV test in case of obtaining an abnormal cytology result (ASC-US, LSIL) as an alternative to a repeat cytology test [7]. There are many methods for detecting HPV infection, which we can divide into three main groups: nucleic acid hybridization assays, signal amplification assays and nucleic acid amplification assays [5]. Until today, the Food and Drug Administration (FDA) has approved 7 tests detecting HPV infection: three signal amplification assays (Hybrid Capture™ II generation, Cervista™ HPV HR, Cervista™ HPV 16/18) and four nucleic acid amplification assays (COBAS® HPV Test, Aptima™ HPV Assay, Aptima™ HPV 16 18/45 and BD Onclarity™ HPV Assay).

HYBRID CAPTURE

The first method for the detection of HPV infection registered by the FDA (2003) is a type of solution hybridization followed by signal amplification, the Hybrid Capture II (HC2) generation method (Qiagen, Canada; former: Digene, USA) [8]. The materials to be examined are cervical swabs and biopsies [9]. The second-generation HC test allows to show the presence of 5 types of the virus with low oncogenic potential (HPV –6, –11, –42, –43, –44) and 13 types of the virus with high oncogenic potential (HPV –16, –18, –31, –33, –35, –39, –45, –51, –52, –56, –58, –59, –68).

Corresponding author:
Slawa Szostek
18 Czysta St., 31–121 Cracow, Poland
Tel.: 12 634 54 00; e-mail: slawa.szostek@uj.edu.pl

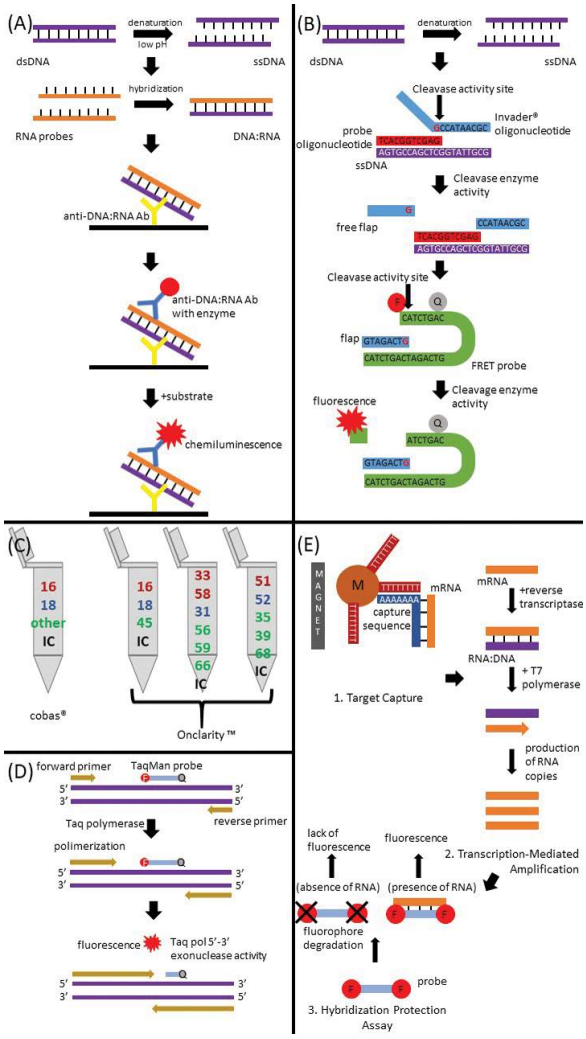


Figure 1. Principles of molecular HPV tests (description in the relevant paragraphs): (A) Hybrid Capture; (B) Cervista; (C) COBAS vs. Onclarity detection and genotyping of HPV types – different colors are different fluorescent dyes; (D) Common steps in COBAS and Onclarity; (E) APTIMA; F — fluorophore; Q — quencher; IC — internal control; M — magnetic microparticle

In the laboratory, the material is denatured in an alkaline environment [9] leading to lysis of cervical epithelial cells, viral capsid damage, release of HPV DNA and obtaining a single strand of viral DNA (Fig. 1A). The single strand of HPV DNA hybridizes with a specific RNA probe and, practically, with a mixture of RNA sequences complementary to HPV DNA of high or low oncogenic potential. The resulting RNA: DNA hybrids are captured and immobilized in wells of a microplate coated with antibodies against RNA:DNA hybrids. Then they are combined with a conjugate of anti-hybrid antibodies with alkaline phosphatase. The addition of a chemiluminescent substrate (dioxetane) to the enzyme reaction triggers the emission of light proportional to the number of hybrids. The luminous intensity is measured in the luminometer and expressed in relative light units (RLU) in relation to the emission of positive control light. In order to eliminate false negative results caused by too little material, the recommended cutoff value is 1.0 pg of viral DNA per 1 mL of the test sample, this value is similar for each of the HPV types detected [9]. This concentration is equivalent to 5,000 viral copies per assay or 100,000 copies/mL [10].

The advantages of the test are: the semi-quantitative evaluation of viral DNA in the infected cell and a relatively high sensitivity of the method, comparable to the amplification reaction [11–13]. The method allows to distinguish virus types with high and low oncogenic potential but does not specify particular genotypes. The disadvantage of the test is also the occurrence of cross-reactions between the probe detecting HPV types with high oncogenic potential and other HPV viruses, the sequence of which do not contain a probe [14]. However, increasing the cutoff value to 10 pg of viral DNA per 1 mL eliminates the majority of cross reactions, except for reactions with HPV –53 and –67 [14]. From a clinical point of view, cross-reactions of the probe detecting types with high oncogenic potential with non-oncogenic types have practically no effect on the treatment of patients with cytological changes in the cervix [15]. Another disadvantage of the test is the possibility of false negative results when using some antifungal creams and contraceptive jelly [9].

The literature also reports on the existence of the Hybrid Capture III test, which was intended to remedy the cross-reactivity problem by using labeled oligonucleotides instead of antibodies against the DNA:RNA hybrids used in the HC2 test [16]. Although it has come into commercial use, the small literature defines it as a "non-commercial test" and states that it is sometimes used in scientific research in combination with the PCR reaction and the HC2 test [17].

CERVISTA

Other methods approved by the FDA (2009) are the Cervista™ HPV HR test and the Cervista™ HPV 16/18 test (Hologic Inc., USA). The materials for examination are cervical swabs [18], as well as biopsies [19].

The Cervista™ HPV HR test is based on solution hybridization and is a qualitative test to detect DNA of all 14 types of HR HPV [18, 19]. The method uses the Cleavase enzyme and consists of two isothermal reactions: the primary one, i.e. the binding of oligonucleotides to the target sequence, and the secondary one, i.e. fluorescence generation [18]. In the primary reaction, two types of oligonucleotides are used: a probe oligonucleotide comprising a sequence complementary only to the 5 'part of the target sequence and a non-complementary region to its further part and Invader® oligonucleotide, complementary to the 3' part of the target sequence (Fig. 1B). These oligonucleotides overlap with at least one nucleotide, so that when bound to the target sequence, a structure is created that is a substrate for the Cleavase enzyme. This enzyme cleaves

the non-complementary region and overlapping nucleotides from the oligonucleotide probe. In the secondary reaction, the cleaved fragment hybridizes to a FRET oligonucleotide with a hairpin structure. FRET oligonucleotide has a fluorophore and a quencher. The presence of the quencher eliminates the phenomenon of fluorescence, because its absorption spectrum coincides with the emission spectrum of the fluorophore [20].

The next sequence is created, which cleaves the Cleavase enzyme, because in this case, nucleotides of the hybridized sequences overlap. Cleavase cleaves the FRET oligonucleotide between the fluorophore and the quencher, which causes fluorescence emission [18]. The internal control of the test is the sequence encoding the histone 2 protein — the mixture of oligonucleotides also contains oligonucleotides that bind to this sequence. For the method to detect the presence of viral DNA and prevent false negative results, 1,250–2,500 copies of DNA are required for virus types –16, –18, –31, –45, –52 and –56; 2,500–5,000 copies of DNA for types –33, –39, –51, –58, –59, –66 and –68; and 5,000–7,500 copies for type –35 [18].

The Cervista™ HPV HR test is characterized by high analytical sensitivity, comparable to the sensitivity of the HC2 test [19]. Compared to the HC2 test, the advantages of the test are: the Cervista includes an internal control, requires lower sample volume and involves hands-free time, because there is a possibility for automation [21, 22]. Because the test requires a small-volume sample, the collected material can be used for a greater amount of analysis, e.g. for testing for other pathogens. The disadvantages of the test are: cross-reactivity with HPV types –67 and –70 and the possibility of false negative results when using contraceptive gels and antifungal creams. Like the HC2 method, Cervista is not specific for particular viral genotypes [18].

The Cervista™ HPV 16/18 test is based on the same reactions as the Cervista™ HPV HR test, however, it contains oligonucleotides complementary only to the two most oncogenic HPV strains: 16 and 18, so it is used to detect only these two types [23]. The test can be used alone or in combination with Cervista™ HPV HR, which is recommended in the case of squamous cells with indeterminate significance (ASC-US) [21]. The advantages of the test are high analytical sensitivity and analytical specificity [24]. In comparison to the PCR method, the overall positive and negative percentages of compliance were 94% and 85%, respectively [24]. The disadvantage is the cross-reactivity with HPV 31; however, it only occurs at high concentrations of this genotype in the sample [21, 22].

COBAS

The COBAS® HPV (Roche Molecular Systems Inc., Switzerland) was approved by the FDA in 2011. The material for examination is an LBC (liquid-based cytology) cervical swab [25]. The test contains primers that are used in the PCR reaction to amplify the sequence of about 200 nucleotides of the gene encoding the L1 protein of 14 HR HPV types. Oligonucleotide primers are fluorescently labeled, allowing the use of quantitative PCR technology (qPCR). The reaction is automated and takes place in the dedicated COBAS x 480 instrument, which reduces the manual work required [25]. There are 4 fluorescent probes used: separate for HPV-16, for HPV-18, for the remaining 12 types, and for the beta-globin gene as positive control of human DNA isolation (Fig. 1C). The test is therefore differentiating only for HPV –16 and –18 genotypes.

If L1 gene sequence of one or more HR HPV types is present in the sample, specific primers attach to the complementary sequences and the amplification reaction takes place (Fig. 1D). Detection is based on oligonucleotide probes [26]. These probes are labeled at one end with a fluorophore and at the other with a quencher. The quencher is so close to the fluorophore that no emission of fluorescence occurs. If the probe binds to a complementary sequence, then it will be degraded during the ongoing qPCR reaction, thanks to 5′–3′ exonuclease activity of polymerase.

Degradation of the probe causes separation of the fluorophore from the quencher, thanks to which the fluorescence can be detected (for each marker at different excitation wave) [26].

The detection limit (LoD) has been specified for 150 copies/mL for type –45, 300 copies/mL for types –16, –31, –33, –39, –51 and –59, 600 copies/mL for types –18, –35 and –58, 1200 copies/ mL for types –56, –66 and –68 and 2400 copies/mL for type –52 [25].

The advantage of the test is its high sensitivity, comparable to the HC2 test [27]. The COBAS test shows lower cross-reactivity with non-oncogenic virus types than Hybrid Capture II (1.2% vs. 2.2%) [28]. The test does not cross-react with other microorganisms or interact with lubricants or antifungal drugs [25]. The COBAS test allows genotyping of only HPV –16 and –18 types. The remaining 12 types give the same signal, so they are detected together, and it is not possible to differentiate the type of virus. Another advantage mentioned above is automation, which reduces the need for manual work. The literature does not report any shortcomings of the test, however, the high price of the instrument used to conduct the test can certainly be regarded as a disadvantage.

APTIMA

The APTIMA (Gen-Probe, USA) test was approved by the FDA in 2011. The materials tested are ThinPrep cervical smears [29]. The tests are designed to detect mRNA of E6/E7 oncoproteins encoded in the viral genome. There are two variants of this test: APTIMA™ HPV and APTIMA™

16 18/45 (approved by the FDA in 2012). APTIMA™ HPV detects an infection with 14 HR HPV types, while APTIMA™ 16 18/45 detects an infection with three HPV oncogenic types: –16, –18 and/or –45. The tests do not allow to distinguish which of the detected types of infection occurred.

The APTIMA test consists of 3 stages, which are carried out in one tube: target capture; target amplification; detection of the amplification products [29]. At the beginning, samples are transferred to the Specimen Transport Medium, in which cell lysis occurs and the mRNA contained therein is released (Fig. 1E). Then, target mRNAs bind to complementary oligonucleotides with (poly-deoxyadenosine) polyA tail. Next, these hybrids are bound by poly-deoxythymidine (polyT) molecules, attached to the magnetic microparticles. This makes it possible to separate the target mRNA with a magnet. The next step, amplification, is associated with using the TMA method, i.e. amplification of RNA using reverse transcriptase and T7 polymerase. The captured mRNAs are transcribed into complementary DNA by reverse transcriptase. The cDNA contains a promoter for the T7 RNA polymerase, which allows this enzyme to join the cDNA and create multiple copies of the complementary RNA strand. Detection of the resulting amplicons is done using the Hybridization Protection Assay. The assay involves hybridization of duplicated sequences with fluorescently labeled oligonucleotide probes. In the absence of hybridization, the probe is degraded by borate buffered solution containing a surfactant. Therefore, the fluorescence signal can be detected only in the presence of multiplication by T7 polymerase. Light emitted by hybrids is measured by RLU using a luminometer [29].

The LoD test, according to the manufacturer's data, is less than 100 copies/reaction for types –16, –18, –31, –33, –35, –39, –45, –58, –59, –66, and –68, and between 100 and 300 copies/reaction for types –51, –52 and –56. The reaction is carried out in a volume of 400 µL +/- 100 µL [29].

The sensitivity of the test is comparable to the sensitivity of the HC2 test (according to Ratman et al., 96.3% for APTIMA vs 94.3% for Hybrid Capture II), so it is high, however, the greatest advantage of the test compared to HC2 is a higher correlation between a positive result of the test and pre-cancer/cervical cancer stages [30]. The test also has a higher specificity compared to the COBAS test [31]. A small disadvantage of the test is cross-reactivity with HPV types –26, –67, –70 and –82, however, it does not show cross-reactivity with other HPV strains or microorganisms. Another disadvantage of the test is no genotyping of particular viral genotypes. The test interferes with some lubricants containing Polyquaternium 15, as well as with some antifungal agents containing tioconazole [29].

ONCLARITY

This test was approved by the FDA in 2018. The BD Onclarity ™ HPV Assay (Becton, Dickinson and Company, USA) is based on qPCR [32]. The materials are cervical swabs collected in a BD SurePath Preservative Fluid. BD Onclarity ™ HPV Assay detects E6/E7 oncogenes of 14 HPV HR types. The test is performed in three separate tubes (Fig. 1C). Onclarity test differentiates infection types –16, –18, –31, –45, –51 and –52 while the remaining 8 genotypes are detected as 3 different groups (–33/–58, –56/–59/–66 and 35/39/68) [32, 33]. The three tubes are necessary because the test uses 15 probes (14 for viral sequences and 1 for the human beta globin gene sequence, as internal control), but only four fluorescent dyes are employed so each tube contains different probes labeled with the markers used.

The test is fully automated and is divided into two stages. The first stage consists in cell lysis and DNA isolation in a high pH environment [32, 33]. The second stage is based on TaqMan oligonucleotide probes, identically to the COBAS test (Fig. 1D).

The LoD of the test is about 250 copies/mL for HPV–16, in the range of 800–900 copies/mL for HPV –31, –52 and –66, in the range of 1,000–1,500 copies/mL for types –18, –45, –56 and –59, in the range of 1,500–1,800 copies/mL for types –33, –35, –39 and –51 and in the range of 2,300–2,400 copies/mL for types –58 and –68 [32].

The advantages of the test are high specificity and sensitivity, which are comparable to the HC2 test [34]. The test provides genotyping information for 6 types of HPV – this is the largest number out of all tests presented here. Thanks to full automation, the test is very easy to use and limits the work required [32]. There was also no cross-reactivity with other types of HPV or any microorganisms. As the only one of all FDA-accepted tests, it differentiates between 6 types of viral infection. The disadvantage of the test is the possibility of obtaining false negative results when using mucin, acyclovir and clindamycin [32]. Another downside is the high price of the BD Viper ™ LT system, which is necessary to perform the test.

SUMMARY

All molecular tests approved by the FDA have high sensitivity and specificity. All tests detect 14 types of HPV HR, except for the HC2 test, which does not detect HPV-66, but does detect 5 low-oncogenic types. Cervista and APTIMA have variants that detect only types with the highest oncogenicity. The COBAS allows genotyping of HPV types –16 and –18, while Onclarity allows genotyping of types –16, –18, –31, –45, –51 and –52. The APTIMA test has the lowermost limit of detection among the tests described. The tests show cross-reactivity with low-risk HPV types, except for the Onclarity test, where cross-reactivity was not found. Cervista has the ability to be automated, and COBAS and Onclarity are compulsorily automated. Automation reduces the need to perform laboratory work, but the one-time expenditure for equipment is high.

The main disadvantage of the described tests is that they do not allow for observation of mixed infections as well as monitoring of persistent infection with all types belonging to the HPV HR group.

In comparison to cytological and histopathological tests, the molecular HPV tests described show different correlations between pathological changes in the cervix and HPV-positive results. Differentiation of correlation between CIN2 + and positive HPV test result is as follows: HC2 test shows 93.4% detection of CIN2+ lesions, Cevrista HPV HR 98.4%, Cervista HPV 16/18 77%, COBAS 95%, APTIMA HPV 89.4%, Onclarity 98% [10, 21, 27, 30, 33, 35]. The literature does not provide this data for the APTIMA 16 16/45 test.

Each of the tests described has both advantages and disadvantages. It is important, therefore, that a laboratory that wants to carry out HPV detection tests selects the most suitable option for itself.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394–424, doi: 10.3322/caac.21492, indexed in Pubmed: 30207593.
2. Human Papillomavirus and Related Diseases Report. Barcelona: HPV Information Centre. http://www.hpvcentre.net/statistics/reports/POL.pdf (2018).
3. Bosch FX, Lorincz A, Muñoz N, et al. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002; 55(4): 244–265, indexed in Pubmed: 11919208.
4. de Sanjosé S, Brotons M, Pavón MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018; 47: 2–13, doi: 10.1016/j.bpobgyn.2017.08.015, indexed in Pubmed: 28964706.
5. Abreu ALP, Souza RP, Gimenes F, et al. A review of methods for detect human Papillomavirus infection. Virol J. 2012; 9: 262, doi: 10.1186/1743-422X-9-262, indexed in Pubmed: 23131123.
6. Krajowa Izba Diagnostów Laboratoryjnych; Polskie Towarzystwo Ginekologiczne. Guidelines for application of molecular tests identyfying HR HPV DNA in the prevention of cervical cancer. Statement of experts from PGS (PTG) and NCLD (KIDL). Ginekol Pol. 2013; 84: 395–399, indexed in Pubmed: 23819408.
7. Olejniczak T, Rabiega-Gmyrek D, Niepsuj-Biniaś J, et al. [Human papilloma virus genotyping in women with abnormal cytology]. Ginekol Pol. 2015; 86(7): 541–546, indexed in Pubmed: 26376534.
8. Wong AA, Fuller J, Pabbaraju K, et al. Comparison of the hybrid capture 2 and cobas 4800 tests for detection of high-risk human papillomavirus in specimens collected in PreservCyt medium. J Clin Microbiol. 2012; 50(1): 25–29, doi: 10.1128/JCM.05400-11, indexed in Pubmed: 22090403.
9. Hybrid CaptureTM II. Gaithersburg: Digene Corporation. https://www.accessdata.fda.gov/cdrh_docs/pdf/P890064S009c.pdf. (2012).
10. Sargent A, Bailey A, Turner A, et al. Optimal threshold for a positive hybrid capture 2 test for detection of human papillomavirus: data from the ARTISTIC trial. J Clin Microbiol. 2010; 48(2): 554–558, doi: 10.1128/JCM.00896-09, indexed in Pubmed: 20007387.
11. Bozzetti MC, Nonnenmacher B, Mielzinska I, et al. Comparison between hybrid capture II and polymerase chain reaction results among women at low risk for cervical cancer. Annals of Epidemiology. 2000; 10(7): 466, doi: 10.1016/s1047-2797(00)00147-2.
12. Castle PE, Schiffman M, Wheeler CM. Hybrid capture 2 viral load and the 2-year cumulative risk of cervical intraepithelial neoplasia grade 3 or cancer. Am J Obstet Gynecol. 2004; 191(5): 1590–1597, doi: 10.1016/j.ajog.2004.05.018, indexed in Pubmed: 15547529.
13. Kulmala SM, Syrjänen S, Shabalova I, et al. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. J Clin Microbiol. 2004; 42(6): 2470–2475, doi: 10.1128/JCM.42.6.2470-2475.2004, indexed in Pubmed: 15184422.
14. Peyton CL, Schiffman M, Lörincz AT, et al. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J Clin Microbiol. 1998; 36(11): 3248–3254, indexed in Pubmed: 9774574.
15. Castle PE, Solomon D, Wheeler CM, et al. Human papillomavirus genotype specificity of hybrid capture 2. J Clin Microbiol. 2008; 46(8): 2595–2604, doi: 10.1128/JCM.00824-08, indexed in Pubmed: 18579716.
16. Castle PE, Lorincz AT, Scott DR, et al. Comparison between prototype hybrid capture 3 and hybrid capture 2 human papillomavirus DNA assays for detection of high-grade cervical intraepithelial neoplasia and cancer. J Clin Microbiol. 2003; 41(9): 4022–4030, indexed in Pubmed: 12958220.
17. Schiffman M, Glass AG, Wentzensen N, et al. A long-term prospective study of type-specific human papillomavirus infection and risk of cervical neoplasia among 20,000 women in the Portland Kaiser Cohort Study. Cancer Epidemiol Biomarkers Prev. 2011; 20(7): 1398–1409, doi: 10.1158/1055-9965.EPI-11-0206, indexed in Pubmed: 21602310.
18. Cervista ™ HPV HR. Madison: Third Wave Technologies. https://www.accessdata.fda.gov/cdrh_docs/pdf8/p080014c.pdf (2008).
19. Kurian EM, Caporelli ML, Baker S, et al. Cervista HR and HPV 16/18 assays vs hybrid capture 2 assay: outcome comparison in women with negative cervical cytology. Am J Clin Pathol. 2011; 136(5): 808–816, doi: 10.1309/AJCPCQD7B7DPVHLH, indexed in Pubmed: 22031321.
20. Marras SAE. Selection of fluorophore and quencher pairs for fluorescent nucleic acid hybridization probes. Methods Mol Biol. 2006; 335: 3–16, doi: 10.1385/1-59745-069-3:3, indexed in Pubmed: 16785616.
21. Alameda F, Garrote L, Mojal S, et al. Cervista HPV HR test for cervical cancer screening: a comparative study in the Catalonian population. Arch Pathol Lab Med. 2015; 139(2): 241–244, doi: 10.5858/arpa.2014-0012-OA, indexed in Pubmed: 25611107.
22. Torres M, Fraile L, Echevarria Jm, et al. Human Papillomavirus (HPV) Genotyping: Automation and Application in Routine Laboratory Testing. Open Virol J. 2012; 6: 144–150, doi: 10.2174/1874357901206010144, indexed in Pubmed: 23248734.
23. Cervista ™ HPV 16/18. Madison: Third Wave Technologies. https://www.accessdata.fda.gov/cdrh_docs/pdf8/P080015c.pdf (2008).
24. Bartholomew DA, Luff RD, Quigley NB, et al. Analytical performance of Cervista HPV 16/18 genotyping test for cervical cytology samples. J Clin Virol. 2011; 51(1): 38–43, doi: 10.1016/j.jcv.2011.01.016, indexed in Pubmed: 21376660.
25. cobas® HPV Test. South Branchburg: Roche Molecular Systems. https://www.accessdata.fda.gov/cdrh_docs/pdf10/p100020s017c.pdf (2015).
26. Rao A, Young S, Erlich H, et al. Development and characterization of the cobas human papillomavirus test. J Clin Microbiol. 2013; 51(5): 1478–1484, doi: 10.1128/JCM.03386-12, indexed in Pubmed: 23447640.
27. Heideman DAM, Hesselink AT, Berkhof J, et al. Clinical validation of the cobas 4800 HPV test for cervical screening purposes. J Clin Microbiol. 2011; 49(11): 3983–3985, doi: 10.1128/JCM.05552-11, indexed in Pubmed: 21880968.
28. Preisler S, Rebolj M, Ejegod DM, et al. Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study. BMC Cancer. 2016; 16: 510, doi: 10.1186/s12885-016-2518-4, indexed in Pubmed: 27439470.
29. APTIMA® HPV Assay. San Diego: Gen-Probe Incorporated. https://www.accessdata.fda.gov/cdrh_docs/pdf10/P100042c.pdf (2011).
30. Ratnam S, Coutlee F, Fontaine D, et al. Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. J Clin Microbiol. 2011; 49(2): 557–564, doi: 10.1128/JCM.02147-10, indexed in Pubmed: 21147950.
31. Castle PE, Eaton B, Reid J, et al. Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. J Clin Microbiol. 2015; 53(4): 1277–1281, doi: 10.1128/JCM.03558-14, indexed in Pubmed: 25653409.
32. BD Onclarity™ HPV Assay. Sparks: Becton, Dickinson and Company. https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160037C.pdf (2018).
33. Ejegod DM, Junge J, Franzmann M, et al. Clinical and analytical performance of the BD Onclarity™ HPV assay for detection of CIN2+ lesions on SurePath samples. Papillomavirus Res. 2016; 2: 31–37, doi: 10.1016/j.pvr.2016.01.003, indexed in Pubmed: 29074183.
34. Bottari F, Sideri M, Gulmini C, et al. Comparison of Onclarity Human Papillomavirus (HPV) Assay with Hybrid Capture II HPV DNA Assay for Detection of Cervical Intraepithelial Neoplasia Grade 2 and 3 Lesions. J Clin Microbiol. 2015; 53(7): 2109–2114, doi: 10.1128/JCM.00246-15, indexed in Pubmed: 25903574.
35. Guo M, Khanna A, Feng J, et al. Analytical performance of cervista HPV 16/18 in SurePath pap specimens. Diagn Cytopathol. 2015; 43(4): 301–306, doi: 10.1002/dc.23221, indexed in Pubmed: 25352375.

VIA MEDICA

REVIEW PAPER / OBSTETRICS

Ginekologia Polska
2019, vol. 90, no. 2, 109–113
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0019

The role of lymphocytes in fetal development and recurrent pregnancy loss

Jan Modzelewski[1, 2], Anna Kajdy[1, 2], Michal Rabijewski[1]

[1]Department of Reproductive Health, Centre of Postgraduate Medical Education, Warsaw, Poland
[2]Centrum Medyczne Żelazna, Warsaw, Poland

ABSTRACT

Fetal survival and development is supported by the maternal immune system. Questions regarding those mechanisms have risen from development of transplantation medicine and observation of graft rejection. Initial theories of anatomic division, fetal immune immaturity and maternal immune system inertia were found incorrect. Rejection of fetal "semi-allograft" by maternal immune system could result in pregnancy loss. Two pregnancy losses of any etiology are considered recurrent and effort should be made to name the probable cause. Immune causes of pregnancy loss are probably multifactorial, thus difficult to research and implement findings in clinical practice. Although a full understating of pregnancy loss is not established, new therapies are being developed. This review summarizes the role of lymphocytes in pregnancy development, presents data from studies on recurrent pregnancy loss patients, evidence of new therapies and ESHRE guidelines regarding immunologic investigations.

Key words: recurrent pregnancy loss; lymphocytes; Treg; Th17; uNK; abortion; habitual

Ginekologia Polska 2019; 90, 2: 109–113

INTRODUCTION

From an immunological perspective pregnancy is an interesting phenomenon. On one hand the fetus requires maternal protection from pathogens, on the other develops tolerance for paternal antigens. Interest in immunology of pregnancy started in the beginning of the transplantation era with the observation of transplant rejection mechanisms and natural fetal protection against such rejection.

First, classic theory was presented in 1953 by Peter Medawar, who is considered the founder of reproductive immunology. He described three mechanisms — anatomic division between mother and fetus by placental barrier, antigenic fetal immaturity, and inertia of the maternal immune system [1]. In the following years all three of these mechanisms where questioned and found incorrect [2].

Human placenta stays in contact with maternal blood. Trophoblast cells invade uterine spiral which is a crucial part of placental development. Pathological placentation plays a major role in development of such pregnancy disorders as placenta precerta, acareta, incerta or preeclampsia [3].

Full contact of maternal and fetal cells, especially extravillous trophoblast (EVT) cells, enforce development of an active tolerance to paternal antigens. Fetal antigens are recognized by maternal innate and adaptive immune systems. Both these elements play role in correct development of materno-fetal interface.

KEY LYMPHOCYTES POPULATIONS IN PREGNANCY AND RECURRENT PREGNANCY LOSS

NK cells are, separate from T and B lymphocytes, lymphocytes with cytotoxic and cytokine producing abilities. Distinct subpopulation of NK called uterine NK (uNK) are present in large numbers in endometrium and decidua [4]. Another lymphocyte populations playing important roles in pregnancy are Treg and Th17 cells. These are two distinct lymphocyte subpopulations of with contradictory roles in the human body. Recent development of reproductive immunology shows that correct balance of these cells may be important in maintaining healthy pregnancy development [5].

Recurrent pregnancy loss (RPL) is defined as loss of two or more pregnancies before reaching viability. Probably 1–2% of couples suffer from RPL [6]. Most commonly described causes are anatomic defects of the uterus

Corresponding author:
Jan Modzelewski
Centrum Medyczne Żelazna, 90 Żelazna St., 01–004 Warsaw, Poland
e-mail: jmodzelewski@cmkp.edu.pl

(e.g. adhesions, myomas, endometrial polyps and congenital anomalies), chronic endometritis, antiphospholipid syndrome, inherited thrombophilia, endocrine pathologies, fetal and parental genetic factors, and immunological imbalance [6, 7]. RPL is not only a medical problem of decreased fertility in couples, but also a large psychological burden [8].

At current we can explain only about 50% of RPL. Probably large proportion of unexplained RPL is caused by immune factors [9]. Research could lead to development of new promising therapies and increase chances for successful pregnancy in couples suffering from RPL.

This paper aims to present a literature review of the role of key lymphocyte populations in RPL, promising therapies of this clinical problem, and available guidelines regarding immunological investigation.

TH17

Th17 cells are adaptive immunity cells characterized by interleukin-17 (IL-17) production. Other important cytokines produced by Th17 are interleukin-22 (IL-22) and granulocyte-macrophage colony stimulating factor (GM-CSF). Physiologically Th17 cells promote inflammation, especially during bacterial and fungal infection [10].

Th17 cells are formed from naïve $CD4^+$ T cells through IL-6 and TGF-β stimulation [11]. These cells possess a certain amount of plasticity that can change cytokine profile to Th1 or Treg. This plasticity is present in vivo during the course of inflammation [12].

TREG

Treg cells are $CD4^+$ lymphocytes characterized by expression of forkhead-box P3 (FoxP3) transcription factor. Foxp3 plays a role in immunoregulation. Its deficiency ameliorates the natural history of severe autoimmune disease such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [13].

Treg cells regulate immune response by direct interaction with antigen presenting cells, mainly by cytotoxic T-lymphocyte antigen 4 (CTLA-4), secretion of TGF-β, IL-35 and IL-10 which are anti-inflammatory cytokines, inducing B cell apoptosis through granzymes and high expression of CD25, IL-2 receptor, which depletes IL-2 from the environment [13].

UTERINE NK

Uterine NK subset is distinct from peripheral NK cells. They have lower cytotoxic and higher immunosuppressive potential. UNK cells are the most abundant lymphocyte subset in human decidua. 60–70% of all uterine lymphocytes are uNK [14]. Numbers of uNK cells differs during menstrual cycle and pregnancy. In the proliferatory phase uNK compose 10% of all endometrial stromal cells, 20% in late secretory phase and even 30% of stromal cells in the first trimester. Mature forms of human chorionic gonadotropin (hCG) with N-linked carbohydrate side chains act through CD206 (mannose receptor) and enhance uNK proliferation [15].

As other NK cells, uNK are $CD56^+$ and $CD3^-$ but lack expression of CD16 which is responsible for antibody dependent cellular cytotoxicity (ADCC). Moreover uNK cells have potential to produce large amounts of cytokines needed in proper development of early pregnancy [15].

Killer immunoglobin-like receptors (KIR) are receptors expressed mainly on NK cells and, depending on type of receptor. KIR binds to trophoblast expressed HLA class C molecules. Certain combinations of KIR and HLA-C haplotypes result in poor uNK activation and increases risk of pregnancy loss. At the same time HLA-G and HLA-E binds to another highly expressed receptor NKG-2A, which result in inhibition of uNK cytotoxicity [15].

IMMUNE SYSTEM INVOLVEMENT IN RPL

Immunological response to pregnancy probably begins even before pregnancy itself occurs. Murine models show that paternal antigens derived during coitus are detected in female lymph nodes. Two days after coitus, Treg lymphocytes reactive to paternal antigens are present in large numbers in lymph nodes draining the uterus, peripheral lymph nodes and spleen [16]. Interestingly, concentration of TGFβ, cytokine needed in Treg differentiation, in seminal plasma is one of the highest detected in biological fluids [17].

Implantation of conceptus requires a delicate game of pro- and anti-inflammatory factors. IL-6 which is a potent proinflammatory cytokine and blocker of Treg differentiation [11], also increases trophoblast invasion [3]. Moreover IL-6 and IL-1β expression is decreased in endometrium of women suffering from pregnancy loss [18] while IL-6 levels in peripheral blood increases [19, 20]. IL-6 and IL-1β are pro inflammatory cytokines. Anti-inflammatory cytokine IL-10, which is produced by Treg cells inhibits trophoblast invasion [3].

Further development of fetus is also upkeeped and regulated by the maternal immune system. Decidualization is a process of endometrium remodeling in response to embryo implantation. This results in proper environment for the developing fetus, which needs nourishment from the maternal vascular system. To provide proper blood flow trophoblast cells invade zona intima of maternal spiral arteries and change their morphology allowing increased blood flow. Surprisingly extra villous trophoblast cells form plugs within maternal spiral arteries, until the end of the first trimester. This causes decrease blood flow and enables proper development of the villous trophoblast and arterial remod-

eling [21]. Process of arterial remodeling is regulated mainly by uNK cells. Immune imbalance is one of the probable etiological factors for idiopathic recurrent pregnancy loss. Such imbalance was detected in several studies. Liu et al. compared lymphocyte numbers from peripheral blood of three groups of patients — unexplained spontaneous recurrent pregnancy loss (URPL), fertile nonpregnant, and pregnant women in confirmed viable pregnancy. Additionally, 6 decidual samples form URPL patients were immunoassayed and compared with samples from elective termination pregnancies. Nonpregnant women and normal early pregnancies had similar number of peripheral Th17 cells. URPL women had significantly higher number of Th17 cells. Number of peripheral Treg cells where similar in nonpregnant and URPL women, while normal early pregnancies had higher number of peripheral Treg cells. Th17/Treg ratio was higher in URPL women than in both other groups. Immunostaining of decidual samples showed higher prevalence of Th17 in decidua of URPL patients than in elective pregnancy termination cases [22].

Lee et al. conducted a study comparing peripheral blood Th17 and Treg ratios in nonpregnant females with URPL and normal fertile women. Study showed statistically higher numbers of Th17 and increased Th17/Treg ratio in URPL women [23].

Study Saifi et al. compared percentage and cytokine profiles of Th17 and Treg lymphocytes in peripheral blood of nonpregnant URPL suffering and fertile women. Fertile women had significantly higher (9.5% ± 0.52) percentage of Treg cells than URPL group (5.66% ± 0.21). Th17 lymphocytes percentage was lower in fertile women (1.82% ± 0.11) than in URPL group (2.8% ± 0.18). Also higher expression of IL-6, IL-17 and IL-23 was found in URPL women [20].

Recent study by Qian et al. compared pregnant and non-pregnant URPL women with pregnant and non-pregnant controls. All groups had similar numbers of peripheral blood mononuclear cells but differ in Treg percentage. Pregnant control group had higher proportion of Treg lymphocytes than nonpregnant control, while there was no significant change between pregnant and nonpregnant URPL patients. Moreover, pregnant URPL patients had lower Treg percentage in peripheral blood than pregnant controls. There was no difference in IL-10 and CTLA-4 expression in peripheral blood between groups. They both take action in Treg activation. No difference in Th17/Treg ratio in peripheral blood was found in this study. Investigators collected also decidual samples from URPL patients and women undergoing elective termination of pregnancy. Decreased proportion of Tregs and increased proportion of Th17 was found in URPL patients. Il-10 expression in Treg did not differ between groups, but expression of CTLA-4 was lower in URPL women [5].

POTENTIAL THERAPIES

With our current knowledge of causes of immune related recurrent miscarriage, arise questions of possible therapies. In fact, there where trials conducted, some with promising results.

Intravenous immunoglobulin (IVIG) administration is proposed to immunomodulate maternal response therefore improving pregnancy outcome. Study of IVIG treatment and NK cell function and levels was conducted by Ahmadi et al. Investigators recruited 78 women with recurrent pregnancy loss, 38 in intervention arm and 40 as a control group. After confirmation of pregnancy, treatment group received 400 mg/kg IVIG iv. each 4 weeks until 32 weeks of pregnancy. Both groups received standard high-risk pregnancy care. Live birth rate was 86.8% in treatment group and 45% in control group ($p = 0.0006$). IVIG treatment also significantly lowered risk of preeclampsia, gestational diabetes and preterm birth. IVIG treatment significantly lowered peripheral NK cell cytotoxicity and frequency. Investigators did not asses uterine NK population [24].

Randomized controlled trials of IVIG treatment yield conflicting results. Most recent metanalysis was performed by Egerup et al. It was conducted according to The Cochrane Handbook for Systematic Reviews of Interventions methodology and strict study protocol. It analyzed outcome of IVIG treatment and scope for calculation of sample size enough to draw definite conclusions. Authors conclude that probably there is a different effect of treatment in primary and secondary recurrent miscarriage patients. Potential beneficial effect of IVIG could be achieved in secondary miscarriage patients, but sample size in both subgroups of metanalysis where too small to establish definite conclusions. Moreover, the treatment group had more maternal adverse effects than placebo group, with no difference in neonatal adverse effects. Authors conclude that there was not enough evidence to give clear clinical recommendations, and IVIG treatment should not be used out of a clinical trial setting. Different conclusions could be made when new evidence will be published [25].

Another widely discussed intervention is paternal or unrelated donor lymphocyte therapy. Most recent metanalysis of this approach was published by Cavalcante et al. It sums up evidence coming from 6 other metanalysis. Four of them found significant improvement in live birth rate, with OR 1.16 (95% CI 1.04–1.34), 1.21 (95& CI 1.04–1.37), 4.02 (95% CI 3.23–5.00), 3.13 (2.56–3.82). One of the metanalyses which shows no improvement with lymphocytes therapy is Cochrane Review published in 2014 [26]. This metanalyses is widely criticized for including one very poor-quality trial showing no effect of lymphocyte therapy. Removal of that study from the Cochrane Review resulted in OR 1.63 (95% CI 1.13–2.35) for live birth. In conclusion the authors remarked

that with improvement of diagnostic and treatment protocols lymphocyte immunotherapy should have its place in RPL treatment [27].

A recently published non-randomized trial by Liu et al. describes successful treatment of 65 patients with low dose ($1x10^7$) lymphocytes. Investigators showed that this kind of therapy alters unfavorable Th1/Th2/Treg ratio and significantly decreases miscarriage rate from 34.78% in the control group to 11.68% in the treatment group. There was no serious adverse events in treatment group, but some patients had reactions in place of administration [28].

Retrospective analysis of 241 patients treated by paternal lymphocyte immunization published by Motak-Pochrzęst and Malinowski showed promising results. Of 241 patients 206 received 2–6 paternal lymphocytes immunization to induce blocking activity measured by mixed lymphocytes reaction test. The control group were 36 patients with high-risk pregnancy care. Investigators showed increased rate of successful pregnancies in treatment group (83.7% vs. 36.1%, $p < 0.05$) [29].

Retrospective study of Cetin et al. analyzing the use of low molecular weight heparin (LMWH) in selected population of patients with methylene tetrahydrofolate reductase (MTHFR) mutation could be of benefit. 121 women with hetero- and homozygotic MTHFR mutations, 53 in the intervention arm with prophylactic dose of LMWH and 68 in control arm, was included in the study. Both groups received folic acid (5 mg/day) and iron (80 mg/day) supplementation. LMWH group had higher live birth rate (69.8% vs. 48.5%, $p = 0.015$) and lower congenital anomalies rate (3.3% vs. 17.6%, $p = 0.022$). Treatment group delivered two weeks later (34.88 vs. 32.75) comparing with supplementation only group but this result did not reach statistical significance ($p = 0.060$) [30].

Recent Bayesian network metanalysis by Lv et al. summed evidence on use of 14 different RPL treatments and placebo. 49 randomized controlled trials and 8469 patients were included. Three different endpoints — miscarriage, live birth and successful pregnancy defined as birth of a viable fetus, were taken into account. Enough data was available to conclude that treatment with corticosteroids + low dose aspirin + unfractioned heparin, low dose aspirin +, and Granulocyte colony stimulating factor (G-CSF) are effective in decreasing RPL rates and increasing live births in both unexplained RPL and RPL with identified cause. There were no statistically significant differences between each of the mentioned above treatments. Additionally authors made analysis for antiphospholipid syndrome patients and concluded that none of the analyzed treatments performed better than placebo [31].

IMMUNOLOGICAL INVESTIGATIONS GUIDELINES

The most up to date available guideline is that published by the European Society of Human Reproduction and Embryology. It is a guideline solely dedicated to recurrent pregnancy loss. The guideline was developed based on evidence published until 31 march 2017. In light of current evidence the authors recommend only antiphospholipid syndrome screening, and HLA class II determination in selected population. No other immunological diagnostic tests should be performed, as it won't lead to better treatment or prognosis. Women who fulfil laboratory criteria of phospholipid syndrome and had 3 or more pregnancy losses should be offered low dose aspirin (75–100 mg) before conception and prophylactic dose heparin at time of positive pregnancy test. IVIG ant lymphocyte therapy are not recommended in light of current evidence and should be used only in clinical trial setting. As a result of lack of randomized controlled trials, recommendations are made based on moderate and low quality evidence [6].

SUMMARY

Reproductive immunology has come a long way since Peter Madawar's first ideas. Yet we are still far from fully understanding exact mechanisms of immunology of embryo development and finding evidence-based treatment for RPL. On the other hand, many trials have brought interesting possibilities and give hope for patients suffering from recurrent pregnancy loss.

Christiansen et al. postulated that because of the evolutionary need for reproductive success most of miscarriages including those of immunological background are multifactorial. A single cause with strong association with recurrent pregnancy loss would be eradicated from gene pool. As a result research and treatment of recurrent pregnancy loss is extremely difficult [9]. Probably there is no universal treatment for women suffering from RPL and combination of therapies tailored for individual patient should be used.

Evidence coming from already conducted trials shows promising results for therapy of immune recurrent pregnancy loss. Most successful therapies seem to be IVIG, paternal lymphocyte therapy and combination corticosteroids + low dose acetylsalicylic acid + unfractioned heparin, GM-CSF, low dose aspirin + low molecular weight heparin.

Although recurrent pregnancy loss research is difficult and full of controversies important progress has been made with hope for finally providing effective care for RPL suffering couples.

Acknowledgements

Possible conflict of interest — JM and AK are ESHRE members.

JM participated in all stages of project. AK and MR designed project, participated in data analysis and reviewed final manuscript. AK proofread the manuscript. MR supervised the project.

This project had no external financial support.

REFERENCES

1. Medawar P. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol. 1953; 7: 320–337.
2. Szekeres-Bartho J. The Role of Progesterone in Feto-Maternal Immunological Cross Talk. Med Princ Pract. 2018; 27(4): 301–307, doi: 10.1159/000491576, indexed in Pubmed: 29949797.
3. Sharma S, Godbole G, Modi D. Decidual Control of Trophoblast Invasion. Am J Reprod Immunol. 2016; 75(3): 341–350, doi: 10.1111/aji.12466, indexed in Pubmed: 26755153.
4. Faas MM, de Vos P. Uterine NK cells and macrophages in pregnancy. Placenta. 2017; 56: 44–52, doi: 10.1016/j.placenta.2017.03.001, indexed in Pubmed: 28284455.
5. Qian J, Zhang Na, Lin J, et al. Distinct pattern of Th17/Treg cells in pregnant women with a history of unexplained recurrent spontaneous abortion. Biosci Trends. 2018; 12(2): 157–167, doi: 10.5582/bst.2018.01012, indexed in Pubmed: 29657243.
6. Atik RB, Christiansen O, Elson J, et al. ESHRE guideline: recurrent pregnancy loss. Human Reproduction Open. 2018; 2018(2), doi: 10.1093/hropen/hoy004.
7. El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017; 9: 331–345, doi: 10.2147/IJWH.S100817, indexed in Pubmed: 28553146.
8. Łuczak-Wawrzyniak J, Czarnecka-Iwańczuk M, Bukowska A, et al. Early and late psychological effects of pregnancy loss. Ginekol Pol. 2010; 81(5): 374–377, indexed in Pubmed: 20568519.
9. Christiansen O, Steffensen R, Nielsen H, et al. Multifactorial Etiology of Recurrent Miscarriage and Its Scientific and Clinical Implications. Gynecologic and Obstetric Investigation. 2008; 66(4): 257–267, doi: 10.1159/000149575.
10. Sandquist I, Kolls J. Update on regulation and effector functions of Th17 cells. F1000Res. 2018; 7: 205, doi: 10.12688/f1000research.13020.1, indexed in Pubmed: 29527301.
11. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006; 441(7090): 235–238, doi: 10.1038/nature04753, indexed in Pubmed: 16648838.
12. Gagliani N, Amezcua Vesely MC, Iseppon A, et al. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. Nature. 2015; 523(7559): 221–225, doi: 10.1038/nature14452, indexed in Pubmed: 25924064.
13. Kawai K, Uchiyama M, Hester J, et al. Regulatory T cells for tolerance. Hum Immunol. 2018; 79(5): 294–303, doi: 10.1016/j.humimm.2017.12.013, indexed in Pubmed: 29288698.
14. Mori M, Bogdan A, Balassa T, et al. The decidua-the maternal bed embracing the embryo-maintains the pregnancy. Semin Immunopathol. 2016; 38(6): 635–649, doi: 10.1007/s00281-016-0574-0, indexed in Pubmed: 27287066.
15. Gong H, Chen Y, Xu J, et al. The regulation of ovary and conceptus on the uterine natural killer cells during early pregnancy. Reprod Biol Endocrinol. 2017; 15(1): 73, doi: 10.1186/s12958-017-0290-1, indexed in Pubmed: 28874155.
16. Robertson SA, Prins JR, Sharkey DJ, et al. Seminal fluid and the generation of regulatory T cells for embryo implantation. Am J Reprod Immunol. 2013; 69(4): 315–330, doi: 10.1111/aji.12107, indexed in Pubmed: 23480148.
17. Robertson SA, Ingman WV, O'Leary S, et al. Transforming growth factor beta--a mediator of immune deviation in seminal plasma. J Reprod Immunol. 2002; 57(1-2): 109–128, indexed in Pubmed: 12385837.
18. Jasper MJ, Tremellen KP, Robertson SA. Reduced expression of IL-6 and IL-1alpha mRNAs in secretory phase endometrium of women with recurrent miscarriage. J Reprod Immunol. 2007; 73(1): 74–84, doi: 10.1016/j.jri.2006.06.003, indexed in Pubmed: 17034864.
19. Zhu L, Chen H, Liu M, et al. Treg/Th17 Cell Imbalance and IL-6 Profile in Patients With Unexplained Recurrent Spontaneous Abortion. Reprod Sci. 2017; 24(6): 882–890, doi: 10.1177/1933719116670517, indexed in Pubmed: 27698192.
20. Saifi B, Rezaee SA, Tajik N, et al. Th17 cells and related cytokines in unexplained recurrent spontaneous miscarriage at the implantation window. Reprod Biomed Online. 2014; 29(4): 481–489, doi: 10.1016/j.rbmo.2014.06.008, indexed in Pubmed: 25154016.
21. James JL, Saghian R, Perwick R, et al. Trophoblast plugs: impact on utero-placental haemodynamics and spiral artery remodelling. Hum Reprod. 2018 [Epub ahead of print], doi: 10.1093/humrep/dey225, indexed in Pubmed: 29955830.
22. Liu YS, Wu Li, Tong XH, et al. Study on the relationship between Th17 cells and unexplained recurrent spontaneous abortion. Am J Reprod Immunol. 2011; 65(5): 503–511, doi: 10.1111/j.1600-0897.2010.00921.x, indexed in Pubmed: 21029245.
23. Lee SK, Kim JY, Hur SE, et al. An imbalance in interleukin-17-producing T and Foxp3 regulatory T cells in women with idiopathic recurrent pregnancy loss. Hum Reprod. 2011; 26(11): 2964–2971, doi: 10.1093/humrep/der301, indexed in Pubmed: 21926059.
24. Ahmadi M, Ghaebi M, Abdolmohammadi-Vahid S, et al. NK cell frequency and cytotoxicity in correlation to pregnancy outcome and response to IVIG therapy among women with recurrent pregnancy loss. J Cell Physiol. 2018 [Epub ahead of print], doi: 10.1002/jcp.27627, indexed in Pubmed: 30317625.
25. Egerup P, Lindschou J, Gluud C, et al. ImmuReM IPD Study Group. The effects of immunotherapy with intravenous immunoglobulins versus no intervention, placebo, or usual care in patients with recurrent miscarriages: a protocol for a systematic review with meta-analyses, trial sequential analyses, and individual patient data meta-analyses of randomised clinical trials. Syst Rev. 2014; 3(10): 89, doi: 10.1186/2046-4053-3-89, indexed in Pubmed: 25128005.
26. Wong LF, Porter TF, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev. 2014(10): CD000112, doi: 10.1002/14651858.CD000112.pub3, indexed in Pubmed: 25331518.
27. Cavalcante MB, Sarno M, Araujo Júnior E, et al. Lymphocyte immunotherapy in the treatment of recurrent miscarriage: systematic review and meta-analysis. Arch Gynecol Obstet. 2017; 295(2): 511–518, doi: 10.1007/s00404-016-4270-z, indexed in Pubmed: 28004193.
28. Liu M, Zhen X, Song H, et al. Low-dose lymphocyte immunotherapy rebalances the peripheral blood Th1/Th2/Treg paradigm in patients with unexplained recurrent miscarriage. Reprod Biol Endocrinol. 2017; 15(1): 95, doi: 10.1186/s12958-017-0315-9, indexed in Pubmed: 29246150.
29. Motak-Pochrzest H, Malinowski A. Polish experiences with paternal lymphocyte immunization in women with recurrent miscarriages. Neuro Endocrinol Lett. 2015; 36(6): 572–577, indexed in Pubmed: 26812293.
30. Cetin O, Karaman E, Cim N, et al. The impact of low molecular weight heparin on obstetric outcomes among unexplained recurrent miscarriages complicated with methylenetetrahydrofolate reductase gene polymorphism. Ginekol Pol. 2017; 88(5): 260–265, doi: 10.5603/GP.a2017.0049, indexed in Pubmed: 28580572.
31. Lv S, Yu J, Xu X. A comparison of effectiveness among frequent treatments of recurrent spontaneous abortion: A Bayesian network meta-analysis. Am J Reprod Immunol. 2018; 80(1): e12856, doi: 10.1111/aji.12856, indexed in Pubmed: 29709100.

VIA MEDICA

CLINICAL VIGNETTE

Ginekologia Polska
2019, vol. 90, no. 2, 114
Copyright © 2019 Via Medica
ISSN 0017–0011

DOI: 10.5603/GP.2019.0020

Exploring the fetal brain: is MRI always better than ultrasound?

Michal Lipa, Przemyslaw Kosinski, Miroslaw Wielgos

The 1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Poland

In many cases of suspected fetal brain abnormalities physicians refer patients for MRI scans to gather more clinical information. Usually this technique is significantly helpful, however in some cases the results may be confusing or even misleading. Despite continuous progress in the field of prenatal ultrasonography, both differential diagnosis and the nomenclature of posterior fossa defects remain challenging. The Dandy-Walker complex is commonly suspected in cases of posterior fossa abnormality or an unusual vermian appearance. However, an upward rotation and allegedly reduced dimension of the cerebellar vermis may be caused by the delayed perforation of Blake's pouch cyst and may occur in an otherwise normal fetal brain. During a routine scan for anomalies, it was therefore possible to visualize the posterior fossa clearly suggesting agenesis of the vermis (Fig. 1). This finding was also confirmed by an experienced reference center sonographer. The patient was then referred for a fetal MRI scan to either confirm or exclude the diagnosis. The MRI was performed at 28 weeks gestation, and the result confirmed the suspicions raised by the ultrasonogram (Fig. 2). The patient was subsequently counselled and provided with information on vermian agenesis. A follow-up scan at 31 weeks gestation revealed normal posterior fossa structures with a clearly present cerebellar vermis and a normal cisterna magna (Fig. 3). The baby was delivered at term, and three months later examined by a pediatric neurologist. The examination revealed a normal neurodevelopment and therefore the quality of life was expected to be normal. To date, only a very few cases of the spontaneous resolution of Blake's Pouch cyst have been reported in the literature. Despite the growing availability of fetal brain MRI scans, it must be emphasized, that in some cases, due to the complex nature of posterior fossa abnormalities, an MRI will not always improve the diagnostic process. In most cases, an ultrasound scan is enough to confirm a diagnosis, but an MRI can still be helpful in cases of poor visualization and with obese patients. Therefore, it is possible that despite the whole process of prenatal abnormality detection, parent counseling, prognosis and additional diagnostic tests being conducted by experienced sonographers using fetal MRI examinations, the initial diagnosis may still be wrong. This confirms that differential diagnosis of the posterior fossa defects may be extremely difficult and sometimes neither an ultrasound nor an MRI can guarantee an unequivocal diagnosis. If there is any suspicion of a posterior fossa defect, a multidisciplinary examination should be introduced. In such cases, careful assessment of the brainstem-vermis and brainstem-tentorium angles may help in forming a prenatal diagnosis. Values below 30 degrees suggest Blake's pouch cyst, while those above 45 degrees point to the Dandy-Walker malformation.

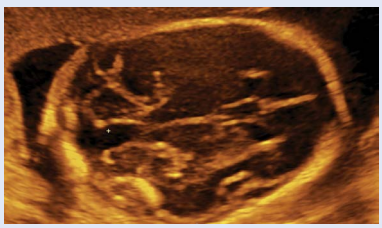


Figure 1. Absent vermis with enlarged cisterna magna at anomaly scan (20 weeks of gestation)

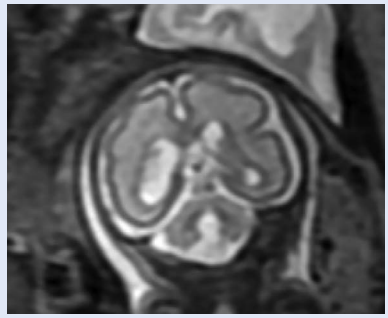


Figure 2. MRI examination at 28 weeks pointing to vermian agenesis

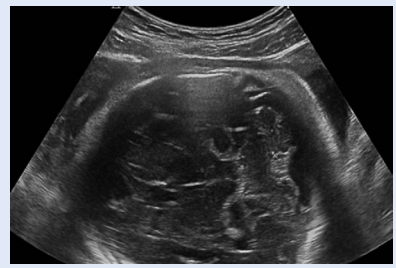


Figure 3. Normal posterior fossa appearance in an ultrasound scan at 31 weeks. Vermis, fourth ventricle and cisterna magna have normal morphology

Corresponding author:
Michal Lipa
The 1st Department of Obstetrics and Gynecology, Medical University of Warsaw, Poland
e-mail: michallipa1@gmail.com