Cent. Eur. J. Med. • 6(1) • 2010 • 49-57
DOI: 10.2478/s11536-010-0049-y

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Central European Journal of **Medicine**

Preconceptional screening of sexually transmitted infections/diseases

Research Article

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Received 15 April 2010; Accepted 4 August 2010

Abstract: Hungarian Preconceptional Care includes the preconceptional screening of sexually transmitted infections/disorders (STD) and vaginal candidosis of potential mothers and pyospermia of potential fathers. The aim of this study was to evaluate the effect of this screening and treatment for the rate of preterm births. Clinical and subclinical vaginal candidiasis (asymptomatic candida colonisation), combination of STD and vaginal candidiasis, STD without vaginal candidiasis, finally women without STD and vaginal candidiasis as references were evaluated in 4,672 pregnant women. The association of STD in pregnant women with higher risk of preterm birth was confirmed. However, an association was also found between clinically diagnosed vaginal candidiasis, asymptomatic candida colonisation, and a higher risk for preterm births. This risk was reduced with clotrimazole treatment. However, pregnant women without recognized STD and/or vaginal candidiasis had a higher risk for preterm birth than pregnant women with STD or vaginal candidiasis after appropriate treatment. Thus the conclusion of the study is that the preconceptional screening of STD and vaginal candidiasis followed by appropriate treatment is important to prevent a certain part of preterm birth but it is necessary to improve the efficacy of the previously used methods for this screening.

Keywords: *Preconceptional care • Preconceptional screening • Sexually transmitted infection/diseases • Vaginal candidosis • Preterm birth*

1. Introduction

In Hungary preconceptional care, consisting of counselling, examination and medical intervention performed by qualified nurses and supervised by medical specialists, was launched in two centres as a model in 1984 [1]. In 1988 a network of 32 regional centres of preconceptional care was established in Hungary. Unfortunately after the change of our political system in 1989, preconceptional care was continued only in some centres, e.g. in the Family Planning Clinic of our Foundation.

The methods and benefits of preconceptional care in Hungary were summarised previously [1-3]. The most important five benefits of preconceptional care are: (i) optimal chance for periconceptional folic acid or folic acid-containing multivitamin supplementation to prevent neural-tube defects and some other congenital abnormalities [4-9], (ii) a good opportunity for an explanatory course to quit smoking and to reduce significantly alcohol intake of female participants [10], (iii) selection of couples at high risk for genetic counselling and for predictive genetic tests [11], (iv) preconceptional screening of *sexually transmitted infections/diseases* (STD) and *vaginal candidiasis* (VC) [12], and (v) a better protection for embryos after the early confirmation of pregnancy immediately after the first missed menstrual period [13].

The methods of preconceptional screening of STD and VC are summarised in the Methods section. However, the results of routine preconceptional care including preconceptional screening of STD/VC are difficult to evaluate according to the expected scientific standards. However, we organized a *randomized controlled trial* (RCT) among the female participants in the coordinating centre of the Hungarian preconceptional care in Budapest, between February 1, 1984 and April

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30, 1992, for the estimation of preventive efficacy of periconceptional folic acid-containing multivitamin supplementation for the first occurrence of neural-tube defects [4-7]. Thus we had extra effort to reduce the drop-out as low as possible and to evaluate rigorously the data of pregnancy outcomes of female participants. As the secondary results of this RCT, the results of preconceptional screening of STD/VC, and the efficacy of their treatment can be evaluated and these data are summarised here.

2. Material and Methods

There are 3 criteria of participation in the preconceptional care: no infertility; not currently pregnant and voluntary cooperation. The preconceptional care consists of three steps: (I) check-up of reproductive health, (II) 3 months preparation for conception and (III) a better protection for embryos (Table 1). In general the preconceptional care includes 4 visits of participants. The first visit includes the check up of reproductive health and the start of a 3 month preparation for conception. The task of the second visit 3 months later is to evaluate the results of clinical and laboratory examinations and/or treatments connected with the above two steps, and to encourage couples to achieve conception if their medical conditions are appropriate (if not, these so-called "second" visits are repeated according to necessary medical interventions). The task of the third visit is to confirm the pregnancy with a sensitive pregnancy test and ultrasound scanning after the first missed menstrual period and to provide optimal circumstances for early pregnancy. The fourth visit is a "farewell" meeting at the 10-12 gestational weeks because pregnant women are informed on the possible screening methods during pregnancy and the necessary lifestyle, finally they are referred to the prenatal care clinics with the discharge summary from preconceptional care.

2.1. Preconceptional screening of female participants

The check-up of reproductive health includes preconceptional screening of females for STD and VC at the first visit in the preconceptional care, and its protocol consists of the following methods according to the different STD and VC based on the internationally accepted recommendations during the study period [14,15]:

1. To obtain the history of genitourinary infections and to evaluate the available medical records.
2. To screen all prospective mothers for STD and VC by

Table 1. The three stages of the Hungarian Preconceptional Care, and activities undertaken at each stage.

1) Reproductive health check-up
a) Family history of prospective mother and father, and obstetric history of females.
b) Case history and available medical records of females, e.g., epilepsy, diabetes,
c) Vaginal and cervical smear screening for sexually transmitted infections/disorders.
d) Sperm analysis to detect subfertility and pyosperm (i.e. pus cells in the semen as indicators of sexually transmitted infections)
e) Psychosexual assessment.
f) Blood screening of women to detect rubella seronegativity, or lack of previous exposure to varicella (vaccination will be offered), or HIV positivity. In addition, carrier screening for cystic fibrosis and, more recently, predictive genetic diagnostic tests are carried out at this stage.
2) The 3-month preparation period for conception
a.) Protection of germ cells: avoidance of tobacco, alcohol or narcotic consumption, and taking of unnecessary drugs.
b) Discontinuation of oral contraception, and removal of IUDs (condoms are provided).
c) Occupational history of females
d) Menstrual history; measurement of basal body temperature for detection of hormonal dysfunction (and commencement of further investigation and treatment, if necessary).
e) Start of preconceptional multivitamin supplementation.
f) Recommendation that dental status be checked.
h) Guidelines for physical exercise.
i) Guidelines for healthy diet.
3) Better protection of early pregnancy
a) Undertaking of all additional investigation/treatment necessitated by conditions and disorders detected at the pre-conception check-up.
b) Appropriate investigation and treatment of women shown to suffer from hormonal dysfunction
c) Optimal timing of conception in relation to ovulation.
d) Early pregnancy confirmation using pregnancy tests and ultrasound scanning.
e) Postconceptional multivitamin supplementation.
f) Avoidance of teratogenic and other risks.
g) Referral of pregnant women to prenatal care clinics.

a) Microscopic examination of fresh vaginal smears using a x400 magnification phase-contrast microscope by the assistants after a special educational course

(i) To check vaginal lactobacillary flora,
(ii) To detect Candida infection based on hyphae and/or spores of different Candida species,
(iii) To diagnose Trichomonas infection based on the usually movement of the flagellae (the evaluation of fresh vaginal smear is important within 10 minutes) combined with the raised vaginal pH level between 4.5 and 7.0 [16],
(iv) To detect mixed bacterial flora to diagnose non-specific vaginitis [17], later called bacterial vaginosis, based on 3 criteria: (a) a homogeneous white adherent vaginal discharge, (b) vaginal fluid pH more than 4.5, and (c) presence of the so-called clue cells, i.e. vaginal epithelium cells covered by bacteria.

b) The diagnosis of chlamydial, mycoplasma and ureaplasma infections is based on direct antigen detection (ELISA kits, Dako) in cervical smears.
c) The blood test is used for the detection of syphilis and HIV infection.
d) The diagnosis of genital herpes and gonorrhoea is based on the history of females and/or inspection-examination of their genital organs followed sometimes with blood test.

3. Females with preliminary diagnosis of STD and VC are referred to the gynaecologist of the preconceptional care for final diagnosis and specific treatment (both members of the couples are treated). The success of treatment is checked in the second visit 3 months later in the preconceptional care.

The protocol for the preconceptional screening of females later was changed according to the recent international recommendations [18-24], but here our method used during the study period (1984-1992) is shown.

2.2. Preconceptional screening of male participants

The preconceptional screening of male partners for STD was also part of the check-up examination of reproductive health and its protocol consists of the following points:

1. To obtain the history of genitourinary infections and to evaluate the available medical records.
2. To screen semen from all potential fathers beyond oligoozospermia and/or asthenospermia for the detection of pyospermia based on the selective orthotoluidine staining of leucocytes [14].
3. Males with preliminary diagnosis of STD are referred to the andrologist of the preconceptional care for final diagnosis and specific treatment (both members of the couples are treated). The success of treatment is checked in the second visit 3 months later during preconceptional care.

2.3. Treatment protocol

Patients with different STDs were treated according to defined treatment protocols, here only the treatment of VC is shown. We suggest the combination of vaginal tablet (Canesten® containing 100 mg) and cream of clotrimazole (Canesten®, 200 mg in 20 g cream, i.e. 1%) before conception for women with clinically diagnosed VC. One vaginal tablet in the morning and one vaginal tablet in the evening (i.e. 200 mg daily) for 3 days are prescribed completed by clotrimazole cream for vulvar and anal irritation applied twice daily. The partners of these females are also treated parallel by clotrimazole cream in their penis twice per day. Women with other STD are also treated by previously defined protocols mainly by clindamycin, metronidazole and doxycycline.

2.4. Evaluation of pregnancy outcomes

All pregnancy outcomes were evaluated [5], however, the primary outcome of this study was preterm birth. The gestational age was calculated from the first day of the last menstrual period, the definition of preterm birth was birth less than 37 completed gestational weeks (or less than 259 days) at delivery.) Folic acid-containing multivitamin supplemented and unsupplemented-placebo groups of females were evaluated together in the study because their periconceptional use had no effect on the rate of preterm births [5].

2.5. Statistical analysis

Statistical analyses were carried-out with the software SAS version 8.02 (SAS Institute Ins., Cary, North Carolina, USA). At the comparison of rates of preterm birth, we used chi square test or odds ratio (OR) with their 95% confidence intervals (CI) were calculated.

3. Results

The number of female participants was 7,905 in the preconception care during the study period, but 140 refused to take part in RCT. Of 7,765 female participants, 5,502 had confirmed pregnancy within one year. The distribution of their pregnancy outcomes is shown in the upper part of Table 2. The pregnancy outcomes could not be identified in 49 females due to either changed addresses or stays abroad. Thus, the total number of females with evaluated pregnancies was 5,453, and 4,747 pregnancies ended with live-birth. However, these 4,747 pregnancies resulted in 4,826 babies because 151 were twins (however, 3 twins had live- and stillborn pairs) and 3 triplets. Sets of twins and one triplet were excluded from this analysis due to their high rate

Table 2. The data set of the study, the pregnancy outcomes of female participants and the characteristics of females who delivered live-born babies.

Data set	No.	%
Total number	5,502	100.0
Dropout	49	0.9
Evaluated number of pregnancies	5,453	99.1
Pregnancy outcomes		
Termination		
First trimester	12	0.2
Second trimester after diagnosis of fetal defect	16	0.3
Fetal death		
Chemical pregnancy*	95	1.7
Ectopic pregnancy	11	0.2
Miscarriages	552	10.1
Stillbirths**	20	0.4
Live-birth***	4,747	87.0
Characteristics of 4,672 females who delivered live-born singletons		
Age, yr (mean + S.D.)	26.9 + 3.4	
Primiparous (%)	89.9	
Educational level (%)		
Primary school	6.4	
Secondary school	33.8	
High (university and college)	59.8	
Prepregnancy body weight, kg (mean + S.D.) 57.2 + 7.5		
Smoking^{o} (%)	7.8	
Drinking^{oo} (%)	0.1	

**Positive pregnancy test without any later clinical symptoms of pregnancy*
***Three stillbirths occurred in twin pregnancies in which the other twin was liveborn*
****Of 4,747 livebirths, 151 were twins and 3 triplets*
^{o}During the study pregnancy
^{oo}More than one drink per week during the study pregnancy

of preterm birth. Thus the rate of preterm births was evaluated in 4,672 singleton live-born babies.

The lower part of Table 2 summarizes the characteristics of female participants. There was a high proportion of primiparae because females preparing their first pregnancy preferred the participation in the preconceptional care. In addition the proportion of females with high education was 3 times higher than that of the Hungarian pregnant population during the study period.

Table 3 demonstrates the results of preconceptional STD screening and the rate of preterm births according to the treatment or lack of treatment. Five groups of females were differentiated.

(i) Of 4,672 females, 215 (4.6%) visited the preconceptional care with known clinical diagnosis of VC due to symptoms and previous laboratory findings. Most females were treated earlier but not successfully, because their VC was confirmed without other STD at the first visit in the preconceptional care.

(ii) Of 4,672 females, 691 females (14.8%) had no recent history or they did complain about the symptoms of VC but the preconceptional screening detected their *asymptomatic vaginal candida colonisation* (AVCC) without other STD.

(iii) Both VC and STD occurred in 466 females (10.0%).

(iv) Of 4,672 females, 795 (17.0%) had STD without VC.

Among STD, chlamidial (16.6%) and trichomonas (15.1%) infections showed the highest occurrence, followed by bacterial vaginosis (11.0%) and genital herpes (6.7%). Mycoplasma hominis (1.1%) and ureaplasma (1.9%) infections were detected rarely, while gonorrhoea, syphilis and HIV infection did not occur. (Some females had more than one STD.)

The gynecologist and andrologist of the preconceptional care recommended our treatment protocol after the confirmed diagnosis of STD and VC for the pairs. However, some women did not want to receive this treatment due to different reasons (the lack of serious symptoms, they were against the drug treatment in general, etc.). Most couples particularly females followed our treatment protocol according to the personal interview in the second visit. Their information was confirmed by the gynecological and laboratory examination in most women. The treatment was continued in 8% of women due to the repeated positive laboratory findings by 2 vaginal tablets of clotrimazole but for 6-12 days completed with clotrimazole cream treatment twice per day.

(v) The fifth and largest group of women had no symptoms of any STD and VC, and this group was used as reference.

We were able to evaluate the pregnancy outcomes of females in the preconceptional care on the basis of discharge summary of their deliveries; all deliveries took place in inpatient obstetric clinics and the birth attendants were obstetricians. Here only the rate of preterm births is shown (Table 3).

First the compliance of female participants was evaluated. The proportion of women who did not follow our treatment protocol was higher in the group of VC than in the group of AVCC (OR with 95% CI: 1.8, 1.3-2.6; χ^2_1=10.3, p=0.001). The compliance of female participants with combination of VC and STD, or STD without VC was much higher than that of females with VC (OR with 95% CI: 8.7; 6.0-12.7; χ^2_1=169.0, p<0.0001).

Table 3. The data of different study groups with or without treatment and the rate of preterm births.

Study groups	Total		Treated		Untreated		Comparison	
	No.	%	No.	%	No.	%	OR	95% CI
Clinical VC	215	4.6	157	73.0	58	27.0		
Preterm birth	13	6.1	7	4.5	6	10.3	2.5	0.8-7.7
Subclinical AVCC	691	14.8	573	82.9	118	17.1		
Preterm birth	34	4.9	23	4.0	11	9.3	2.5	1.2-5.2
Combination of VC-AVCC and other STD	466	10.0	456	97.9	10	2.1		
Preterm birth	25	5.4	22	4.8	3	30.0	8.5	2.0-34.9
Other STD without VC-AVCC	795	17.0	771	97.0	24	3.0		
Preterm birth	58	7.3	47	6.1	11	45.8	13.0	5.5-30.7
No STD, no VC-AVCC	2,505	53.6	-	-	2,505	100.0	-	-
Preterm birth	214	8.5	-	-	214	8.5	-	-
Total	4,672	100.0	1,957	41.9	2,175	58.1		
Preterm birth	344	7.4	99	5.1	245	11.3	2.4	1.9-3.0

VC = vaginal candidiasis
AVCC = asymptomatic vaginal candida colonisation
STD = sexually transmitted infection/disease

Secondly, the rate of preterm births was analysed in the different study groups. The reference group of females without STD and VC had a high rate of preterm births (8.5%). However, it is corresponded to the very high Hungarian figure during the study period (about 9%), if we consider that our participants had a higher socioeconomic status. The highest preterm rate was seen in the group of untreated women with STD but without VC.

Thirdly, our findings provide evidence for the preterm preventive effect of clotrimazole treatment in women with VC (4.5% vs. 10.3%) and AVCC (4.0% vs. 9.3%). Untreated VC (10.3%) and AVCC (9.3%) associated with a nearly similar high rate of preterm births (OR with 95% CI: 1.1, 0.4-3.2). There was a very obvious difference in the rate of preterm births (4.8% vs. 30.0%) in treated and untreated females with both STD and VC. The preterm protective effect of other drugs used in females with STD, but without VC was also shown, though a higher rate of preterm births (6.1%) was found in this treated group of the study.

Finally an unexpected finding of this study was the previously mentioned high rate of preterm births in women without diagnosed VC and STD who therefore were untreated. This rate was higher than the rate of preterm births in the treated females with VC and AVCC together (OR with 95% CI: 0.5, 0.3-0.7; χ^2_1=15.9, p<0.0001). This difference is also obvious between the total group of women with treated STD and VC (OR with 95% CI: 0.57, 0.44-0.73; χ^2_1=20.5, p<0.0001).

Our study was not appropriate to evaluate the possible associations between VC-AVCC/STD and other pregnancy outcomes due to the limited number of offspring in the different groups of fetal deaths. Nevertheless it is worth noting that the rate of ectopic pregnancies was 0.2% (Table 2) though the Hungarian population figure was 0.9% during the study period. Thus the effective treatment of STD may contribute to the reduction of ectopic pregnancies.

4. Discussion

The findings of our study confirmed the well-known association between women with STD and a higher risk for preterm birth. However, our study showed an association of pregnant women with VC-AVCC and a higher risk of preterm birth as well. In addition a preterm preventive effect of clotrimazole treatment was found in the preconceptional period of women who are preparing their pregnancy. Finally the unexpected high rate of preterm births in female participants without diagnosed VC and STD may raise the suspicion that a certain part of these females had undetected VC-AVCC or STD in the preconceptional screening or had a new infection in early pregnancy.

Our study has many strengths: (i) women were participants in the preconceptional care (instead of the selected patients in genitourinary clinics); (ii) all women were screened preconceptionally for STD and VC-AVCC; (iii) about 80% of women with VC-AVCC were treated by the similar protocol of vaginal and topical clotrimazole; (iv) about 90% of females were primiparae without previous preterm birth; (v) their male partners were also examined and treated; (vi) pregnancy outcomes including preterm births were medically recorded, and

gestational age was confirmed by ultrasound scanning during pregnancy.

However, there are many weaknesses of our study. (i) The social selection of participants was obvious in the preconceptional care. (ii) The major drawback that our preconceptional screening of STD and VC-AVCC was based on the routine methods without the use of the recently introduced most sensitive tests [18-27], thus many women with STD might not be detected. (iii) Microscopic examination is not sensitive enough for the diagnosis of VC, to discriminate between colonized and non-colonized women; in addition this approach is not sensitive enough to control the efficacy of antimycotic treatment. (iv) We were not able to differentiate the lactobacillary grades [26]. (v) The recently classified pathological manifestations of vaginal infections such as aerobic vaginitis [27] and cytolytic vaginosis [28] were not considered. (vi) There were a limited number of untreated females with different STD and VC-AVCC in our study. (vii) We did not differentiate the three groups of preterm birth, i.e. spontaneous (about 50%), preterm premature membrane rupture (30%) and medically induced preterm birth due to maternal and genital complications (20%) [29]. (viii) We had no chance to examine our pregnant women at the time of delivery regarding the clinical symptoms of STD and VC (e.g. amniorrhexis), in addition to the laboratory detection of VA or AVCC.

The rate of preterm births was very high (9.2%) in Hungary during the study period, therefore preterm birth is the most common cause of infant mortality and morbidity [30]. Several causes of preterm births are known but genital tract infections are believed to account for 40% of spontaneous preterm births [31-34]. Microorganisms from vagina and cervix during pregnancy can gain access to the uterine cavity and infect the placenta, membranes and fetus due to subclinical endometritis [35]. Several studies and RCT indicated the beneficial effect of antimicrobial drugs in the prevention of preterm delivery, particularly when it was caused by premature rupture of the membranes due to concomitant infections [e.g. 36,37]. However, Cochrane Systematic Review [38] and meta-analysis [39] of available data showed conflicting results on the benefits of routine screening and treatment for vulvovaginitis and bacterial vaginosis.

The occurrence of pregnant women with vaginal candida colonisation is high (up to 40%), two fold higher than in non-pregnant women [12]. This phenomenon may be connected with increased levels of circulating oestrogens, deposition of glycogen and other substrates in the vagina, and the well-known immunological changes in pregnant women [40]. Screening for VC was not recommended during pregnancy because moderate to heavy candida colonisation did not associate with preterm birth in a large observational study [41]. However, clotrimazole treatment of vulvovaginitis and VC in pregnant women associated with a lower rate of preterm births in the two data sets of population-based Hungarian Case-Control Surveillance of Congenital Abnormalities [42-44]. In addition, Kiss et al. [45] organized a RCT in Austrian pregnant women based on their antenatal screening of STD and VC between 15th and 20th gestational weeks. Of 289 women with VC treated by clotrimazole intravaginally 100 mg daily for 6 days, 8 (2.8%) while of 291 women with untreated VC, 22 (7.6%) had preterm births (OR with 95% CI: 0.35, 0.14-0.84, p=0.009). A similar beneficial preventive effect was not found after the treatment of bacterial vaginosis.

These controversial findings stimulated us to evaluate our available material which seemed to be appropriate to provide new data for this debate. In addition, Okun et al [46] recommended further controlled studies of antibiotic therapy initiated early in pregnancy to achieve sufficient power to asses clinically important findings for the reduction of preterm birth in pregnant women affected with STD. Here we summarized the main results of this preconceptional screening on the basis of an available but previously not evaluated material.

The explanation for the above controversial results may be the various times of screening and treatment. The previous observational Hungarian studies showed the efficacy of early treatment of VC before 20th gestational week of pregnancy [42-44]. Austrian pregnant women were screened and treated between 15 and 20 gestational weeks [45]. The time of screening and treatment was in the preconceptional period in this study. However, the screening was performed at 24-28 gestational weeks of pregnancy in the study of Cotch et al. [41]. Thus, the earlier in gestation the abnormal flora of pregnant women is treated, the better is the chance that the intervention may reduce the incidence of preterm birth. In addition it is worth considering the possible genetic differences of pregnant women as well [40,47].

The importance of time is that very early spontaneous preterm births are more likely to be of infectious etiology than preterm birth just before term [48,49]. Thus the optimal time for the screening and treatment of abnormal vaginal flora including VC-AVCC is the prepregnancy-preconceptional period or early pregnancy. If the longer abnormal colonisation remains untreated, the greater is the chance of microorganisms ascending through the cervix into the decidua and initiating the inflammatory process which may lead to labour. The explanation for unsuccessful results in several studies was that

antimicrobial drug treatments were administered too late in pregnant women with genital infections [50].

The possible preterm birth preventive effect of clotrimazole is not a direct effect but it may be connected with the reduction of abnormal candida colonization of the female genital organs. In addition, clotrimazole may help in the restoration of the abnormal vaginal flora, and clotrimazole has some antibacterial and antiprotozoal effects as well [51,52]. Finally the predominance of candida species in the vagina may help the development of bacterial vaginosis or the disease-inducing effect of pathogenic microorganisms. However, further studies are needed to better understand the interaction between candida and other microbes in vaginal infections [53].

An unexpected finding of our study is the lower rate of preterm births in newborn infants born to mothers with VC-AVCC and/or STD after appropriate treatment than in the babies of mothers without these recognized genital tract infections and – of course – without treatment. Thus a certain part of these women may have unrecognized genital tract infections including AVCC, and this finding indicates the limited efficacy of our preconceptional screening methods of STD and VC-AVCC. Thus it is necessary to introduce the recent, more effective laboratory methods for preconceptional screening of STD and VC-AVCC in the preconceptional care.

In conclusion, our study confirmed the well-know association of maternal STD and a higher risk for preterm birth, but showed an association between maternal VC-AVCC and a higher risk of preterm births as well. However, a certain part of these preterm births is preventable by use of clotrimazole at the appropriate time.

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