ORIGINAL ARTICLE

Correlation between cervical infection and preterm labor

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ABSTRACT

Aim To investigate a correlation between cervical canal infection and imminent preterm labor and to identify most frequent pathogens.

Methods A prospective study was conducted in obstetrics/gynecology departments of Health Center and the University Clinical Center Tuzla, and General Hospital Tešanj (Bosnia and Herzegovina, B&H) between October 2013 and May 2014. An examined group included 50 healthy pregnant women with singleton pregnancy of the gestation age between the 28th and 37th week, with cervical changes that are related to imminent preterm labor. Changes were detected by ultrasound biometry of cervix and modified Bishop score. A control group included 30 healthy pregnant women with singleton pregnancy of the gestation age between the 28th and 37th week of pregnancy without signs of imminent preterm labor. Cervical mucus was microbiologically analyzed for identification of pathogens.

Results The infection in cervical canal was proven in 35 (70%) examinees and four (13%) patients from the control group (p=0.015). In seven (20%) cases each *Ureaplasma* and *Mycoplasma* were detected followed by *E. coli* in five (14%) cases (p=0.001).

Conclusion Cervical canal infection is associated with changes on cervix and premature rupture of fetal membranes, i.e. preterm labor and imminent preterm labor. Screening for infection before pregnancy should be the main task of family doctors as well as gynecologists.

Key words: uterine cervicitis, premature birth, pregnancy complication

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INTRODUCTION

Preterm labor is defined as labor that begins before 37 completed weeks of pregnancy (1-3).

More than 12% of infants born in the USA are preterm (1). Being one of most serious medical problems, it causes 28% of all early neonatal deaths which, are not related to congenital malformations (deaths within the first 7 days of life) (2). Preterm birth rates have been reported to range from 5% to 7% of live births in some developed countries, but they are estimated to be substantially higher in developing countries (10-13%), even more, in some countries (Pakistan, Indonesia, Mauritania) very high rates (15-16%) were reported (1-4). Unfortunately, there are no official data about prematurity prevalence for Bosnia and Herzegovina. Preterm birth is the major cause of neonatal morbidity and mortality in developed countries. Sequelae of preterm birth are common in the neonatal period, they may persist into adulthood and are inversely related to gestational age (1-5).

There are numerous causes of preterm labor, however, infection of the birth passageway is one of the most important causes due to its high participation in the total number of preterm labors and also due to the fact that it poses a double burden and threat for newborns (1-5). At least 40% of preterm births are associated with intrauterine infection (3-5). In individual cases it is often difficult to determine whether infection is the cause or consequence of the processes leading to preterm delivery (5).

However, infection and inflammation generated by infection are primary causes of a substantial proportion of preterm births evidenced by the facts that amniotic fluid of patients with preterm labor has higher rates of microbial colonization and levels of inflammatory cytokines than preterm patients not in labor and term patients in labor; intrauterine or systemic administration of microbes or microbial products to pregnant animals can result in preterm labor and delivery, extrauterine maternal infections such as pyelonephritis, pneumonia and periodontal disease have been associated with premature parturition, subclinical intrauterine infections are associated with preterm labor and delivery, patients with intra-amniotic infection or intrauterine inflammation (i.e. elevation of amniotic fluid cytokines and matrix-degrading enzymes) identified as early as the mid-trimester are at risk for subsequent preterm delivery (4-10).

Infection in the birth canal, primary in cervical canal, is connected with changes on cervix, premature rupture of fetal membranes and prematurity as well as with direct impact on a newborn who is immunologically insufficiently protected from infection to which it is exposed (8-11). A timely detection of the infection and the treatment in preconception and early conception period would certainly decrease the prematurity rate thus reducing perinatal morbidity and mortality, while overall health status of pregnant women would have a favorable course (8-12).

In developing countries the incidence of lower genital tract infections during pregnancy is rather high and ranges between 40-54% including pregnant women with no signs of infection (1,2, 5-10). Such infections include vulvovaginitis (colpitis), cervicitis, bartolinitis. Intrauterine infection is often associated with preterm delivery. There are numerous reports showing the role of infection in initiating the mechanism of preterm labor (13-18). Infections such as pyelonephritis or pneumonia are often associated with preterm delivery, yet the treatment of asymptomatic bacteriuria can prevent the activation of the mechanism of preterm labor (7,11, 18-21). Non-manifest (subclinical) and manifest intrauterine infections also lead to the occurrence of preterm delivery, while the treatment of ascending intrauterine infection with antibiotics can prevent prematurity. Non-manifest (subclinical) or clinically manifest infection of the genital tract is responsible for at least one third of preterm deliveries (7,11,18). Experimental studies have proven that intrauterine infections or systemic administration of products of microorganisms to pregnant animals may result in preterm labor (11, 18-21).

In Bosnia and Herzegovina the incidence of lower genital tract infections during pregnancy is not known. Also, no studies dealing with this issue have been conducted.

The aim of the study was to investigate the presence of cervical canal infections in threatened preterm labor.

PATIENTS AND METHODS

Study design and examinees

The prospective study was conducted in obstetrics department of obstetrics/gynecology departments of Health Center in Tuzla and the Gyne-

cology and Obstetrics Clinic of the University Clinical Centre in Tuzla, and General Hospital Tešanj, in the period September 2013 - May 2014. The selection of medical institutions for the investigation was based on population similarity which they cover.

The examined group included 50 healthy pregnant women with singleton pregnancy of the gestation age between the 28th and 37th week, who had cervical changes related to threatened preterm labor detected by transvaginal ultrasound measurements of the cervix (Cervical score, CS) and modified Bishop score (BS), without any other known cause of preterm delivery, maternal of fetal, including uterine anomalies or fetal anomalies.

The control group included 30 healthy pregnant women with singleton pregnancy of the gestation age between the 28th and 37th week of pregnancy who had no changes indicating threatened preterm labor, without any other known cause of preterm delivery, maternal or fetal.

Approvals of the Ethics Committees of all institutions participating in the study were obtained (Health Center Tuzla, General Hospital Tešanj).

Methods

Modified Bishop score used in this study includes assessment of cervical length, cervical softening, canal dilatation, amniotic membrane integrity. To be related to imminent preterm delivery, there had to be a minimal number of changes or minimal degree of changes measured by Bishop score marked with Bishop score 2 or more, i.e. at least two minimally changed parameters or one significantly changed parameter (Table 1).

A condition of uterine cervix was checked by transvaginal ultrasound and elements were monitored and scored according to cervical score (CS) (4,11), which includes measurement of cervical length, dilatation of cervical canal and openness of internal ostium: closed internal ostium (T), partially (moderate) open ostium (Y), and wide open ostium (U). Ideal Cervical Score includes cervical length of ≥40 mm, closed cervical canal and "T" shape of internal ostium (Table 2).

Table 1. Assessment of the condition of cervix based on cervical length, dilatation, consistency and amniotic membranes integrity

	Bishop score (50 patients)			
Parameters	0	1 16 (32%)	2 30 (60%)	3 4 (8%)
Dilatation of cervical canal (cm)	closed	1 – 2	3 – 4	5 and more
Cervical length (cm)	3	2	1	0
Cervical consistency	hard	middle	tempered	soft
Amniotic membrane integrity	existing	-	-	ruptured

Table 2. Assessment of the condition of cervix by transvaginal and transabdominal ultrasound based on measurement of cervical length, dilatation and internal ostium shape

	Cervical score		
	Score		
Parameters	0	1	2
Cervical length (mm)	30 and more	20	less than 20
Dilatation of cervical canal (mm)	0	3 -5	more than 5
Shape of internal ostium	T	Y	U

T, closed internal ostium; Y, partially (moderate) open ostium; U, wide open ostium (U)

Each patient was followed during regular obstetric check-ups in intervals of 15-21 days.

All patients had cleanliness of their cervical mucus determined by taking a smear of cervical canal for microbiological analysis with identification of microbiological agent. Microbiological analyses were done at the Department of Microbiology of the General Hospital Tešanj. Smears were cultivated at blood agar for detection of gram positive cocci, endo agar for detection of gram negative bacilli, liquid Sabouraud agar for detection of *Candida* spp. For detection of the presence of *Streptococcus agalactiae* (Group B) or *Enterococcus fecalis* (Group D), Mastsrep test (Mastgroup, Germany) was used. For detection of the presence of *Staphylococcus* spp. Maststaph test (Mastgroup, Germany) was used. Sensitivity or resistance of bacteria to antibiotics is examined. *Mycoplasma hominis* (*M. hominis*) and *Ureaplasma urealyticum* (*U. urealyticum*) were detected and identified by specific API tests (Biomerieux, Marcy l'Etoile, France) as well as their sensitivity/resistance; the procedure takes up to 48 hours.

For detection of *Chlamydia trachomatis in vitro* diagnostic Rapid test (of high sensitivity) Accubiotech Co. Ltd., China, Mainland) was used.

Detection of *Trichomonas vaginalis* was done by examination of a native preparation, where only mobile trophozoites of *Trichomonas* were noted.

Statistical analysis

Results are shown in the form of contingency tables (numbers with two decimal places). The χ^2 test, standard deviation, Fisher test, Student

Table 3. Distribution of microbiological agents in single/mixed infections of preterm patients according to Bishop score (BS)

Isolated microbiological agent	No (%) of patients							
	BS 1		BS 2		BS 3		TOTAL	
	Single infection	Mixed infection	Single infection	Mixed infection	Single infection	Mixed infection	Single infection	Mixed infection
U. urealyticum	3 (19)	1 (7)	3 (22)	1 (8)	1 (17)	0	7 (20)	2 (6)
M. hominis	2 (13)	1 (6)	2 (15)	0	1 (17)	0	5 (14)	1 (3)
E. coli	1 (6)	1 (6)	1 (8)	1 (8)	0	0	2 (6)	2 (6)
Trichomonas	1 (6)	0	1 (8)	0	0	0	2 (6)	0
Streptococcus haemoliticus group B	0	1 (6)	0	1 (8)	1 (17)	1 (17)	1 (3)	3 (8)
Chlamydia trachomatis	1 (6)	0	1 (8)	0	0	0	2 (6)	0
Enterococcus faecalis	1 (6)	1 (6)	0	0	0	0	1 (3)	1 (3)
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0
Staphylococcus aureus	1 (6)	1 (6)	1 (8)	1 (7)	1 (16)	1 (16)	3 (8)	3 (8)
Total	10 (62)	6 (38)	9 (69)	4 (31)	4 (67)	2 (33)	23 (66)	12 (34)

T test, and relative risk (RR) were used for the analysis. The significance level was $p \leq 0.05$.

RESULTS

The presence of infection in cervical canal was proven in 35 (70%) examinees and four (13%) patients from the control group (p=0.015).

Presence of the infection in cervical canal in examinees with Bishop score 1 was found in 16 (32%) cases, in examinees with Bishop score 2 in 30 (60%) and examinees with Bishop score 3 in four (8%) cases without statistically significant difference (p=0.618) (Table 1).

Most often the infection had one pathogen isolated, in 23 (66%) cases, while in 12 (34%) more than one pathogenic agent were found in cervical canal smear (p=0.001) (Table 3).

In the most cases *U. urealyticum* and *M. hominis* were detected, in seven (20%) cases each (p=0.001), followed by *E. coli*, in five (14%), *Trichomonas* in four (11%), *Streptococcus haemoliticus* group B and *Chlamydia trachomatis* in three (9%) cases each; other infectious agents made a total of six (17%) cases (*Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) (Table 4).

In patients from the control group infections of cervical canal were found in four (13%) patients with equal distribution of *E. coli, U. urealyiticum, Trichomonas vaginalis,* and *Enterococcus foecalis* in one (3.3%) case each, while infections caused by *Mycoplasma*, *Chlamydia* and *Streptococcus* were not found (Table 4).

Table 4. Pathogens isolated from cervical mucus of examinees and controls

Isolated pathogen	No (%) of patients	
	Examinees	Controls
Ureaplasma urealiticum	7 (20)	1 (3.3)
Mycoplasm hominis	7 (20)	0
Escherichia coli	5 (14)	1 (3.3)
Trichomonas vaginalis	4 (11)	1 (3.3)
Streptococcus haemoliticus group B	3 (9)	0
Chlamydia trachomatis	3 (9)	0
Enterococcus faecalis,	2 (6)	1 (3.3)
Pseudomonas aeruginosa	1 (2)	0
Staphylococcus aureus	3 (9)	0
Total	35 (100)	4 (13)

DISCUSSION

Preterm labor is a huge health problem in perinatology. Thus, there have been continuous efforts of obstetricians to prevent preterm labor, because the selection of pregnant women who are at risk and prevention of prematurity would significantly decrease the rate of perinatal morbidity and mortality (1-5, 10-13). The link between infection and preterm labor has been confirmed by many authors (5-10, 17-30).

This study has shown that infection of cervical canal healthy pregnant women with singleton pregnancy of the gestation age between the 28th and 37th week was frequently associated with changes (70%) on uterine cervix, which could be easily detected by Bishop score and cervicometric score, with statistically significant difference when compared with the control group. Similar results are presented in numerous studies (7, 18-30). Romero reported 42% of preterm birth patients with positive amniotic fluid culture (7). Verma at al. concluded that urogenital infection was 2.1 times (36.54 %) frequently noticed in women with preterm labor compared to the patient with term birth (14). In our study one-agent was most frequently isolated (66%), but in 34% of cases there was an infection with several microbiological agents. When compared to other reports we did not find any study investigating mixed versus isolated infections. It could be explained by the

fact that only one pathogen is sufficient to cause tissue damages and preterm membrane rupture or cervical changes (4-7, 20).

Analyzing the condition of cervix, no connection between the severity of a change or higher Bishop index or cervicometric index and the number of different microbiological agents was found. It could be explained by the fact that toxicity of only one agent is sufficient to cause changes on uterine cervix, which can be registered either by the Bishop score or cervicometric screening (1, 6-7, 20-27). In 4% of examinees in this study the infection was connected with premature rupture of fetal membranes and preterm labor.

It is important to note that even 40% of examinees had infection caused by *M. hominis* or *U. urealyticum*, which places those microbes in the center of attention. Mycoplasma and ureaplasma are conditionally pathogenic bacteria having in mind that they make a part of normal flora of female lower genital tract (20-27). Similar results are reported by numerous authors (20-27) suggesting a need for testing of pregnant women for this infection. Averbach (21) reported that prevalence of *mycoplasma* colonization at the first prenatal visit was 8.4%, while the incidence of preterm delivery was 16.7%. The incidence of preterm delivery did not differ with respect to *mycoplasma* colonization. The crude odds ratio for preterm delivery among women with *mycoplasma* colonization versus those without it was 1.27 (95% confidence interval, 0.02–14.78). A high percentage of infection of upper respiratory tract of premature newborns was found (20). In a recent cohort study of infants under 33 weeks gestation, Sung reported that *Ureaplasma* spp. was detected during the first week of life in tracheal aspirates or nasopharyngeal aspirates in 35% of infants (25). Kwak at al. reported that prevalence of positive vaginal fluid cultures for genital mycoplasma was 62.5% in the group that included 99 patients carrying only ureaplasma and 13 carrying both (ureaplasma and mycoplasma) organisms, while no patients were found to carry only mycoplasma. Compared to patients positive only to ureaplasma, patients with both organisms showed significantly decreased gestational age at birth (26).

E. coli was found in 14% of examinees, which makes it the second most frequent pathogen in cervical mucus that can be associated with preterm delivery. Similar results have been published by other authors too (23-29). Rasa reported prevalence of *E. coli* vaginal colonization of 19% (29). Krohn evaluated the relationship of vaginal *Escherichia coli* colonization to birth weight <1500 g and other perinatal complications in a cross-sectional study of 2646 women and reported that vaginal *E. coli* colonization was more strongly associated with delivery at <34 weeks (relative risk 1.7; 95% confidence interval 1.3-2.3) (26).

According to Koumans, bacterial vaginosis is the most common cause of vaginal symptoms among women. The prevalence in the United States is estimated to be 21.2 million (29.2%) among women aged 14–49 (28).

Trichomonas infection was registered in 11% of examinees and *chlamydia* infection in 9% of examinees in this study. Krashin found *T. vaginalis* in 19% examinees (30), while reports of chlamydia infections varied from 3-13% (13-15). Low rate of those two pathogens in the presented study was less expected but the explanation could be found in frequent usage of vaginal antibiotics containing metronidazole and tetracycline, which are commonly used for the treatment of vaginal discharge even without doctor's prescription (6,19).

In conclusion, an infection of the birth passageway, primarily the existing infection in cervical canal, is associated with changes on cervix and preterm rupture of fetal membranes, i.e. preterm labor and threatened preterm labor. Screening for infection before pregnancy should be the main task of family doctors as well as gynecologists. Detection and treatment of infections in early pregnancy are already compromised due to limited usage of antibiotics, but the treatment should start as soon as possible.

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TRANPARENCY DECLARATION

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Infekcija cervikalnog kanala i prijevremeni porođaj

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SAŽETAK

Cilj Ispitati povezanost infekcije cervikalnog kanala i prijetećeg prijevremenog porođaja.

Metode Prospektivna studija provedena je u ginekološko-akušerskim ambulantama Doma zdravlja i Univerzitetskog kliničkog centra u Tuzli te Općoj bolnici u Tešnju, u periodu od oktobra 2013. do maja 2014. godine. Ispitivanu skupinu činilo je 50 zdravih trudnica s jednoplodnom trudnoćom, gestacijske dobi između 28. i 37. nedjelje, kod kojih je na osnovu ultrazvučne biometrije i modificiranog Bishop-skora utvrđeno stanje prijetećeg prijevremenog porođaja, dok je kontrolnu skupinu činilo 30 zdravih trudnica s jednoplodnom trudnoćom, gestacijske dobi između 28. i 37. nedjelje, kod kojih nisu pronađene promjene koje bi upućivale na stanje prijetećeg prijevremenog porođaja. Kod svih pacijentica je utvrđeno stanje čistoće cervikalne sluzi uzimanjem brisa cervikalnog kanala i izolovanjem mikrobiološkog agensa.

Rezultati Prisustvo infekcije u cervikalnom kanalu dokazano je kod 35 (70%) ispitanica i 4 (13%) pacijentice kontrolne skupine. Najčešće su otkrivane *Ureaplasma* kod 7 (20%), *Mycoplasma* kod 7 (20%) i *E. coli* kod 5 (14%) ispitanica (p=0.001).

Zaključak Infekcija porođajnog kanala udružena je s pojavom promjena na cerviksu i prijevremenim prskanjem plodovih ovojnica, odnosno s prijevremenim porođajem i prijetnjom prijevremenog porođaja. Vodeći zadatak porodičnih liječnika, kao i ginekologa, morao bi biti probir na infekcije cervikalnog kanala prije nastanka trudnoće.

Ključne riječi: cervicitis, komplikacije u trudnoći, prijevremeni porođaj