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INFECTION MAJOR RISK FACTOR MATERNAL AND FETAL

ABSTRACT OF DOCTORAL THESIS

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SUMMARY

Introduction

This paper brings to the fore the relationship between preterm birth and infection. Preterm birth is frequently associated with intra-amniotic infection, even if this cannot be targeted at the outset. Microbiological studies have shown that intra-amniotic infection is responsible for 25% to 40% of preterm births, although this percentage is underestimated due to the conventional bacterial detection methods used.

The difficulty in this area is that there are numerous studies, statistics, case reports with conflicting results. A significant percentage of preterm births are associated with the presence of an infectious agent and a severe inflammatory process in the placenta and amniotic fluid.[1,2] The mechanism by which bacteria can be detected and how they manage to cause preterm birth is an area that is being explore.[2]

Most studies have focused on infections caused by group B Streptococcus or Chlamydia trachomatis, but not so for infections or rather vaginal colonisation with Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum and Ureaplasma parvum. We know that these are opportunistic pathogens and are frequently identified in the vaginal smear of healthy pregnant women.

These observations have led to the motivation to carry out a study on this topic.

Given the complexity of the topic investigated, we have structured the paper in the following sequence:

- Synthesis of opinions regarding the current state of knowledge in the field of preterm birth, inserting observations, rectifications and additions, or selecting and/or repeating many of the statements analysed (general part).
- Identification of the demographic and clinical factors involved in the pathogenesis of preterm birth and the role of genital infection with Ureaplasma spp. on pregnancy outcome and prognosis of the newborn. (special part)

I. GENERAL PART

1. Preterm birth

According to the World Health Organisation and the International Federation of Gynaecology and Obstetrics (FIGO), preterm birth is defined as birth occurring before 37 completed weeks[1].Thus, spontaneous preterm birth, prematurity and low birth weight have been associated with increased risk of neonatal mortality and morbidity (85%).

1.1. Epidemiology

According to the National Institute of Statistics, the preterm birth rate in Romania is between 8-10% (8.3% of 180735 preterm births in 2021).

1.2. Etiopathogenesis of spontaneous preterm birth

1.2.1. Mechanisms of spontaneous preterm birth

Both preterm and term labour share common pathways, which include: increased uterine contractility, cervical ripening, and premature rupture of membranes[3].

Studies debate the role and importance of triggers of preterm birth and identify the sequence of mechanisms involved.

Prostaglandins play an important role in the mechanism of onset of preterm labour in association with infection, but their impact on the onset of preterm labour in the absence of infection remains an area for further research.[4] They can induce: myometrial contractility, maintenance of proteolysis at the cervical level and at the extracellular matrix level of the amniotic membranes, thus inducing cervical ripening, as well as fetal membrane rupture, stimulate decidual/membranous activation .[5]

1.2.2. Genetic factors in preterm birth

Recent research suggests that genetic factors contribute to the risk of preterm birth, particularly through variants in the maternal genome. There is evidence for an environmental factor-gene interaction in infection-associated preterm birth.[6]

1.2.3. Inflammation, stress and term/preterm birth

Both term and preterm labour are associated with inflammation; this mechanism involves leukocytic infiltrate in the myometrium, cervix, decidua and fetal membranes[7].

During labor peripheral leukocytes invade uterine tissues and secrete bacterial mediators such as proinflammatory cytokines that initiate uterine activity and labor. When these pathophysiological mechanisms occur too early in pregnancy they are responsible for preterm birth.[4]

1.2.4 Role of inflammatory mediators in premature rupture of fetal membranes

Premature rupture of membranes is one of the main risk factors for preterm birth. The amniochorion has a unique physiology characterized by distinct molecular, enzymatic and biomechanical transformations.[7] A decidual infiltrate dominated by neutrophils, which are a rich source of proteases, elastases that degrade the extracellular matrix and MMPs, is associated with premature membrane rupture induced by membrane detachment. [52,53] This phenomenon occurs in the presence or absence of infection. .[7,8]

1.3 Infectious implications in preterm birth

1.3.1 Association between intrauterine infections and preterm birth

Based on the results of cultures taken from pregnant women with infections associated with preterm birth, the most common microorganisms identified in fetal membranes and amniotic fluid are Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis, Gardnerella vaginalis, Group B Streptococcus, Bacteroides and Escherichia coli.[4] The amniotic cavity is normally considered to be sterile and thus isolation of any microorganism from the amniotic fluid is clear evidence of microbial invasion. This may be present in the absence of clinical signs and symptoms of infection.

Cultures underestimate the frequency with which microbial pathogens are involved [4].

The placental microbiome is different from other microbiota in the body, showing great phylogenetic similarity to the oral flora of the non-pregnant woman, raising the hypothesis that the fetus does not develop in a sterile environment.[4] The implication of altered vaginal microbiome in preterm birth remains a controversial topic.

1.3.2 Pathophysiology

The mechanism by which microorganisms enter the amniotic cavity is not fully understood. Four routes of invasion of the amniotic cavity by pathogenic microorganism are

described in the literature. The most common route by which bacteria reach the decidua, placenta and fetal membranes is the ascending route from the lower genital tract (vagina, cervix). [4,9]

The mechanism of premature rupture of membranes may be associated with invasion of bacteria via the ascending pathway from the lower genital tract. The location of infection at the choriodecidual junction may also be responsible for premature rupture of membranes [5].Microbial invasion of the amniotic cavity may occur as a result of dissemination of microorganisms from the choriodecidual level or directly from the vagina via the membrane continuity solution. Eventually, these micro-organisms affect the fetus, succeeding in infecting the product of conception. [5]

Emerging theories suggest that microorgansims are identified by components of the immune system, triggering a cascade of events that will culminate in premature birth. [10]

1.3.3 Role of proinflammatory agents in preterm birth

Infection is responsible for the initiation of labour through different cellular mechanisms; the main pathways intricate in this process are contraction-associated proteins (CAP), prostaglandin E2 (PGE2), Rho factor-associated protein kinase and matrix metalloproteinases (MMPs). [12,16] Arachidonic acid (AA), toxins, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), nuclear factor κ-activated B-cell chain enhancer (NF- κB) induced infection mediate the occurrence of PGE2-induced myometrial contraction. PGE2, Rho/ROCK, NF- κB and infection-induced MMPs act directly at the myometrial fibre level initiating labour.[5] Biosynthesis of prostaglandins (IL-1,IL-6, IL-8 and IL-10) can be stimulated either by bacterial invasion or by the host organism through mediators it synthesizes in response to bacterial presence.

1.3.4. Neonatal consequences of preterm birth

The trigger for neonatal complications is inflammation, which can lead to bronchopulmonary dysplasia, necrotising enterocolitis, retinopathy, intracerebral haemorrhage. [4] The concept of sustained inflammation contributing to both short-term and long-term complications is becoming increasingly important and studied. There have been numerous observational studies demonstrating the association between impaired immune adaptation and long-term vulnerability for infections, bronchial asthma, neurodevelopmental disorders.[11,12]

1.3.5. Diagnosis of preterm birth

The diagnosis of preterm labour is challenging because the sequence of events preceding preterm labour and the timing of their occurrence are incompletely understood. Because the progression from subclinical labour to preterm birth is gradual, the traditional criteria for diagnosing preterm labour (painful uterine contractions, cervical changes) are usually imprecise. Thus, overdiagnosis of preterm labour occurs, leading to treatment of pregnant women with tocolytics in order to stop preterm birth in the absence of true preterm labour.[4]

Clinical markers of increased risk of preterm birth include: rupture of membranes, vaginal bleeding, cervical dilatation over 2 cm. Also, the presence of sludge in the amniotic fluid near the internal cervical os on transvaginal ultrasound examination has been associated with an increased risk of preterm birth within 48 h and intraamniotic infection in pregnant women with preterm labour symptoms. [3]

2. Chorioamnionitis

2.1 Definition

Chorioamnionitis is defined as inflammation of the fetal membranes, chorion and amnion and is the consequence of the maternal body's inflammatory response to an insult that may be infectious or non-infectious (sterile intra-amniotic inflammation, occurring in the absence of demonstrable micro-organisms, it may be induced by danger signals released under conditions of cellular stress, cell apoptosis). [3] A large number of cases of chorioamnionitis are subclinical and are diagnosed retrospectively by histopathological examination of the placenta. [13,14]

2.2.Prevalence of chorioamnionitis and impact on pregnancy and the newborn

Clinical chorioamnionitis and histological chorioamnionitis affect 1-4% and 23.6% of term births, respectively, while 94.4% are found among preterm births.[13]

2.3. Etiology

A multitude of microorganisms, ranging from bacteria, viruses and (less commonly) yeasts and fungi, have been implicated in chorioamnionitis, thus we can conclude that it is the result of polymicrobial infection. The bacterial pathogens that are most commonly isolated in cases of chorioamnionitis include the human Ureaplasma species (Ureaplasma parvum and Ureaplasma urealyticum), Fusobacterium spp., Streptococcus spp. and, less commonly, Gardnerella spp., Mycoplasma spp. and Bacteroides spp.. [14]

2.4. Pathophysiology

The decidua is of maternal origin, whereas the chorioamniotic membranes and villous tree are of fetal origin. The precise origin of the inflammatory process (maternal versus fetal) can be determined by the origin of the maternal or fetal neutrophilic infiltrate.[14] Normally, neutrophils in the maternal circulation are present in the intervillous space and absent in the chorioamniotic membranes and are presumed to migrate from the decidua into the membranes in cases of acute chorioamnionitis.[14] Migration of neutrophils from the intervillous space to the chorionic plate of the placenta occurs only under certain conditions, when there is a chemotactic gradient that attracts neutrophils to the amniotic cavity. Thus, inflammation of the chorionic plate is considered a maternal inflammatory response.[14] Inflammation of the

umbilical vessels begins in the umbilical vein (phlebitis) and is followed by involvement of the arteries (arteritis) and then infiltration of neutrophils at the level of the Wharton's jelly.[15]

2.5. Diagnosis of chorioamnionitis

The diagnosis of chorioamnionitis is the sum of clinical signs, symptoms and histopathological and microbiological examinations of the placenta after birth. Clinical signs of chorioamnionitis are maternal pyrexia (fever 37.5-38C), uterine contractility, maternal tachycardia (> 100 beats/minute), fetal tachycardia (> 160 beats/minute), altered vaginal discharge, leukocytosis (15000 cells/mm3) and premature rupture of membranes. However, the clinical diagnosis of chorioamnionitis is not always confirmed by histopathological or microbiological examinations.Histological classification of the placenta is considered the gold standard for the diagnosis of chorioamnionitis; thus, we are talking about a retrospective diagnosis that is not useful in the development of therapeutic management during pregnancy, especially in the absence of clinical signs.

2.6. Triggering of birth in normal pregnancy versus chorioamnionitis

In patients with chorioamnionitis, delivery may be accelerated by a maternal and/or fetal inflammatory response, which is thought to be mediated by Toll-like receptor (TLR). Bacterial endotoxins, such as lipopolysaccharides (LPS) and live microorganisms, have been shown to increase placental/chorioamnion TLRs, which are expressed by amnion epithelial cells, decidual cells, chorionic intermediate trophoblasts, macrophages, and neutrophils.[14]

2.7 Host defence mechanisms/ Inflammatory response to microbial invasion of the amniotic cavity

The chorioamnion, the main physical and immunological barrier to the developing fetus, expresses TLRs, which detect pathogen-associated molecular patterns and intervene in modulating cellular immune responses.[14] It also secretes numerous natural antimicrobial peptides and defensins that protect against microbial invasion. [16] Knowing that histological chorioamnionitis is associated with microorganisms considered to have low virulence, we can assume that commensal microorganisms in the placenta and fetal membranes represent a hitherto unknown source of bacteria that under certain conditions can initiate an inflammatory response [14].

2.8. Acute chorioamnionitis - sterile inflammation

Romero and co-workers, in recent studies, have highlighted a new type of intraamniotic inflammation called sterile inflammation, and which is found to be much more common than intraamniotic infection in preterm labor patients with intact membranes, preterm MRS and an asymptomatic short cervix.[12]

2.9 Fetal inflammatory response syndrome

Fetal inflammatory response syndrome (FIRS) is defined as a severe inflammatory condition that is associated with significantly increased concentrations of inflammatory cytokines in fetal plasma, particularly IL-6 (an important marker of acute phase response) and increased fetal plasma leukocyte counts.[14] FIRS is associated with multiorgan damage and severe neonatal morbidity and mortality.[14]

3. Elements of microbiology involved in preterm birth

3.1. SPP MYCOPLASMA AND SPP UREAPLASMA - General

Mycoplasmas constitute a large group of organisms, but not all are pathogenic to humans, those that are considered to have a negative impact on humans are Mycoplasma and Ureaplasma species. [14] Species isolated from the mucosal surface of the genitourinary tract are Mycoplasma hominis , Ureaplasma urealyticum, Ureaplasma pavum and the most recently discovered, with the smallest genome Mycoplasma genitalium. [14]

Ureaplasma is considered part of the normal genital flora because it is isolated from the surfaces of the vaginal mucosa or cervix in 40 to 80% of sexually active women.[14] There is now a growing body of scientific evidence that these low-virulence microorganisms are not so harmless.

3.2. Pathogenicity factors

Ureaplasma spp. manipulates host cells by suppressing innate defense mechanisms, reduces antimicrobial gene regiogenesis through epigenetic changes, thereby establishing a chronic persistent infection. [4]

3.3 Virulence factors

Virulence factors are: multiple band antigen (MBA), phospholipase A and C, IgA protease and the urease gene.126 Virulence and persistence of infection are influenced by the ability of microorganisms to form biofilms. Most clinical isolates of Ureaplasma spp. form biofilms. [14] Ureaplasma parvum can be internalized into HeLa cells and avoid host cell autophagosomal degradation by disrupting intracellular organisms by an unknown mechanism. [18]

3.4 Host response to infection with Ureaplasma spp.

Most studies have focused on the association between Ureaplasma spp. during pregnancy and morbidity of the preterm newborn based on the presence of these bacteria in the amniotic fluid or respiratory tract of the newborn. [20] Studies have demonstrated that the host immune response may be a key factor modulating the pathogenesis of Ureaplasma spp. infection.

3.5. Laboratory diagnosis

Culture techniques have traditionally been used to identify these microorganisms, but molecular diagnostics using PCR are much more sensitive.

3.6. Therapeutic options in infections triggering preterm birth

Antimicrobial agents are indicated to eradicate subclinical intra-amniotic infections and to prevent the ascent of pathogens from the vaginal or cervical level to the amniotic cavity.[21]

Treatment is particularly problematic in human Ureaplasma spp. They can cause chronic, asymptomatic intrauterine infections that modulate the host immune response to prevent significant pathological events, but are still associated with adverse outcomes.[14] Macrolides are bacteriostatic agents recommended by ISIDOG (The International Society for Infectious Diseases in Obstetrics and Gynaecology) for the treatment of genital mycoplasma infections. They can be administered during pregnancy as they have no proven teratogenic effects.[19]

Antibiotic resistance

We cannot neglect the current trend in increasing antibiotic resistance as it poses a great challenge in eradicating bacterial infection both during pregnancy and especially in women with recurrent infectious

II. PERSONAL CONTRIBUTIONS

4. Working hypothesis and specific objectives

The working hypothesis of our research is secondary to a careful analysis of the numerous studies addressing the issue of preterm birth and is in line with the trend of recent international concerns. We found that there is not yet a common, well-established guideline, despite the increasing incidence of preterm birth. We also observed a limited number of studies on the role of Ureaplasma spp. infections in our country. There is no guideline for screening and treatment of these infections in pregnancy, with currently recommended antibiotic treatment addressing cases with premature ruptured membranes and confirmed genital infection, not pregnancies with preterm birth symptoms and unconfirmed infection (as is most common with Ureaplasma spp. infections). Establishing correct and complete management would increase the period of temporization and improve the prognosis of mother and newborn.

General objectives

In view of the above, our research has the following general objectives:

1. To identify the demographic and clinical factors involved in the pathogenesis of preterm birth and their impact on the timing of delivery;
2. The role of genital infection with Ureaplasma spp. on pregnancy outcome and prognosis of the newborn;
3. Role of genital infection with Ureaplasma spp. on pregnancy outcome and prognosis of the newborn in pregnant women with spontaneously ruptured membranes at 24-34 weeks gestational age.

5. Methodology

The PhD thesis is composed of two studies with different groups of patients, following the same diagnostic protocol, but with different objectives, highlighted by statistical analysis.

Study I - Demographic and clinical factors involved in the pathogenesis of preterm birth

Between January 2018 and October 2021, we conducted a prospective study on a sample of 157 pregnant women selected from the Obstetrics and Gynaecology Clinic and from the Specialty Outpatient Clinic, both belonging to the University Hospital of Emergency Bucharest.

The sample of 157 pregnant women was structured as follows:

- - group A (cases) - consisting of 115 pregnant women who were admitted for threatened preterm birth;
- - group B (control) - made up of 42 pregnant women who showed no signs of preterm birth and gave birth at gestational ages greater than 34 weeks of pregnancy. These were selected from pregnant women presenting for regular pregnancy follow-up check-ups in the specialist outpatient clinic.

Study II Maternal factors that may influence the course of pregnancy and their impact on the newborn

Between January 2021 and October 2021 we conducted a prospective, non-randomized study on a sample of 30 pregnant women with spontaneously ruptured membranes, diagnosed and treated in the Obstetrics and Gynecology Department of the University Emergency Hospital, Bucharest, Romania. The local ethics committee approved the study design and protocol.

Upon enrolment in the two studies, respecting the inclusion and exclusion criteria, pregnant women completed a consent form accepting participation in the study. Patients were also given a thorough history, clinical and paraclinical examination in order to establish the diagnosis according to the criteria of the Romanian Society of Obstetrics and Gynaecology guidelines.

It should be noted that all selected pregnant women had cervical cultures taken to detect the presence of Ureaplasma spp. infections using the kit "Urinogenital and resistance" ver.06, manufacturer Aus Diagnostics, Australia. The kit (IVD, EC) uses the manufacturer's

proprietary Multiplex Tandem Polymerase Chain Reaction (MT-PCR) method, with testing performed on the High-Plex 24 System platform (Aus Diagnostics).

The management of the patients admitted, both those included in study I (group A) and those in study II, for the threat of preterm birth, i.e. premature ruptured membranes, involved monitoring a series of parameters in order to achieve the proposed objectives, among which we list the following: the presence of genital infections with Ureaplasma spp., assessment of the presence of maternal systemic inflammation or infection at admission by taking the following tests: complete blood count (we followed the leukocyte count), C-reactive protein (in study I these were assessed only at admission, and in study II they were followed dynamically, collected after completion of antibiotic treatment on day 10), presence of clinical/histological chorioamnionitis, institution of antibiotic treatment as indicated in guidelines and previous studies that followed this issue, age of pregnancy at delivery, timing of delivery.

We also assessed the status of the newborn in both studies, weight and sex of the newborn, Apgar score at 1 minute and at 5 minutes, need for respiratory support, newborn's protein C value, administration of antibiotic therapy, length of stay to achieve the proposed goals.

Study results

6. Study 1: Demographic and clinical factors involved in the pathogenesis of preterm birth

6.1 Results

The study was structured in two parts: the first part includes the comparative analysis between the two groups, and in the second part only group A was analysed where we looked at a number of variables with possible impact on the timing of birth.

- **Demographic and clinical factors involved in the pathogenesis of preterm birth**

Pregnant women admitted to the study were aged between 18-24 years, with a mean age of 29 years, with no differences of clinical significance in terms of age between the two groups of pregnant women. The mean gestational age at inclusion in the study was 30 weeks (group A) and 28 weeks (group B), respectively. The majority of patients were from urban areas, as they were selected from among the pregnant women who presented at the emergency room and outpatient department of the University Emergency Hospital of Bucharest.

Infections diagnosed in the first trimester of pregnancy:Regarding infections diagnosed in the first trimester of pregnancy, we note an increased incidence of infections in group A (about 7% higher). Ureaplasma urealyticum infection in the first trimester was also more frequently diagnosed in pregnant women at risk of preterm birth. In group B, there were 9 cases of Ureaplasma spp. infection in the first trimester of pregnancy.

Referring to group A (pregnant women with symptoms of preterm birth) we observe that about 24% were diagnosed with genital infections in the first trimester of pregnancy. Of these, almost 1/3 gave birth prematurely, the most frequently implicated agents in our group were Ureaplasma urealyticum and Chlamydia trachomatis.

We note that, Ureaplama urealyticum infection in the first trimester of pregnancy was associated in our study with gestational ages at birth ranging from 28-29 weeks.

We can also observe that genital infections in the first trimester of pregnancy with Klebsiella pneumoniae, E. Colli and Enterococcus faecalis were associated with gestational ages at birth between 27-34 weeks.

Number of full-term births in patient history: No clinically significant differences were observed.

Number of early and late miscarriages in patients' personal pathological history

Regarding obstetrical history, early miscarriages (<16 weeks) and late miscarriages (>16 weeks) we found no differences with clinical significance between the two groups. Regarding the association between history of early abortion (< 16 weeks) and preterm birth in group A, 3 or more abortions are associated in more than 50% of cases with preterm birth.

Concerning the impact of late miscarriage history (> 16 weeks) on preterm birth, we observe that pregnant women in the case group with this type of obstetric history gave birth prematurely at ages below 34 weeks.

In group B (control) there were 3 pregnant women with a history of late miscarriage, of which one pregnant woman had Ureaplasma urealyticum infection at enrolment and one pregnant woman had Ureaplasma parvum and Ureaplasma urealyticum co-infection, we note that both pregnant women gave birth at 34 weeks of pregnancy.

In group A there were 6 pregnant women with a history of late miscarriage, of which: 3 pregnant women were diagnosed at admission with Ureaplasma parvum infection, one pregnant woman with Ureaplasma urealyticum and one pregnant woman with Ureaplasma parvum and Ureaplasma urealyticum coinfection.

Number of preterm births in patients' personal obstetric pathological history

No differences of statistical clinical significance were observed between the two groups, however, the incidence of number of preterm births was higher in group A.

In the case group there were 11 pregnant women with a history of preterm birth, of which 5 pregnant women gave birth in the current pregnancy at gestational ages between 25-32 weeks of gestation. In group A there were 16 patients with a history of preterm birth, of whom almost 1/3 gave birth at gestational ages less than 36 weeks in the current pregnancy.

How pregnancy occurs:

Fisher's exact test result, $p>0.05$ shows differences without statistical significance. Pregnancies obtained by assisted reproduction methods were more common in group A.

We observed that patients who achieved pregnancy by assisted reproductive methods in group A were diagnosed in the first trimester of pregnancy with genital infections: Ureaplasma urealyticum (2 cases), Chlamydia trachomatis (2 cases) and in the current pregnancy with coinfection between Ureaplasma urealyticum + Ureaplasma parvum, Ureaplasma parvum + Mycoplasma hominis; and in group B pregnant women had genital infections with Ureaplasma

urealyticum in the first trimester, coinfection with Ureaplasma urealyticum + Mycoplasma hominis in the first trimester and Ureaplasma parvum in the current pregnancy.

Gestational age at study entry (group B) or admission (group A)

The result of the Welch two-way t-test for two independent means, T (2.86, 81.32), $p<0.01$ indicates that there are statistically significant differences between the two groups, the following graph shows the distribution of the variable between the two groups.

Positive diagnosis of bacterial vaginosis

The result of the Fisher's exact test, $p>0.05$ shows that the differences observed between the two batches are not statistically significant. In group A, 19 cases of bacterial vaginosis were reported, of which 10 cases were associated with Ureaplasma spp. infection and one case associated with Mycoplasma homminis infection. In group B (control) there were 4 cases of bacterial vaginosis, of which only 2 pregnant women associated infection with Ureaplasma parvum.

A number of 3 patients diagnosed with Ureaplasma urealyticum at admission had Bacterial Vaginosis, noting that one of the 3 cases also had Ureaplasma parvum infection simultaneously.

7 patients in group A with Ureaplasma parvum infection were also diagnosed with bacterial vaginosis, thus obtaining the most frequent association of vaginosis with an infectious agent of the Ureaplasma spp. family.

Positive diagnosis of genital infections on admission:

The incidence of infections in the study is:

- Mycoplasma genitalium 1.3%
- Ureaplasma urealyticum 6.4%
- Mycoplasma hominis 3,8%
- Ureaplasma parvum 34.4%

The prevalence of infections in the group of pregnant women with preterm birth symptoms is:

- Mycoplasma genitalium 0,9%
- Ureaplasma urealyticum 7,8%
- Mycoplasma hominis 3,5%
- Ureaplasma parvum 39,1%

Genital infection with Mycoplasma genitalium: No differences with statistical clinical significance are detected.

Genital infection with Ureaplasma Urealyticum: Fisher's exact test result, $p>0.05$, reveals that the differences were without statistical significance. Regarding the gestational age at admission of patients with preterm birth symptoms (lot A) and diagnosed with Ureaplasma urealyticum, it was observed that 63% of them were admitted at 31 weeks or less.

Genital infection with Mycoplasma hominis: no statistically significant differences.

Genital infection with Ureaplasma Parvum: The $\chi 2$ test result for two independent proportions, $\chi 2$ (4.27, 1), $p<0.05$, shows statistically significant differences between the two groups. Ureaplasma parvum was most commonly diagnosed in pregnant women in group A. 3% of pregnant women with Ureaplasma parvum infection were admitted for preterm birth symptoms at less than/equal to 31 weeks gestational age.

Positive diagnosis of histological chorioamnionitis

By calculating Fisher's exact test, $p < 0.001$, we observe statistically significant differences. A strong association was observed between infection with Ureaplasma urealyticum and Ureaplasma parvum and the presence of chorioamnionitis, i.e. 57% of chorioamnionitis cases were associated with the presence of Ureaplasma spp..

As regards pregnant women in group A (cases) diagnosed with Ureaplasma parvum infection at admission, 14 out of 45 pregnant women were associated with chorioamnionitis.

▷ **ositive diagnosis of Funisitis:**

The test result, $p=0.063,1$, is at the limit of statistical significance, there is evidence but not strong enough to say that the difference is statistically significant. Relative to the whole sample studied the incidence was 6.4%, and comparing the two groups we obtained differences at the limit of statistical significance ($p=0.063.1$), but still significant: 0% in the control group and 8.7% in the case group.

Gestational age at birth

The result of the two-way Welch t-test for two independent samples T (-7.27, 141.19), $p < 0.001$, shows that the differences are of statistical significance, the mean gestational age at birth being almost 3 weeks lower in patients in group A.

Newborn weight

Two-way Welch's t-test for two independent samples, T(-4.366, 129.53), $p<0.001$, shows statistically significant differences, with the fetuses of pregnant women in group A weighing almost 500g less than the fetuses of patients in group B.

Newborn APGAR score at 1 minute

The two-way Welch t-test result for two independent samples, T(-6.22, 143.21), $p<0.001$, reveals that the differences are statistically significant, with the APGAR score immediately after birth being about 1 point lower for the newborns of pregnant women in group A.

Newborn APGAR score at 5 minutes

The two-way Welch t-test result for two independent samples, T(-5.75, 132.67), $p<0.001$, shows that the differences are statistically significant, with the APGAR score at 5 minutes after birth being 0.65 points lower for the newborns of patients in group A

- **Predictors impacting on the timing of birth**

The objective of the second part was to identify factors impacting on the timing of birth in patients in group A (case group - pregnant women with preterm birth symptoms).

In this part of the study, a number of demographic and clinical variables were followed up in the patients of group A (case group).

Descriptive statistical analysis:

o 74% of pregnant women had painful uterine contractions at admission,

o 21% were admitted with spontaneously ruptured membranes

o 39% had cervical length < 25mm.

o 5% (6 cases) of pregnant women in group A had cervical cerclage at admission.

o Gestational age at admission of pregnancies with spontaneously ruptured membranes at admission was between 24 - 34 weeks of pregnancy.

o 86% of pregnant women had a delay in delivery of more than 7 days. 11 out of 16 cases where the delay was less than 7 days had associated genital infections from the bacterial spectrum studied.

o Ureaplasma urealytium + Ureaplasma parvum, Ureaplasma parvum + Mycoplasma hominis association correlates with a timing period of less than 7 days.

It is noted that Ureaplasma urealyticum infection in the first trimester of pregnancy is associated with a 2 week decrease in the timing period and personal obstetric history of preterm birth correlates with a 1.6 week decrease in the timing period. Both are without statistical significance,

Patients in whom pregnancy was achieved spontaneously, the timing period is 3.6 weeks longer, a statistically significant effect. The shorter delay period in patients with IVF pregnancies may be a consequence of genital infections, as described above.

Pregnant women admitted with spontaneously ruptured membranes have a 5.4 week shorter latency period. The effect being statistically significant.

Association with spontaneously ruptured membranes and chorioamnionitis/funnitis

Of the 24 patients with spontaneously ruptured membranes, 16 were diagnosed with genital infections with Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis (2 cases), and 13 had associated Chorioamnionitis and 8 Funisitis.

Predictors:

- One unit higher CRP value is associated with a 0.09 week decrease in the timing period. The effect is statistically significant
- A 1000-fold increase in leukocyte count is associated with a 0.15-week decrease in the time delay.
- The presence of chorioamnionitis is associated with a 1.5 week reduction in the time delay. The effect is marginally insignificant.
- The presence of Funisitis is associated with a decrease of 4 weeks in the temporisation period, the effect is statistically significant.
- Pregnant women who were not diagnosed with Ureaplasma urealyticum infection had a longer time delay, but the effect is statistically insignificant, probably due to the small sample.

Influence of delaying birth for more than one week on the newborn's birth status

Comparisons between the two groups were made using two-way comparison tests for two independent samples

The result of the two-way Welch T-test for two independent samples, T (6.57, 17.81), $p < 0.01$, shows that the differences were statistically significant. The Apagar score at 1 minute being more than 2 points higher in patients in whom the timing was more than one week

Comparison APGAR score at 5 minutes:T-test result(6.24, 17.60), $p<0.001$, indicates that the differences are with statistical significance. The APGAR score at 5 minutes is 1.5 points higher in patients in whom the delay was more than one week.

Comparison for proportions of newborn protein C positivity:$\chi 2$ test (chi-square) for two independent proportions $\chi 2(15.06, 1)$, $p<0.001$, shows that the differences are statistically significant, with the proportion of CRP-positive newborns being more than 3 times higher in newborns of pregnancies in the group where the time delay was less than one week.

Comparison of proportion of newborns with a length of stay of more than 7 days

Fisher's exact test result, $p<0.001$ reveals that the differences were statistically significant. The proportion of newborns requiring more than 7 days of hospitalization was more than 3 times higher in pregnant women with a delay of less than one week.

Comparison of proportion of newborns requiring antibiotic therapy:

Fisher's exact test result, $p<0.001$ shows that the differences were statistically significant. The percentage of newborns requiring antibiotic treatment is more than 4 times higher in pregnant women with a timing of less than one week.

Comparison of proportion of newborns requiring CPAP:

Fisher's exact test result, $p>0.05$ shows that the differences were statistically insignificant.

Comparison proportion of newborns who required IOT:

The $\chi 2$ (chi-square) test for two independent proportions $\chi 2$ (15.06, 1), $p<0.001$, tells us that the differences are statistically significant. The proportion of newborns requiring IOT is 10 times higher in newborns of pregnant women in the group where the timing was less than one week.

7. STUDY 2 Maternal risk factors that may determine the predictability of fetal impact in cases of premature rupture of membranes

7.1 Results

Two univariate simple or multiple linear regressions were used to achieve the main objective, with the dependent variable Apgar Score at 1 minute and Apgar Score at 5 minutes respectively and the independent variables (predictors) - a series of demographic-clinical parameters followed in the study.

Descriptive analysis (maternal and fetal demographic-clinical parameters):

- The mean age of patients admitted to the study was 28.3 years, with 2/3 of patients aged 26-35 years.
- BMI of the patients, 22 patients, representing 73.3% of them, had BMI between 35-39.99 (obesity grade II).
- The majority of patients were from urban areas (70%).
- 23.3% of pregnant women had a history of genital infections in the first trimester of pregnancy. Of these 85.7% (6 patients) were diagnosed with Ureaplasma urealyticum infection and 14.3% (one patient) with Bacterial Vaginosis.
- The history of early miscarriages (< 16 weeks) of the SRM pregnancies in the study, 8 of them had a history of miscarriage and 2 other patients with 2 episodes of miscarriage. As for the number of patients with late miscarriages, 4 pregnant women with 1 miscarriage and 3 pregnant women with 2 miscarriages >16 weeks.

Bacterial spectrum in the study sample. Ureaplasma spp. infection was diagnosed in 63.6% of pregnant women (Ureaplasma parvum 40%, Ureaplasma urealyticum 13.3%, Mycoplasma hominis 13.3%). Microbiological examination (Gram stain) revealed two cases of Escherichia and two cases of GBS. There were six cases of bacterial vaginosis.

Gestational age distribution at birth with mean (SD) of 32 weeks and 4 days.

The number of days of delay to delivery was on average (SD) 13.1. 11 pregnant women had a delay to delivery of less than 7 days, while 19 pregnant women (63%) were delayed for more than one week.

Maternal and fetal complications followed up in the study we observed that 13 pregnant women were diagnosed with histological chorioamnionitis, in 6 cases funisitis was detected and 4 pregnant women developed postpartum endometritis.

Newborn Apgar score at 1 minute and at 5 minutes: Median Apgar score of newborns at 1 minute was 7 and 8 at 5 minutes respectively. It is noted that the Apgar score at 1 minute in 9 newborns was 5, which is a significant percentage, almost 30% of the sample studied. At 5 minutes only one newborn had an Apgar Score 6, while the rest had Apgar Scores between 7-9.

After data collection and characterization of the pregnant sample we continued the study with statistical analysis in order to achieve the main proposed objective, to examine the correlation between neonatal mortality or morbidity and potential maternal risk factors.

Simple univariate linear regression model with dependent variable APGAR score at 1 minute:

- Independent of other predictors, a one unit increase in maternal CRP value at admission is associated with a 0.07 decrease in APGAR score at 1 minute;
- Independent of the other predictors, a fetus delivered by caesarean section has an APGAR score at 1 minute that is on average 0.81 higher;
- Independent of the other predictors the presence of chorioamnionitis is associated with a mean decrease of 1.2 points in the APGAR score at 1 minute.

All predictors are statistically significant, with simultaneous effects shown in the adjacent graph:

Simple univariate linear regression model with dependent variable APGAR score at 5 min:

Independent of the other predictors, a one unit increase in CRP value is associated with a 0.04 decrease in APGAR score at 5 minutes;

Independent of the other predictors, a 1000-fold increase in the leukocyte count at admission is associated with a 0.05 decrease in the APGAR score at 5 minutes;

Independent of the other predictors, newborns born by caesarean section have a 0.45 higher 5-minute APGAR score than those born naturally; Independent of the other predictors, newborns of pregnancies diagnosed with chorioamnionitis have a 0.43 lower score than those of pregnancies without the condition.All effects have statistical significance.

8.Conclusions

Identification of demographic and clinical factors involved in the pathogenesis of preterm birth

- One of the factors involved in the pathogenesis of preterm birth is the history of infections diagnosed in the first trimester of pregnancy.
- Approximately 1/4 of pregnant women with a history of genital infection in the first trimester of pregnancy delivered preterm.
- The most commonly diagnosed infectious agent was Ureaplasma urealyticum.
- The association of a history of previous preterm birth with the presence of genital infection with Ureaplasma spp. increases the risk of preterm birth.
- Genital infection is a major cause of preterm birth and the frequency with which the bacteria studied were identified was as follows: Mycoplasma genitalium 1.3%, Ureaplasma urealyticum 6.4%, Mycoplasma hominis 3.8%, Ureaplasma parvum 34.4%.
- The impact of infections on gestational age at birth was significant, 66% of pregnant women diagnosed with Ureaplasma urealyticum infection and 45% of those diagnosed with Ureaplasma parvum gave birth before 36 weeks.

Assessment of the impact of predictors on the timing of delivery

- In terms of the impact of infection diagnosed in the first trimester of pregnancy we found a decrease in the timing period by approximately two weeks for Ureaplasma urealyticum positive pregnancies in the first trimester of pregnancy.
- Pregnant women who were not diagnosed with Ureaplasma urealyticum infection at admission had a longer delay period.
- Obstetric history of preterm birth is associated in our group with a decrease in the birth delay period by approximately 1.6 weeks.
- Vaginal bleeding at admission increases the risk of preterm birth and decreases the timing period by about 1.4 weeks.
- The presence of ruptured membranes at admission decreases the birth delay by 5.4 weeks.

Determining the importance of delaying birth for more than one week on the status of the newborn at birth

Newborns of pregnant women with less than one week's delay had lower Apgar scores, required longer periods of hospitalization, received antibiotic treatment at a much higher rate, and required respiratory support.

Maternal factors that may influence the course of pregnancy and their impact on the newborn:

- Presence of genital infection in the pregnant woman (diagnosed in the first trimester of pregnancy and at admission);
- gestational age at diagnosis of spontaneously ruptured membranes;
- maternal inflammatory marker status at admission (values above the upper limit are associated with poor fetal prognosis);
- the occurrence of complications (chorioamnionitis, funisitis) is associated with a decrease in Apgar score and an unfavourable clinical-paraclinical evolution of the newborn;

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