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MATERNAL-FETAL TRANSMISSION OF HUMAN PAPILLOMA VIRUS - NEONATAL IMPLICATIONS

ABSTRACT OF DOCTORAL THESIS

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Table of content

INTRODUCTION 10

1. GENERAL PART 13

1.1. Human papilomavirus 14

1.1.1. Genomic structure 14

1.1.2. Human papilloma virus life cycle 15

1.1.3. HPV classification 17

1.2. Patogenesis 19

1.2.1. Anatomical distribution 19

1.2.2. Epithelial and cellular localisation at host level 19

1.2.3. Differentiating between high-risk and low-risk HPV types 19

1.2.4. Clinical manifestations and effects of HPV infection 20

1.3 Epidemiology and routes of transmission 22

1.3.1 Age and type of infection 22

1.3.2 Risk factors 23

1.3.3 HPV and pregnancy 24

1.3.4 HPV in children 25

1.3.5 Routes of transmission 25

1.3.6 Caesarean section versus vaginal birth 30

1.3.7 Detection of HPV DNA in the placenta 31

1.4 Immunological processes involved in HPV infection 33

1.4.1 Antigen-presenting cells 33

1.4.2 T-type lymphocytes 33

1.4.3 Types of immunity 35

1.4.4 Antibodies 37

1.5 HPV vaccination 38

1.6 Paraclinical investigations 42

1.6.1 Morphological methods 42

1.6.2 Detection methods based on nucleic acids 43

1.6.3. Serological methods of HPV detection 44

1.7 Neonatal implications 46

1.7.1 HPV-associated prematurity 46

1.7.2 Intrauterine growth restriction associated with HPV infection 51

1.8 Paediatric implications 52

1.8.1 Recurrent respiratory papillomatosis 52

II. PERSONAL PART (ORIGINAL CONTRIBUTION) 57
2.1. General objectives of the PhD thesis 58
2.2. Research working hypotheses 59
CHAPTER 3. METODOLOGIA GENERALĂ A CERCETĂRII 60
3.1. Study type 60
3.2. Study population 60
3.2.1. Inclusion criteria 61
3.2.2. Exclusion criteria 62
3.3. The examination protocol 62
3.3.1. Objective clinical examination 62
3.3.2. Microbiological examination of cervico-vaginal discharge 62
3.3.3. Papanicolaou cytological examination 62
3.3.4. HPV genotyping 64
3.3.5. Laboratory diagnostics 64
CHAPTER 4. EVALUATION OF THE ENROLMENT LOT OF PREGNANT WOMEN 70
4.1. Introduction 70
4.2. Material and method 70
4.2.1. Study type 70
4.3. Results 71
4.3.1. General data 71
4.4. Discution 97
4.5. Conclusion 107
CHAPTER 5. MATERNAL-FETAL TRANSMISSION OF HPV INFECTION 108
5.1. Introduction 108
5.3. Results 109
5.4. Discution 115
6.5. Conclusion 121
CHAPTER 6. HPV INFECTION AND PREMATURITY 123
6.1. Introduction 123
6.2. Material and method 125
6.3. Results 125
6.4. Discution 133
6.5. Conclusion 137
CONCLUSIONS AND PERSONAL CONTRIBUTIONS 138
References 147
Annex 1. HPV survey questionnaire 166
Annex 2. Informed consent 167
List of publications and presentations in the PhD topic 168

INTRODUCTION

The human papilloma virus (HPV) is known to be the main aetiological factor involved in the acquisition of cervical cancer, but it can also cause benign condylomatous lesions. Thus, we consider the possibility of classifying the virus in two categories: low oncogenic risk and high oncogenic risk. The higher risk is usually associated with the presence of strains 16 and 18 respectively, which can induce the development of dysplastic lesions and, later, in the absence of drug or surgical treatment, excisional lesions, which can become neoplastic lesions.

Possible transmission routes are largely dependent on sexual contact, but vertical (maternal-fetal) transmission routes have been described that can occur both prenatally (during pregnancy or preconception) and peripartum (during labour or delivery), and horizontal transmission routes (through breastfeeding).[1]

In addition to the neoplastic risk caused by the virus in question, the impact and outcome in pregnancy were also considered. This is based on the physiological changes that the pregnant woman has to undergo in order to maintain the pregnancy to term. Thus, the hormonal environment and the immune system of the pregnant woman can be considered as determinants and thus as favouring factors in viral persistence. Potential risks of HPV in pregnancy include prematurity, risk of premature rupture of amniotic membranes, increased risk of miscarriage, maternal complications in the spectrum of hypertension which may lead to lower fetal birth weight and intrauterine growth restriction.

Thus, by understanding and deepening our understanding of the pathophysiological mechanisms used by HPV, mechanisms that can play a detrimental role during pregnancy, we can determine a holistic approach to these cases that can potentially offer us a better way to counteract the negative effects and optimize obstetric and perinatal outcome.

It should be noted that the literature does not provide us with a wealth of information on HPV infection and its implications for both the pregnant woman and the product of conception.

Regarding the current state of knowledge, I mention studies that have evaluated the involvement or association of viral infection in preterm birth (studies that I will detail during the research thesis). Thus, statistically significant associations have been described both in pregnancies with premature preterm rupture of amniotic membranes (PPROM)[2] and in pregnancies with term prelabor rupture of amniotic membranes (TPROM).[3]

Studies assessing the risk of maternal-fetal transmission of HPV infection will also be detailed throughout the thesis.

Our study is a prospective study, which shows the differences between the two groups of pregnant women (HPV negative control group and HPV positive study group). It was conducted over a period of 3 years and includes 49 pregnant women with a recent history of HPV infection acquired prior to the present pregnancy, altered Pap smear and condylomatosis in the anogenital region in the context of HPV infection, and 49 HPV negative pregnant women. I mention that these clinical landmarks and the characteristics of the working group are similar to the group in the HERITAGE study, the results of which will be mentioned in the thesis.

Regarding the preliminary results of our study, I mention that I divided the study in order to study comparatively both the epidemiological data of the two groups of pregnant women and the risk of maternal-fetal HPV transmission, i.e. the risk of preterm birth in the two groups.

Thus, I would like to specify that we did not determine a higher association of HPV infection with hypertensive disorders, or with overall duration of labour in the working group compared to the control group.

On the other hand, in our study we found a percentage superiority in the risk of miscarriage determined both by the presence of oncogenic strains (especially a determinant risk of strain 16) and by the marital status of the pregnant women regardless of the nature of the strain in relation to this last parameter.

Furthermore, when the association between the risk of preterm birth and the presence of the virus at the genital level was analysed, this association was not statistically significant and did not lead to a higher risk of preterm birth for infections caused by oncogenic strains compared to non-oncogenic strains (studying at this level also the particular determinant risk of strain 16).

In contrast, when analysing the risk of preterm birth among patients with PROM, we found a 100% risk in the working group, and a higher risk for PROM in the case of the association of an oncogenic strain.

The limitations of the study are primarily based on its single-center nature, the study being conducted exclusively in the Obstetrics-Gynecology and Neonatology Clinic of the University Hospital "Elias".

Secondly, I mention the expected low prevalence of HPV infection in pregnant women included in the study, which can be explained by the method of collecting samples for genotyping the virus only from the cervix and not from the vaginal secretion.

Thirdly, the determinations were performed periconceptional (before conception or in the first trimester of pregnancy), thus only qualitatively determining the presence of HPV.

Fourthly, I mention that postpartum follow-up of newborns only took place clinically to objectify certain signs or symptoms specific to viral infection. We note at this point that through exclusive breastfeeding they can acquire HPV infection from the mother horizontally.

By specifying the limitations of the present PhD thesis, I thus open the way to new ideas and opportunities in terms of research level.

CHAPTER 1. GENERAL PART

Papillomaviruses have a wide worldwide distribution, with more than 200 types that have been identified in both animals and humans, with a predominant tropism for squamous epithelial cells of the mucocutaneous level.[4]

HPV infection causes, depending on the genotype involved, both benign proliferative lesions such as vegetations or papillomas, and the formation of preneoplastic lesions that may evolve and subsequently lead to the development of cervical cancer.

HPV is a DNA virus, which, despite the heterogeneity that Papillomaviruses exhibit, the genomic organisation is similar for most HPV types. They are simple non-enveloped viruses that are approximately 55 nm in size and consist of double-stranded DNA capped by an icosahedral viral capsid, which consists of 72 capsomeres. Expression of genes belonging to this virus family is mediated by viral promoters, two of which are found within the high-risk HPV genome: the p97 - HPV 16 promoter located in the CSF and the p670 - HPV 16 promoter located in the E7 oncogene.

HPV is a host cell-dependent virus because it does not contain the polymerase enzyme or other types of enzymes involved in viral replication, which is why it is synthesised using proteins that contribute to host cell replication. [5,6]

In the cervix, during the period of embryogenesis, the mullerian epithelium is gradually replaced by stratified squamous epithelium until near the external cervical os where the congenital (original) squamocolumnar junction is evident, delimiting the two types of epithelium. This process of cellular squamous metaplasia is under hormonal stimulation, the location of the squamocolumnar junction varying according to age; thus, during adolescence,

pregnancy and combined oral contraception, there is an eversion of the junction at the level of the exocervix, which is followed by a regression at the level of the endocervical canal during menopause, when breastfeeding is prolonged and when minipillules are administered (progestogenic contraception). The process of squamous metaplasia is ongoing, which makes this transition zone susceptible to oncogenic development, which is why the cervix and anal canal are known preferential sites for high-risk HPV types. [7]

The virus penetrates the tissue through excoriation or microtrauma or, in particular cases, through insufficient epithelial maturation, creating optimal conditions for replication. More prone areas facilitating viral adhesion have been described, such as the hair follicle or the epithelial transition zone of the cervix, but also the anal canal, upper respiratory tract and bronchi. Thus, hair follicles can be considered an important viral reservoir in the ano-genital region.

Once the virus enters the body, it crosses the superficial epithelial barrier and attaches itself to the host cell with the help of receptors, including heparan sulphate glycosaminoglycan, -6 integrin or laminin 5. Subsequently, the virus is transported to the endocytosomes, where it will leave when a complex forms between the viral genome and the L2 protein of the viral capsid and integrates into the nucleus.

Mitosis will begin to take place at the deepest layer, the basal layer of the squamous epithelium, which is represented by a single cell layer sitting on a thin basement membrane. At the basal level, the number of copies of the genome will be low, ranging from 10-200 copies/cell, all under the influence and control of viral proteins E1 and E2. During S phase viral replication will take place, a process that occurs synchronously with that of the host cells, depending entirely on their differentiation. Thus, the life cycle of HPV occurs strictly in a fully differentiated and intact squamous epithelium.[8]

When infected cells leave the basal layer and advance to the superficial layer, proliferation begins, with the virions dividing into daughter cells. This will increase the expression of the E6 and E7 genes, which amplify the copy number of the genome, reaching up to 1000 copies/cell, at which point kertinocyte differentiation is slowed. The synthesised genome is assembled into capsids, composed of the viral proteins L1 and L2, this being the final form in which virions will be released. Subsequently, the activity of the E6 and E7 genes is reduced, allowing complete differentiation of the host cells, forming new surface layers.

The time from infection to viral release takes an average of three weeks, and for the appearance of clinical lesions this interval can vary from weeks to months. [9-12] HPV, being a

strictly epithelial tropism virus, is most commonly found in the cutaneous tissue and anogenital tract, but cases have been described with localisation in the oro-pharyngeal mucosa, laryngeal mucosa and, less commonly, oesophageal, tracheal, bronchial, nasal sinus, conjunctival and urinary tract mucosa. [13]

Clinically, HPV types are classified on the basis of oncogenicity as well as close association with cervical neoplasia into high-risk HPV and low-risk HPV. The low-risk category mainly includes genotypes 6 and 11, which are responsible for the majority of papillomatous lesions, and which are rarely associated with oncogenic risk. On the other hand, the high-risk category includes genotypes 16, 18, 31, 33, 35, 45 and 59, which account for about 95% of preneoplastic and neoplastic cervical lesions. Of particular note would be type 16 which presents the highest oncogenic risk, a risk determined by the tendency of the virus to cause persistent infection unlike other high-risk genotypes. Progression to neoplasia, even in the presence of high-risk strains, is not a mandatory consequence, and additional factors are required for this, which are host immunocompetence and environment.[7]

Structurally, the differentiation between the two categories is mainly achieved by the two viral proteins E6 and E7, as follows:

Naturally, there is the E2 gene that suppresses E6 and E7 transcription, thus preventing cell transformation and, by extension, the development of invasive lesions. However, when the viral genome integrates into the host cell, the E2 gene is inactivated, causing a consequent increase in the levels of E6 and E7 proteins, facilitating cancer initiation. [14]

The E7 protein acts by binding and degrading proteins belonging to the retinoblastoma family (pRb), which are central regulators of oncogenic steps. For high-risk viral types, E7 degrades the majority of pRb's, whereas for low-risk viral tuples, only one pRb protein - 130 - is affected.

On the other hand, there is also the tumour suppressor protein, p53, one of the important DNA regulatory and apoptosis initiation factors especially for cells with aberrant progression of invasive lesions. There is also the telomerase complex, which once activated keeps the telomeric ends of chromosomes open to repetition. These normally, as they are consumed from the finite number of cell divisions, cause apoptosis. In HPV infection, the E6 protein inactivates the p53 protein and activates the telomerase complex, preventing cell apoptosis. [11, 15-18]

HPV infection is one of the most common sexually transmitted infections, which is why most studied have been those with genital localization, especially because of the correlation with cervical cancer. Clinically, 3 types of infections have been described:

- Latent: generally asymptomatic infections without cytological or histological changes. In this case, viral DNA can only be identified by more sensitive molecular methods.
- Subclinical: Generally shows minor changes on cytological and histological examination, with no clinically apparent lesions.
- Clinical: Abnormal cell proliferations, such as epithelial acanthosis, koilocytosis and dyskeratosis, and clinically from vegetations and condylomata to low- to high-grade dysplasia have been described. [19]

However, although the incidence is increasing, most infections are transient and become undetectable within 1-2 years. Infections that do not fall within the definition of "transient" are referred to as persistent, but there is no clear definition, being considered on the one hand infections that are detected at least twice consecutively at an interval of 4-6 months, and on the other hand infections that last longer than the average duration of infection. In short, persistent infections are those that the host's immune system has been unable to clear. This pattern of infection has been particularly correlated with high-risk HPV strains.

It has also been shown that the risk of HPV infection persistence increases with age. [20-25]

In recent years, multiple studies have been conducted to identify the incidence of HPV infection in mothers and newborns. A randomised study was conducted in Poland over a 2-year period involving 152 pregnant women and their newborns. Oral mucosal samples from newborns and oral and genital samples from pregnant women were collected to identify HPV infection. The results showed that there is an 11-fold increased risk that samples collected from the oral mucosa of newborns from HPV-positive mothers also associate a positive result, which should be considered in terms of transmission risk if the pregnant woman is tested positive. [26]

In addition to viral determinants of infection, other factors have been considered that are mostly host-dependent. Among these, age at onset of sexual contact, parity, immunocompetence versus immunosuppression status, use of combined oral contraceptives, smoking, a history of sexually transmitted diseases or the existence of HPV-specific vegetations, previous results of the Babies-Papanicolau test were studied.

The higher incidence of infection in young women may be due on the one hand to a higher risk of inappropriate sexual behaviour (increased number of sexual partners, lack of

barrier contraceptive methods) and on the other hand to a lower immune status, but also to a physiological vulnerability due to a more pronounced maturation of the cervix in this age group, largely influenced by the use of cigarettes or combined oral contraceptives, a process also observed during pregnancy. [27-32]

Pregnancy is considered to be a risk factor for HPV infection partly due to hormonal changes that cause increased vascularity and increased squamous metaplasia of the cervix, and partly due to poor immune status. [32-34]

One of the severe complications in pregnancy is pregnancy-induced hypertension (PIH), so one element of the study was the association of HPV infection with the risk of hypertensive spectrum disorders. Pre-eclampsia or PIH is a potentially severe complication that can occur both from the 20th week of pregnancy and in the postpartum, post-leukemia period, and can have lethal consequences on vital maternal organs. It is important to note that maternal-fetal outcomes are directly proportional to the severity of the hypertensive condition, with patients additionally at risk of later developing cardiovascular and/or renal disease during life. [35] In a systematic review, approximately 5% of pregnancies were found to be complicated by pre-eclampsia.

A case-control study of 108 patients found a similar prevalence of HPV DNA in placental samples between the 2 groups studied compared 46, and a retrospective cohort study found a doubling of the risk of pre-eclampsia in patients diagnosed with HR HPV infection 47 in contrast to 2 studies that found no negative impact on pregnancy, one of which was conducted on a cohort of 15,357 patients. [2,38]

HPV-infected child status is possible because routes of transmission of infection other than sexual have been identified. In these children, vegetations and papillomas characteristic of HPV infection have been frequently identified and have an increasing incidence, of which the rarest but also the most serious is juvenile recurrent respiratory papillomatosis (JORRP), the cause of which is HPV viral strains 6 and 11. [39]

In the newborn, lesions have been identified in the oral mucosa, conjunctiva, genitals and skin. Clinical manifestations include cutaneous and ano-genital condylomata but also oral, conjunctival and laryngeal papillomas.

HPV has been highlighted as a sexually transmitted infection, mainly because of this way of contracting the disease, but recent studies have shown this viral presence even in patients who have not had a sexual debut, as well as in children. This has eventually led to the presumption that there are other ways of transmitting and contacting the disease. The following pathways have been described:

- Vertical: Acquisition of infection occurs during pregnancy and at the time of birth, following passage through the pelvic-genital filiation. In this case, the presence of maternal genital condylomata has been considered a reservoir of infection that can cause respiratory impairment in children by determining JORRP.
    - Periconceptional: Transmission occurs during the fertilisation process, HPV being detected in the organs involved in the human reproductive process such as the endometrium and ovaries in the case of females, and the seminal fluid, vas deferens and urethra in the case of males. The ability of spermatozoa to carry the virus to the oocyte, where elements of the viral genome were also detected after fertilisation, was demonstrated.[40-42]Viral DNA has been identified in varying proportions of 8 to 64% in seminal fluid and spermatozoa. [43]
    - Prenatal: Infection is contacted during pregnancy, in utero, on the one hand via placental circulation, although no studies have yet been carried out to prove with certainty the presence of viremia. On the other hand, the ascending route of transmission from the genital tract has been considered. Contamination in the prenatal period is also supported by the presence of lesions present in the newborn immediately after birth. [44]
    - Perinatal: This route is more likely in the case of vaginal birth, as the newborn comes into direct contact with maternal endocervical and vaginal tissues. This theory is supported by the presence of nasopharyngeal or genital viral DNA in the newborn. However, it is not believed that delivery by caesarean section completely eliminates the risk of acquiring infection in the newborn. [44, 45]
- Horizontal: This occurs through direct contact of the integument with the infecting lesion, [46] through breast milk [47] or from surfaces.

Studies have been conducted on transmission through breast milk, but results are not unanimous due to variability in viral DNA detection methods.

Transmission via surfaces has been considered and so studies have been carried out. A 2014 study shows that the virus can survive in the external environment and retain its infectivity if common disinfectants are used to clean surfaces.

As for HPV transmission through breast milk, it ranges from 2-8%.[43]

- Autoinoculation.

CHAPTER 2. GENERAL OBJECTIVES AND WORKING HYPOTHESES

General objectives of the PhD thesis

This paper has focused on the approach to premature birth among patients with HPV infection, an infection with curable potential, which is secondarily associated with the avoidable mortality of cervical neoplasia achieved by early detection of both HPV infection and cervical dysplasia.

As specified in the general part, given that HPV infection remains the main aetiological factor and therefore the necessary condition for the development of cytologically determined squamous or glandular cell abnormalities that induce progression to cervical neoplasia, we can emphasise the extremely important role of genotyping involvement in primary prevention programmes.

Our research work aimed to highlight the importance of early detection of HPV infection by analyzing the clinical, cytological, virological status in a group of pregnant women for a 3-year interval. It also aimed to follow up clinically and biologically the conception product resulting from the birth in both the HPV infected pregnant group and the control group.

The general objectives of the research thesis are represented by the following sub-points:

a. To assess personal and demographic characteristics and the prevalence of specific risk factors for HPV infection.
b. To assess the clinical, cytological, microbiological and virological status of pregnant women at study inclusion.
c. Assessment of the presence of HR and LR HPV genotypes at enrolment.
d. Virological assessment at the time of delivery by virus collection from amniotic fluid and placenta respectively.
e. Prospective assessment of infant development by monitoring clinical parameters up to 6 months after birth.

Working hypotheses of the research

a. The present research started from the following hypotheses:
b. Low level of adherence to a full HPV vaccination schedule of eligible populations, contrary to medical recommendations.
c. Behavioural risk factors for dysplastic and neoplastic cervical lesions, respectively, which are more prevalent in younger age groups.
d. Factors favouring the development, persistence and worsening of lesions through tobacco use, use of oral hormonal contraception (hence the lack of an effective barrier method of contraception), increasing number of sexual partners associated with an early age of sexual debut.
e. Increasing proportion of HR HPV types in the female population at risk.
f. Decreasing HPV clearance during pregnancy.
g. Studies in the literature associating HPV with obstetric outcome and altered perinatal prognosis.

CHAPTER 3. GENERAL RESEARCH METHODOLOGY

Study type

The study is a prospective non-interventional study.

In terms of the rationale for the notions used above, the prospective nature is determined by the way patients were included in the study in my study, they were followed and assessed during pregnancy and then, for the second study, infants were followed for 6 months postpartum.

The non-interventional particularity is supported by the fact that both subjects included in the control group and those included in the working group in this study did not undergo drug or interventional testing. They were given appropriate medical therapeutic measures based on clinical and paraclinical findings, based on updated practice protocols.

Study population

The study population consists of a group of 49 pregnant women with HPV infection, who presented to the Obstetrics-Gynaecology and Neonatology Clinic of Elias University Emergency Hospital on the basis of a referral note issued by the territorial family doctor or by other medical professionals of another specialty, for a gynaecological consultation.

All pregnant women were examined by the Obstetrics-Gynaecology specialist and samples of cervico-vaginal secretion were collected (culture and microscopic examination on stained smear from vaginal discharge, culture from cervical discharge, culture on growth medium for mycoplasma hominis, ureaplasma urealyticum, testing for Chlamydia trachomatis and Neisseria Gonorrhoeae, conventional cervical cytology Papanicolaou).

Statistics: the results obtained from these tests and the clinico-biological data of each case were entered and centralized in a Microsoft Excel 2010 table, which was the data base for our study. After data entry in Microsoft Excel 2010, they were statistically processed in IBM SPSS statistics v.20. The graphical part was done in IBM-SPSS Statistics v. 20 and Microsoft Office-Excell (2010).

Patient data were collected using the study questionnaire method and the personal files and pregnancy records of the pregnant women included in the study.

Several parameters or variables were followed: age, education level, marital status, duration of relationship, tobacco use, history of immunosuppression, type of lesion caused by HPV, location of lesions, HPV strain, type of HPV infection (single, double, multiple), presence of cervical excisional surgery, previous immunization against HPV, co-infection with Mycoplasma hominis, Ureaplasma urealyticum or Chlamydia trachomatis, number of miscarriages, personal history of preterm birth, type of delivery, gestational age at diagnosis of infection, lenght of labour with or without rupture of amniotic membranes, general lenght of labour, presence of lesions at time of delivery, presence of PIH.

In the database, the following variables were also entered for the newborn: sex, weight, appearance of the skin and mucous membranes, presence of lesions characteristic to HPV infection at birth, as well as evolution during the first 6 months of life (health status, nutrition, medication administered). These variables were also included in the postpartum and follow-up questionnaire.

Pregnant women signed an informed consent to participate in this research study, and medical staff were trained in sample collection according to a protocol.

Laboratory diagnosis:

Sample collection and storage: In the newborn, the following were collected in the delivery room during the first minutes of life: oral swab, gastric aspirate - amniotic fluid - and cord blood.

Cord blood was collected in the EDTA tube and sent urgently to the laboratory for centrifugation 2400 rpm in 10 minutes, and the resulting plasma was kept in the freezer.

Gastric aspirate in the amount of 2-3 ml was collected in exudate tube in which 2-3 ml of virus transport medium was added.

Jugal swab was collected in exudate tube to which 2-3 ml of virus transport medium was added.

Samples were labelled and stored in the freezer at minus 80 degrees Celsius and then sent to the Stefan S. Nicolau Institute of Virology.

Statistical analysis

The statistical analysis used was of two types: descriptive statistics and inferential statistics.

Descriptive statistics were used to analyse and describe the data by indicators: mean, standard deviation, median, percentiles, proportions.

Deductive (inferential) statistics were used to make general inferences about the populations from which the samples were drawn. The p-value was used for statistical conclusions. The threshold for statistical significance was $p < 0.05$.

The variables identified in this study were: continuous (numeric), dichotomous (yes/no), nominal (e.g. gender, signs and symptoms), ordinal (categories in a particular order relative to each other, e.g. date of admission).

To calculate the CI95% we used the online calculator.

Twelve HPV genotypes are recognized as high oncogenic (HR-HPV): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, correlated with the frequency of their presence in association with malignancies. Some classifications also include genotypes: 66, 68, 73 and 82 as highly oncogenic. In this study, HPV 16, 18, 31, 33, 45, 51, 58 are considered HR-HPV and HPV 6, 11, 32, 42, 53, 54, 70, 81 are low oncogenic risk strains (LR-HPV).

CHAPTER 4. EVALUATION OF THE ENROLMENT LOT OF PREGNANT WOMEN

We evaluated a group of patients clinically, cytologically and virologically at the time of enrolment in our study.

Results and discussion

In our study, we mention that we selected an identical number of both HPV confirmed (n=49) and unconfirmed/ negative patients (n=49) following HPV genotyping.

Analysis of the epidemiological profile of each group of patients by age group showed that the mean age of the participants included in this study was 29.16 years in the HPV-negative group and 31.73 years in the HPV-positive group, which was statistically significant ($p = 0.006$).

In the literature, the estimated mean age of causal HPV infection was 23.9 years, with the caveat that about 42.7% of these infections occurred at the age of at least 27 years.

Analysis of the education level of the patients included in this study revealed a higher percentage of the group of patients with higher education who associated HPV infection compared to the group of patients with high school as their last form of education (50.6% versus 47.4%), the differences not being statistically significant. Mansouri et al. also found in their analysis that, although a higher level of education and therefore higher awareness was expected, only 10% (Morocco), 17.7% (Nigeria), [48] 21.7% (Malaysia), [49] 50.6% (Ethiopian medical student group), [50] respectively 55.4% (Portugal) [51] of this group of patients were aware of HPV infection and methods of infection prevention. The field with the highest level of knowledge of risks as well as methods to prevent infection and secondary cervical dysplasia remains medical (general medicine, biology). [52]

Analysis of the groups in terms of marital status, among those with a positive HPV genotyping result and we found a percentage superiority in the group of married patients who associated a positive result in a percentage of 52.4% compared to the group of unmarried patients (35.7%). The percentage difference was not statistically significant.

Analysing the longevity of intimate relationships of the patients included in the study, both groups (HPV positive and HPV negative) showed relatively comparable results (4.18 years as opposed to 5.58 years).

At this level it is important to bring into discussion the information obtained by Thompson et al. regarding the importance of vaccination in terms of the type of couple relationship the women included in their study had. Thus, they observed that women who lived with a partner or those who had never been married showed more interest in the topic of HPV vaccination than married women. [53]

Analysis of vicious smoking behaviours was similar in the HPV-positive group (40.8% versus 40.8%), with no statistical significance.

We also assessed the percentage ratio of patients with HR HPV infection to LR HPV infection among pregnant smokers. Thus, out of a total of 20 pregnant smokers we observed a percentage of 25% (n=5) associating HR HPV infection, respectively a percentage of 75% (n=15) associating LR HPV infection.

In 2008, Vaccarella et al. concluded that smokers were associated with a moderately significant increased risk of HPV infection prevalence, with the risk of being HPV positive being directly proportional to the increase in the number of cigarettes consumed during a day, and women who reported smoking a minimum of 15 cigarettes per day had a 2-fold increased risk of being positive compared to women who never smoked. [54]

In terms of viral clearance there is some contradiction in the influence of tobacco use, [55] but the regression of low-grade intraepithelial lesions over 2 years is significantly reduced in smokers compared to non-smokers. [56]

Analysis of the HPV strain type identified among positive patients revealed a numerical superiority of cases with a single infecting genotype (36 out of 49) and a numerical superiority of infections with oncogenic strains (49%).

When we consider the literature data, it mentions that genotypes 51 (HR) and 18 (HR) were identified predominantly in the 14-25 age group, reducing further in the 26-46 age group.

Analysis of the infectious risk of patients acquired from antiviral vaccination determined a non-significant percentage superiority of negative results (62.5% compared to 37.5%) compared to the percentage inferiority of these negative results observed among patients who did not undergo the vaccination scheme (48.9% compared to 51.1%), both results without coefficient of statistical significance.

Regarding concurrent HPV infections, Mycoplasma hominis and Ureaplasma urealyticum are recognised as risk factors for rapid progression of preneoplastic cervical lesions along with persistence of HPV infection, i.e. increased viral replication. These bacteria were found in 30 ± 80% of the urogenital tract of women as commensal microorganisms, but have pathogenic potential. [56, 57] In our study, we found a 6.1% difference between the 2 groups of patients (diagnosed with or without co-infection), a statistically insignificant difference ($p = 0.375$).

Regarding the risk of Chlamydia trachomatis infection, no patients with genital-origin Chlamydiosis were found in our study.

HPV infection associated with pregnancy

In addition to the neoplastic risk of HPV infection, morbidity secondary to infection observed during the course of pregnancy has also been suggested. As the maternal hormonal environment and immune system undergo significant changes during pregnancy, the persistence of the virus is arguably favoured. Literature data on HPV infection in pregnancy and its implications for the pregnant woman and her unborn child are not numerous.

Various studies have shown an increased risk for adverse pregnancy outcome among HPV-positive women, with the clinical impact of infection including a range of conditions including prematurity, spontaneous abortion, pregnancy-induced hypertensive disorders, intrauterine growth restriction and low birth weight relative to gestational age, premature rupture of amniotic membranes and intrauterine fetal death. Therefore, understanding the pathogenic mechanisms of HPV that may negatively impact pregnancy outcome and establishing potential approaches to counteract them is of interest both in an attempt to optimise pregnancy and obstetric outcome and to improve survival and health of the newborn.

In the comparative evaluation of our groups we did not find an association of hypertensive disorders among positive patients compared to negative patients. Also, Subramaniam et al. did not observe HPV infection as a risk factor by itself for determining hypertensive disorder in pregnancy after evaluating a cohort of 2321 HPV positive patients. Differences in HTAIS rates in this study were not statistically significant (17.0% HPV positive versus 16.4% HPV negative; adjusted RR 1.0, 95% CI 0.7-1.5). [58]

The analysis of preterm birth risk was performed from several perspectives. Thus, firstly, in our study, the association between genitally detected HPV and the risk of preterm birth was found to be statistically significant (22.4% versus 8%, $p = 0.005$).

At the same time, in our groups no higher risk of preterm birth was found in patients infected with oncogenic strains compared to infection determined by non-oncogenic strains (21.2% in the HPV LR group versus 25% in the HPV HR group, $p = 0.766$).

As for the link between HPV and PROM, several studies have examined the involvement of the virus in this pathology. Also, following a targeted analysis of the incidence of PROM in the evaluated group, Cho et al. specifically analysed the incidence rates of PROM in two population groups (HPV-positive vs HPV-negative patients), finding percentages very close to the results of our study (27.3% of HPV-HR-positive women were associated with PROM compared to 14.2% of HPV-HR-negative women ($p = 0.029$)). [2]

Analysis of the involvement of HPV infection in the overall duration of labour in our studied groups does not show statistically significant differences either in patients presenting with intact amniotic membranes (9 hours in case of positives compared to 8 hours in case of negatives) or in patients admitted with spontaneously ruptured membranes (7.25 hours compared to 6.6 hours).

We assessed the percentage number of caesarean births compared to vaginal births in the study group (HPV positive). Thus, among HPV-positive pregnancies ($n = 49$), 77.6% of

births (n = 38) were completed by caesarean section, while 22.4% (n = 11) were completed vaginally.

We also assessed the percentage of pregnancies presenting to the emergency room for admission to the obstetrics-gynaecology ward for spontaneous rupture of amniotic membranes in the study group. Thus, we found that the HPV positive group included 12 pregnant women (24.5%) who were admitted with early spontaneous rupture of membranes, and 37 pregnant women (75.6%) who did not present this sign at the time of admission.

In the group of HPV positive patients we compared the risk of preterm birth according to the type of HPV strain. Thus, among HPV positive patients with premature rupture of membranes (n = 12) we found that 100% had a preterm birth (<37 weeks).

We wanted to compare the percentage of pregnant women admitted with spontaneously ruptured membranes and in the control group (HPV negative). Thus, we found that in this group only 26.5% (n = 13) were in this scenario, compared to 73.5% (n = 36) of pregnant women without PROM.

We examined the number of patients with HR versus LR HPV infection among pregnancies admitted for amniotic fluid loss. Thus, among the 12 HPV-positive pregnant women with ruptured membranes, HPV LR was found in 58.3% (n=7) of them, compared to 41.7% (n=5) who associated HPV HR.

Also, **personal pathological history of excisional procedures** performed at the cervical level in positive patients associated, in our study, preterm birth in 45%, without statistical significance

Analysis of the **risk of miscarriage** showed a higher percentage in the group of married patients regardless of the presence of the virus (34.5% compared to 7.1%, p = 0.040).

Also, in our study we found a higher risk in case of positives (44.9% compared to 16.3% for negatives, p = 0.002).

In the present study we also observed a higher risk in case of presence of oncogenic strains (60.6% compared to 12.5% for LR, p = 0.001).

Determinant risk of infection with HPV strain HR 16

HPV 16 was found to be the most prevalent HPV genotype overall.

In our study we analyzed the degree of association of genotype 16 with risk of miscarriage and observed percentage differences of 72.2% compared to 27.8% (p=0.003).

In our study, we did not detect a significant risk for preterm birth among patients with HPV 16 (22.2% versus 77.8%, p=0.977).

In our study, analysis of the possible association of infection with strain 16 and the risk of developing pregnancy-induced ETS, the proportion was identical, not detecting a risk in this area.

CHAPTER 5. MATERNAL-FETAL TRANSMISSION OF HPV INFECTION

Introduction

Human papilloma virus infection is known to be the leading cause of cervical cancer. The increasing incidence and prevalence of HPV infection is a consequence of the development of HPV detection mechanisms and the identification, through screening programmes, of people at risk of developing malignant lesions.

The main route of transmission of this envelope-less DNA virus is sexual, with tropism for epithelial cells in the skin and mucosa.

HPV infection can also be transmitted periconceptional, via infected sperm, and the virus is also found in seminal fluid in a percentage ranging from 8-64%.

Intrauterine transmission occurs either upstream from the maternal genital tract through microlesions in the membranes or via placental blood. In amniotic fluid, viral DNA has been identified in 15-65%.[43]

Perinatal transmission occurs through direct contact of the newborn with infected cells in the cervix or vagina at the time of vaginal delivery or through caesarean section in pregnancies with rupture of amniotic membranes. .[1]

Results and discution

Perinatal transmission of HPV has already been demonstrated in several studies in small groups of patients, but also in a large prospective study: the HERITAGE study. 167 HPV-positive pregnant women were recruited in this study (HPV-DNA present in cervical-vaginal secretion in the first trimester, HPV-DNA subsequently repeated in the third trimester).

In the studied group, we included 49 patients, HPV positive pregnant women, diagnosed at the first visit to the obstetrician or known with HPV infection in the preconceptional period, some of them having in their personal pathological history excisional procedures performed on the cervix for dysplastic cervical lesions. The baseline characteristics of the study participants are summarised below in Table 5.1.

Our study compares these variables between the two groups of pregnant women - the study group and the control group - both consisting of 49 patients.

Regarding the analysis of the study group, we observe a mean age of 31.73 years (statistically significant difference, p = 0.006), a higher educational level (no statistical significance), a marital status of marriage, in which we observe a predominance of HR HPV strains, as well as a tendency of association of a cervical lesion, i.e. a lower level of vaccination.

The types of HPV strains and their incidence in the study group were also studied and grouped.

Results of the biological samples

From the analysis of the results obtained for the gastric aspirate - amniotic fluid samples, out of the total number of samples tested, 4 samples (4, 10 ,12, 13) did not show amplification for beta-globin and thus did not qualify for further determination.

Following visualization of amplicons for beta-globin in oral swab samples, 5 samples were found to be negative in the PCR reaction (1,2,4,5,6) and were not used for further testing.

Concerning the blood samples, it was observed that all samples investigated were positive for beta-globin gene amplification confirming the presence of quality DNA in the samples tested.

Following Nested PCR testing of the investigated samples, a method with increased sensitivity, all samples were negative for HPV-DNA.

Perinatal transmission of human papillomavirus has been the subject of several studies in relatively small groups of patients. The results suggest that newborns are exposed to and can become infected with HPV in the perinatal period. The first case of perinatally transmitted HPV infection was identified in 1956 as a case of juvenile laryngeal papillomatosis. [59]

In studies confirming the presence of HPV in the oral cavity or genital region in newborns from HPV-positive mothers in pregnancy, the infection rate ranges from 4 to 79%. [60-70]

Regarding concordance between HPV strain type in parents and newborns, a study of a large group of subjects (n = 574) published in 2004 shows low concordance (<1%), with the rate of HPV-DNA in the genital area of newborns not influenced by gestational age, sex and birth weight of the newborn, neonatal pathology or associated malformations. [67]

To better understand the vertical mode of transmission of HPV infection, a group of researchers in Montreal, Canada, designed the HERITAGE study. [43] The study initially included 167 pregnant women tested in the first trimester of pregnancy for 36 HPV genotypes, with subsequent repeat testing in the third trimester of pregnancy for pregnant women who initially tested positive. Placental samples were also collected for HPV-DNA testing at the time of birth. In newborns of HPV-positive mothers, samples were collected from the conjunctiva, oral and oropharyngeal cavity and genital region, with subsequent repeat testing at 3-6 months to 2 years. Blood was taken from the mother and newborn for HPV antibodies.

Variables

Age (years)	Mean	31,73
Education: n (%)	High school	9 (18,4)
	Higher education	40 (81,6)
Marital status: n (%)	Single	5 (10,2)
	Married	44 (89,8)
Relationship length (years)	Mean	4,18
Smoker: n (%)	No	29 (40,8%)
	Yes	20 (59,2%)
Type of HPV strain: n (%)	HR	24 (49)
	LR	16 (32,7)
	HR+LR	9 (18,4)
Type of HPV infection: n (%)	Unică	36 (73,5)
	Multiplă	13 (26,5)
Type of HPV induced lesion: n (%)	Cervicală	46 (93,9)
	Altă localizare	3 (6,1)
HPV vaccinated: n (%)	No	46 (93,9)
	Yes	3 (6,1)
Miscarriage: n (%)	No	27 (55,1)
	Yes	22 (44,9)
Miscarriage: n (%)	LR	2 (12,5)
	HR	20 (60,6)
Miscarriage + HPV 16: n (%)	No	9 (40,9)
	Yes	13 (59,1)

PIH: n (%)	No	45 (91,8)
	Yes	4 (8,2)
Length of labour (hours)	Mean	9,29
PROM: n (%)	No	37 (24,5)
	Yes	12 (75,5)
Type of delivery: n (%)	Vaginală	11 (22,4)
	Cezariană	38 (77,6)

Table 5.1. Variables of the studied lot

The results of this prospective study reported an increased prevalence of HPV in pregnant women, 45%, with 14% positive placental tests. The percentage of positive tests in the newborn and at 3 months of age was 11%. This is the first study to identify the presence of HPV in the conjunctiva, with 5% testing positive.

Our study is a prospective, control study, conducted over a 3-year period and includes 49 pregnant women with a history of recent HPV, prior to pregnancy, modified Pap smear and genital condylomatosis in the context of HPV, with the characteristics listed above. These characteristics of the working group overlap with those of the HERITAGE study.

The high prevalence of HPV in the Canadian study may be due to several factors: young age of women tested (18-30 years), present sexual activity - the most important risk factor for HPV, low HPV vaccination - the vaccination campaign in Quebec started 2 years before this study.

The low prevalence of HPV in pregnant women in our cohort can be explained by the fact that HPV sampling was from the cervix, not from vaginal secretion where the expected HPV prevalence is higher. Also, this study was conducted exclusively in the Obstetrics-Gynecology and Neonatology Clinic of the "Elias" SUU.

Risk factors for perinatal transmission are identified as: **viral load**, [74] **presence of maternal genital tract lesions**, [75] respectively **maternal infection with multiple HPV genotypes**. [60]

The dynamics of HPV transmission between parents and children were prospectively studied in Finnish families. The conclusion of the researchers from the University of Turku was that the risk of HPV infection in children correlates with the persistence of maternal infection and not with the presence of the virus in pregnancy in the vaginal secretion. [68]

Referring to the risk factors for HPV infection identified in the literature, the results of our study are as follows:

- the diagnosis of HPV infection was established before pregnancy in the majority of cases, 40 (81.6%), and during the first trimester in 9 cases (18.4%);
- lesions present at the time of birth were present in 8 of the HPV positive pregnant women, i.e. 16.3%; 4 pregnant women (50%) of the 8 with lesions present at the time of birth had at least 2 HPV strains identified;
- 22 of the pregnant women (44.8%) in the study group were primiparous, 6 of them, 27%, positive for at least 2 different HPV strains; there was also 1 case positive for 6 HPV types;
- most HPV-positive pregnancies were delivered by caesarean section (38 - 77.6%), with vaginal delivery possible in 11 of the study group (22.4%).
- of those who delivered vaginally, 2 were primiparous (13.6%), in one case 2 different HPV strains were identified;
- there were 2 cases of vulvar condylomatosis, 1 case in primipara with 3 HPV strains, 1 case in secundipara with a single HPV strain.

I mention that all HPV tests were qualitative, performed prior to pregnancy or in the first trimester of pregnancy, correlated with the modified Pap smear, without determination of viral load or viral clearance in pregnancy.

All oral mucosal samples collected from newborns at the time of birth were negative for HPV, thus waiving the subsequent collections originally planned in the study protocol from 1 month to 6 months postpartum.

In an attempt to elucidate the mode of perinatal transmission of HPV infection, studies/research have aimed to identify HPV-DNA in amniotic fluid, umbilical cord blood samples and placental samples.

In the study published in 2008, part of the Finnish Family Study, the presence of HPV-DNA was identified in 3.5% of 311 cord blood samples and was correlated with modified maternal cytological examination. The only independent predictor of HPV presence in cord blood was a history of vulvar condylomatosis. [76] Presence of the virus in cord blood, as well as in the placenta, increases the risk of oral and genital carriage in the newborn 4-fold, but was not correlated in this study with positive maternal peripheral blood samples.

In most cases studied, evidence of HPV in the cord or placenta is not consistent with the presence of HPV in genital culture or oral mucosa before pregnancy. This can be explained by the fact that HPV infection of the placenta occurred early in pregnancy and genital viral clearance is completed by the time of delivery.

In the literature, studies are also published in which the viral identification rate for HPV is low, in some even zero, for blood samples collected from the umbilical cord and amniotic fluid. Low HPV detection rates in cord blood have been reported from studies that excluded pregnant women with HPV lesions present from the sample.

In this regard, Wonda et al. tested a large group of clinically asymptomatic pregnant women for the presence of viral DNA in placenta, amniotic fluid and cord blood. Of the 153 pregnant women, 56 (36.6%) had HPV-positive cervico-vaginal tests collected prior to cesarean section surgery. HPV-DNA was detected in 8 placental samples but was absent in all cord blood and amniotic fluid samples.189

In my study, I opted to identify HPV-DNA in cord blood and newborn gastric aspirate (amniotic fluid) immediately postnatally.

It should be noted that 8 patients in the study group, representing 16.3%, had genital lesions and Pap smear with altered cellularity at study entry, i.e. immediately before pregnancy or in the first trimester of pregnancy. In the study group, there were also 2 cases of vulvar condylomatosis, considered an independent predictive factor for the presence of HPV in cord blood.

In all blood samples investigated, the presence of high-quality DNA was confirmed, with samples tested positive for beta-globin gene amplification.

Nested PCR testing, a method with increased sensitivity, of samples investigated and confirmed with the presence of quality DNA, all samples were negative for HPV-DNA.

These results could be explained by the persistence of the cellular abnormalities identified on Pap smear for another 1-2 months after HPV-DNA clearance and the possibility that they were present before birth. [78]

From the analysis of the results obtained on the gastric aspirate - amniotic fluid samples, out of the total number of samples tested, 4 samples (4, 10 ,12, 13) did not show amplification for beta-globin and thus did not qualify for further determinations. As with the

buccal swab and cord blood samples, Nested PCR testing of amniotic fluid samples was negative for HPV-DNA.

These results are supported by several studies in the literature that did not detect HPV-DNA in amniotic fluid samples. [77-79] These studies aimed to identify HPV in amniotic fluid in pregnant women asymptomatic for HPV infection189 or with an indication for amniocentesis for prenatal diagnosis in suspected genetic abnormalities with intact membranes. [79,80]

Ruffin et al. publish in 2006 the results of their study of 146 pregnant women with an average age of 35.2 years, mostly Caucasian 78%, but also African American 5%, Asian 14% and Hispanic 2%. The population sample chosen is similar to other studies suggesting that 12-36% of pregnant women have HPV infection, but although they contained high-quality DNA - positive for beta-globulin, samples collected from amniotic fluid were tolerably negative for HPV. [79]

A mention related to the age of the pregnant woman considered an important factor in transplacental transmission. Some authors consider that 75% of HPV-positive women are aged 15-24 years. [81] Note the higher average age - 31.73 years, with a range of 21-42 years - of pregnant women in our study group, which is close to that of pregnant women enrolled (35.2 years) in studies in which amniotic fluid samples were entirely negative for HPV-DNA (average 35.2 years). [79]

CHAPTER 6. HPV INFECTION AND PREMATURITY

Introduction

Prematurity is an important cause of neonatal morbidity and mortality, and the latest studies show that HPV infection also impacts the newborn. Changes in pregnancy related to HPV infection, inflammatory changes in the trophoblast and histopathologically identified secondary placental abnormalities increase the risk of obstetric complications such as premature or early rupture of amniotic membranes, prematurity, pre-eclampsia and spontaneous abortion.

The persistence of infection in pregnancy can be explained by increased levels of steroid hormones that alter the immune status of the pregnant woman with the aim of maternal immunological tolerance of the product of conception, but also of increasing the risk of infection. [82]

Persistence of infection correlates with genotype and viral load, [83] an important factor being the particularity of HPV to alter local immunity. [84,85] Host-dependent factors influencing HPV persistence are well known as age, smoking, number of sexual partners and age of sexual debut, socioeconomic status, associated cervico-vaginal infections, hormonal status, host immunocompetence and oral contraceptive use. [86-88]

The results of a retrospective study examining HPV status and placental histopathological samples collected from pregnant women with pregnancy complicated by prematurity were published in 2011 and demonstrated that infection with HR-HPV strains is a risk factor for prematurity and placental abnormalities. [89]

HPV infection causes placental dysfunction through trophoblast cell damage with cell apoptosis and compromised placental adhesion and circulation.

The interaction between trophoblast and virus leads to an exaggerated inflammatory response, with collagen degradation and fetal membrane rupture. 46, 48, 49 In the first trimester of pregnancy, the trophoblast secretes pregnancy-associated protein A; low values of this correlate with increased risk of PROM and prematurity and may be an indirect indicator of trophoblastic damage in HPV-positive pregnant women. 46

The impact that HPV infection can have in pregnancy, the increasing interest of researchers in this topic, and the controversial results recently published, led me to analyze, in our study, the risk of prematurity associated with HPV infection.

Material and method

We conducted an observational comparative study with control group in the Obstetrics-Gynecology and Neonatology Clinic of the SUU Elias, the study group consisted of HPV positive pregnant women in the first trimester of pregnancy, the control group of negative or unconfirmed pregnant women with HPV infection. We prospectively evaluated whether vaginal HPV infection in pregnancy is associated with preterm birth, independent of other factors.

Preterm birth was defined as birth before 37 weeks gestation.

Results and discussion

In our study, we followed 49 HPV-positive pregnant women, made correlations between HPV infection and risk of preterm birth and compared the results with the control group of HPV-negative pregnant women.

In the study group, HPV infection was associated with preterm birth in 22.4% of cases (n = 11). In the control group, preterm birth was present in 8.2% of cases (n = 4), the result being statistically significant (p = 0.05).

Data in the literature are controversial. In 2008, Gomez et al. present the results of their study identifying more frequent HPV-DNA in the placenta of preterm delivery pregnancies compared to control group placentas (p = 0.03). HPV infection in the trophoblast induces cell apoptosis, placental dysfunction with consequences on pregnancy outcome, including preterm birth. [36] Another study evaluating HPV infection as a risk factor for preterm birth, published in 2016, identifies no significant difference in preterm birth rates between HPV-positive and HPV-negative women (16.5% compared to 12.2%).[58]

Another risk factor associated with preterm birth is excisional cervical surgery resulting in a shortened cervical length. In this regard, Miler et al. recently (2016) describe an increased risk of prematurity in excisional cervical dysplasia. [90] Diathermic loop excisional surgery induces cervical collagen remodeling that is associated with preterm birth. It should be noted that the study does not take into account other factors that may influence outcome, such as: genital HPV-associated infections, HPV strain type.

In our study group, of the 11 pregnant women with preterm birth, 5 (45%) had cervical interventions, 3 of them (60%) were HPV 16.

Currently, viral infection is thought to influence the immune shaping role of the trophoblast and trigger an exaggerated immune response to infection in the presence of bacteria. [91]

Genital HPV infection accentuates susceptibility for ascending infections, consequently, coinfections with Ureaplasma urealyticum, Mycoplasma hominis and Chlamydia trachomatis are associated with immune system changes and preterm birth (6 Inf Diseases). In a 2013 study of mice, Racicot et al. demonstrate that viral infection affecting Ureaplasma urealyticum like) can ascend from the maternal-fetal interface to the gravid uterus. This reduction in self-defense mechanisms is driven by decreased anti-inflammatory and inflammatory cytokines in the virally infected cervix. [92]

In our study, there were 8 HPV-positive pregnant women (16.3%) who associated other genital infections and 5 pregnant women (10.2%) from the control group. Co-infections were with Ureaplasma urealyticum and Mycoplasma hominis, but there was no case of Chlamydia trachomatis infection.

In the most recent multicentre study conducted in Canada on 899 pregnant women, the risk of prematurity is correlated with the presence of HPV16/18, independent of other factors. [93]

The HERITAGE prospective study was conducted in 3 academic centres in Montreal from November 2010 to October 2016. The objective of the study was the association between HPV infection and preterm birth, thus vaginal HPV-DNA was determined in the first and third trimester of pregnancy, as well as at the placental level, The results of this study indicate a significant correlation between the persistence of HPV 16/18 strains at the vaginal level and preterm birth. Results were similar in pregnancies without a history of neoplastic cervical lesion.

In our study, we also looked for the association between preterm birth and HPV strain type, LR-HPV, subsequently correlating: preterm birth - HPV 16. The results were not statistically significant (p=0.766); among pregnant women with LR-HPV, 25% (n=4) associated preterm birth, respectively 21.2% (n=7) of the HR-HPV group.

Specifically following the correlation between preterm birth and the presence of HPV 16, only 4 cases (22.2%) had a history of preterm birth, other 14 cases (77.8%) were not associated with prematurity.

The results of our study can be justified by the limited number of cases included in the study group, as well as by the fact that HPV testing of pregnant women was performed before pregnancy or in the first trimester, all tests being qualitative, without determination of viral load or viral clearance during pregnancy.

For the newborn, HPV-DNA has been identified in the genital mucosa, oral mucosa, and conjunctiva, where it causes up to 10% of conjunctival tumors. [94]

In the literature, there are several studies that identify -HPV-DNA in buccal swab samples in newborns aged 1 to 4 days, with a high variability rate of between 0.9% and 56%.[74,95,96]

Variability is determined by the mode of collection, the sensitivity of viral DNA determination methods, the very different number of patients entered into the study. HPV types identified were both LR (6, 11) and HR (16, 18 - most common).

The same studies identify the presence of HPV-DNA 6, 11, 16, 18 with a wide variability also at 6 weeks and 6 months: 62%/59%,[98] respectively 0%/0%. [99, 100] In the Finnish HPV Family Study, at 6 months 21% of oral samples associate a positive result by nested PCR method. [36, 68]

In the oral cavity, HPV appears to persist longer than in the genital mucosa, with the most common strain in asymptomatic infections being 16, followed by LR - 6 and 11. [13]

In our study, all samples collected at birth from the oral mucosa were negative for HPV-DNA by Nested PCR, a method with increased sensitivity. Clinically, at birth, none of the newborns showed lesions characteristic of HPV infection, cutaneous or mucosal.

Subsequently, these children were clinically evaluated at 1 month and 6 months and found to have no skin or mucosal lesions, but respiratory disease, upper respiratory tract infections and bronchiolitis, which were more common among those from HPV-positive mothers. Thus, 29 children (59.2%) in the sample of HPV-positive mothers had respiratory infections in the first 6 months of life, compared with 11 children (22.4%) in the sample of HPV-negative mothers.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The conclusions drawn from this study are:

1. The study group, consisting of 49 HPV positive pregnant women; respectively the control group consisting of 49 HPV negative pregnant women.
2. We analysed the epidemiological characteristics of the investigated group and identified the following particular elements:
   - Mean age is 31.73 years.
   - Patients with higher education were more frequently associated with HPV infection (50.6% versus 47.4%).
   - Married patients associated a higher percentage, 52.4%, with a positive HPV genotyping result.
3. We assessed the distribution of risk factors for cervical lesions, namely: duration of relationship, smoking status of the patient, type of infecting HPV strain, co-infection with species such as Mycoplasma hominis and Ureaplasma urealyticum.
   - The mean relationship duration among positive patients was 4.18 years.
   - Female smokers accounted for 40.8% of patients who were associated with a positive HPV test result, while non-smoking patients were positive in an identical proportion of 40.8%.
   - Of the total number of pregnant smokers (n=20), we observed 25% (n=5) associating LR HPV infection and 75% (n=15) associating HR HPV infection.
   - We observed that there was a higher incidence of patients infected with a single HPV strain (n=36), followed by patients infected with 2 strains (n=7). One case associating 6 HPV strains was also detected.
   - Depending on the infecting HPV genotype, strict association of infection with non-oncogenic HPV strains was observed in 32.7% of cases (n = 16), with oncogenic HPV strains was detected in 49% of cases (n = 24), while association of both types (both oncogenic and non-oncogenic) was noted in 18.4% of cases (n = 9). The highest incidence was shown by HPV strain 16, both in single and associated infections, followed by HPV 6 and HPV 18.
   - Regarding co-infection with species such as Mycoplasma hominis and/or Ureaplasma urealyticum, in the working group we identified 8 patients out of 49 (a percentage of 16.3%), and in the control group we found 5 patients out of 49 (a percentage of 10.2%).

- Another co-infection we considered was Chlamydia trachomatis infection, but in our groups we did not identify any patient with chlamydiosis.

4. We also analysed the HPV vaccination status of patients compared to their passage through HPV infection.
   - Of the HPV vaccinated patients only 37.5% (n = 3) tested positive for HPV genotyping, the remaining 62.5% (n = 5) were negative. Of patients not vaccinated against HPV, 51.1% (n = 46) were positive, while 48.9% were negative (n = 44).
5. Another aspect analysed was the type of lesion with which the patients included in our study were diagnosed. Thus, 93.9% (n = 46) of positive patients were diagnosed with cervical lesions, the remaining 6.1% (n = 3) had vulvovaginal condylomatosis lesions.
6. Following evaluation we found the presence of pregnancy-induced hypertension in 4 HPV-positive and 4 HPV-negative patients, respectively. We thus aimed for identical proportions in both groups of patients.
7. We assessed the duration of labour in the group of positive and negative patients. We observed a comparable mean duration of labour between the 2 groups studied: 9 hours for the positive patients and 8 hours for the negative patients. When analysing the groups of positive versus negative patients who went through labour with ruptured membranes, we found that the mean duration of labour was approximately equal, i.e. 7.25 hours among positive patients compared to 6.6 hours among negative patients.
8. Thus, among HPV-positive pregnancies (n=49), 77.6% of deliveries (n=38) were completed by caesarean section, while 22.4% (n=11) were completed vaginally.
9. We also evaluated the percentage of pregnancies presenting to the emergency room for admission to the obstetrics-gynecology ward for spontaneous rupture of amniotic membranes among patients with HPV infection.
   - Thus, we found that the HPV positive group included 12 pregnant women (24.5%) who were admitted with early spontaneous ruptured membranes and 37 pregnant women (75.6%) who did not show this sign at the time of admission.
   - We examined the number of patients with HPV infection HR compared to LR in pregnancies admitted for amniotic fluid loss.
   - Thus, among the 12 HPV-positive pregnant women with ruptured membranes, HPV LR was found in 58.3% (n=7) of them, compared to 41.7% (n=5) who associated HPV HR.

10. Another aspect assessed was the risk of premature birth.

- We compared the groups by marital status to identify the background preterm birth risk of the patients and found that 16.7% of married patients (n = 14) were associated with preterm birth compared to 6.7% (n = 1) of the unmarried group.
- We also compared the group of patients with HPV infection and without HPV infection to identify the background preterm birth risk of the patients and observed that in the group of HPV positive patients 22.4% (n = 11) associated preterm birth compared to the group of HPV negative patients of which only 8.2% (n = 4) associated preterm birth. The difference was statistically significant (p = 0.050).
- We targeted 25.0% (n = 4) of the group of patients infected with low-risk HPV strains and 21.2% (n = 7) of the group of patients infected with high-grade HPV strains who associated preterm birth.
- We paralleled the presence of HPV strain 16 with the risk of preterm birth among these patients. Thus, among HPV genotype 16 positive patients, only 4 cases (22.2%) had a history of preterm birth, as opposed to 77.8% (14 cases) who had no associated preterm birth history.
- We also investigated the risk of preterm birth among patients with HPV infection who underwent surgical intervention by diathermy loop electrosurgery. Of 49 HPV-positive patients, 11 had preterm births. Of this small group, only 5 cases (45%) had cervical surgery.

11. We found a higher percentage of miscarriages among married patients, i.e. 34.5% (n = 29) as opposed to the group of unmarried patients where only 7.1% (n = 1) were associated with miscarriage. These percentage differences were found to have statistical significance (p = 0.040). We objectified that 60.6% of HPV-positive patients who associated at least one miscarriage (n = 20) were diagnosed with high-risk strains, as opposed to 12.5% (n = 2) representing patients with miscarriage diagnosed with low-risk HPV infection. The percentage differences determined were statistically significant (p = 0.001). We identified that among patients with HPV 16 strain, 13 cases (72.2%) had associated miscarriages, compared to 5 cases (27.8%) who had no history of missed pregnancies. The percentage differences were statistically significant (p=0.003).
12. In our study, we aimed to identify viral DNA for HPV in newborns of HPV-positive mothers, supporting the idea of intrauterine and perinatal transmission of HPV infection. Thus, during the first minutes of life, in the delivery room, mouth swabs,

gastric aspirate - amniotic fluid - and cord blood were collected from newborns. In the biological samples collected, viral DNA isolation was followed using specific extraction kits.

13. We tested all biological samples for quality viral DNA content using the high sensitivity method - Nested type PCR, but the results were negative.
14. We clinically evaluated newborns, in the first minutes of life, and did not identify lesions characteristic of HPV infection, cutaneous or mucosal. At 1 month and 6 months reassessment, we found no skin or mucosal lesions, but the presence of respiratory disorders, upper respiratory tract infections and bronchiolitis, more common in those from HPV positive mothers. Thus, 29 children (59.2%) in the sample of HPV-positive mothers presented with respiratory diseases in the first 6 months of life, compared to 11 children (22.4%) in the sample of HPV-negative mothers. The difference is statistically significant, $p<0.001$.

Personal contributions

We conducted a prospective study on a group of 49 HPV-positive pregnant women with a numerically identical control group of HPV-negative pregnant women in order to highlight the maternal-fetal transmission of HPV infection and to analyze the association of the risk of preterm birth, miscarriage and pregnancy-induced hypertension with HPV infection.

In the present study we demonstrated a statistically significant correlation between the risk of preterm birth and the presence of HPV in pregnant women, regardless of strain type, HR or LR.

Regarding the association between HPV and the risk of miscarriage, we found a higher percentage of miscarriages in HPV positive patients compared to the HPV negative group and also demonstrated a higher percentage of miscarriages in married versus unmarried patients, both differences being statistically significant.

We included in the study the analysis of the group of newborns from HPV-positive and HPV-negative pregnant women in order to prove maternal-fetal transmission of HPV infection. We monitored and clinically reassessed the infants over a period of 6 months. Statistically, in the group of children from pregnant women in the study group, respiratory infections predominated, differing with statistical significance.

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