Sexuality, sexually transmitted diseases by oncogenic viral genotypes and importance of molecular screening methods

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Abstract

Viral infections bearing oncogenic potential play a vital part in cancer lesions identified in the heterosexual, homosexual and bisexual relationships.

Links have been set out between the genotypes of the human papilloma virus, HPV, a virus that has non-enveloped icosahedral capsids and a high oncogenic potential, those being 14 strains, and the herpes simplex virus (HSV2), meaning the form of premalignant and malignant lesions in the case of anogenital and oral-pharyngeal-laryngeal condition pathology, by HPV viruses mainly contacted by fellatio and cunnilingus, as well as by other infection sources.

In fact, the genome enters the transcription site from the nucleus of the infected cell (Longworth MS and Laimins L.A. 2004), the HPV receptor being integrin Alfa $_6$.

Key words:

koilocytes, genome, genotype, oncoprotein p16 Ink4a, Ki-67, HPV, HSV2, fellatio, cunnilingus.

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Main text:

Introduction:

Scope:

- to set the significance of screening by immunochemical tests, in this case by simultaneously detecting the proteins p16 INK4a and Ki-67 for early identification of the evolving forms of intraepithelial cervical lesions prone to lead to cancer, in a situation involving an infection with oncogenic genotypes of HPV.

- can the molecular screening methods replace the histopathology examination?

- what is the value of the dual immunocytochemical test, simultaneously for proteins p16 Ink4a and Ki-67, as molecular screening method, before and after the treatment?

- what is the treatment to follow in the case of HPV oncogenic genotypes in the structure of the cervix?

- form of removing the oncogenic genotypes at the time of finding them?

- importance of the *immunocytochemical* tests for correctly assessing the lesions.

Methods:

The epithelial lesions caused by viral infections with HPV and Herpes Simplex Virus Type 2, with oncogenic potential, transmitted by sexual intercourse, can be

1. Anogenital (fig.1.a, b, c)

- anal: anus, rectum, perineum

- genital: pubic region, vulva, vagina, cervix

2. Penis (fig.2.a, b, c)

3. Oral-pharyngeal-laryngeal: lips, mouth, pharynx, larynx

In this case, the most common oncogenic HPV types are 6, 11, 16 and 18.

The best researched, given its pathology and frequency of its severity, is the infection of the cervix with oncogenic genotypes of HPV.

Viral infection of the cervix with oncogenic genotypes

- The screening program, initially by the Babeș-Papanicolau cytology test (Pap smear), now by employing the Bethesda System, for early finding and treating the precancerous and cancerous lesions, has significantly diminished the death rate caused by the cervical carcinoma, the second most frequent cancer type in females after breast cancer.

HPV affects the mucosa in various ways:

- without determining a cervical lesion, HPV infiltrating the mucosa (fig.3.a,b); the infection is certified by a cytology test and genotyping. The valve speculum examination does not indicate a cervical lesion, especially in the case of endocervical cancer forms, which renders impossible to see the lesion in its early stage; sometimes, abnormal postcoital intermenstrual post-menopause vaginal bleeding occurs, vaginal purulent discharge, and foul smell. If HPV oncogenic genotypes were found on a macroscopic non-lesion cervix, which was modified as indicated by colposcopy (leucoplakia, mosaic, acetowhite, punctuation, atypical vessels, cuffed glands, iodine negative epithelium, invasive carcinoma), proven by HPV DNA and HPV DNA genotyping, I employed the molecular screening methods systematically, thus following the value of the progression risk of the intraepithelial cervical lesion to neoplasm. When needed, the histopathology examination was performed.
- it can cause cervical lesion or peripheral ulceration,(fig. 4.a, b, c, d, e, f, g) which easily bleed when performing a valve speculum examination (fig.5a, b), and the damaging of the cervix and the upper and lower Douglas pouch following even the smallest trauma, when the cancer lesion has not invaded the Douglas pouch and the vagina;
- virus enters by the existing damaged mucosa (fig.6), which is an aggravating factor in connection to the speed of virus inoculation and

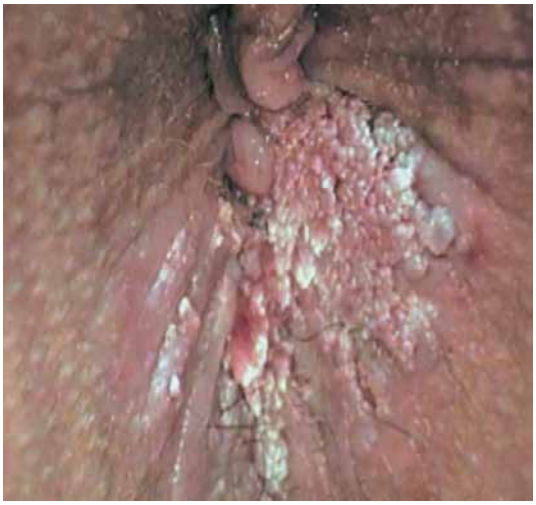

Fig. 1a

Fig. 1b

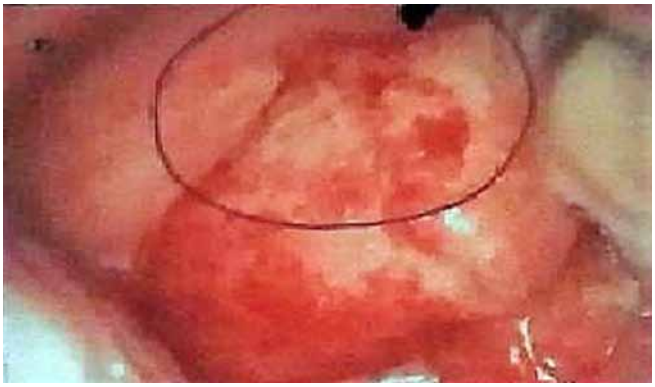

Fig. 1c

Fig. 1a -Anogenital warts by infection with HPV genotype 6; **b** - Condylomata acuminata positioned beneath the clitoral gland; **c** Cervical neoplasia; HPV infection, genotype 18

Fig. 2a

Fig. 2c

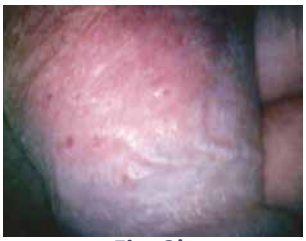

Fig. 2b

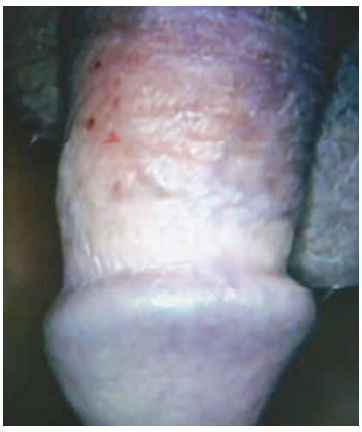

Fig. 2a - Herpetic vesicles located on the body of the penis **b, c**- Penile infection with HPV genotype 11 and HSV 2

disease progression, by breaking the DNA chains, which then leads to mutations by fast mitosis cell division.

The presence of koilocytes (Meisels and Fortin) in the biologic sample taken from the cervical surface has explained the existence of the HPV infection in which 65-77% of the cervical cancer is determined by genotypes 16 and 18 (Munoz NM et al. 2004). The rapidity of setting a diagnosis by RNAm detection for the viral oncoproteins E6 and E7, by molecular screening, renders a differentiation of the precancerous and cancerous lesions, thus resulting in a correct treatment. The viral load of a cell with genotype 16 or 18, by adding other HPV oncogenic or non-oncogenic genotypes, or with HSV type 2 increases the oncogenic risk of the infection and the risk that the intraepithelial lesion of the infected cervix would evolve, a fact proven by the sensitivity and specificity of the molecular methods, such as Real Time PCR and PCR Multiplex. Those are "in situ" hybridization or double detection techniques, which involve the qualitative simultaneous identification of proteins p16 and Ki-67.

Genital infection with oncogenic HPV genotypes is contracted in the first year after the onset of sexual activity, according to most authors, by 80% of females, while reaching a peak around 25-30 years, after which the prevalence decreases (Kjaer SK at al. 2000). That is because the organism of the infected person synthetizes specific antibodies, which directly affect the vital protein L1 of the infecting genotype in which the anti-oncogenic genes (against the vital oncoproteins HPV E6, E7) block the gene 53 (tu-

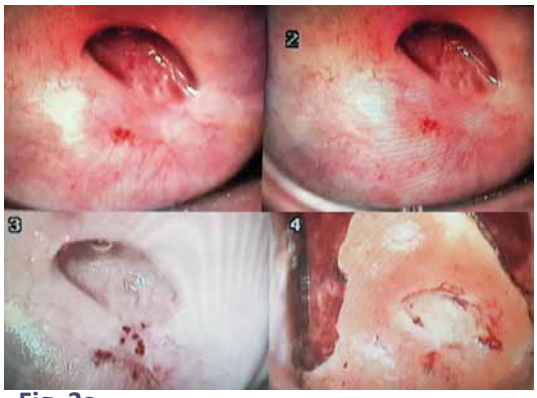

Fig. 3a

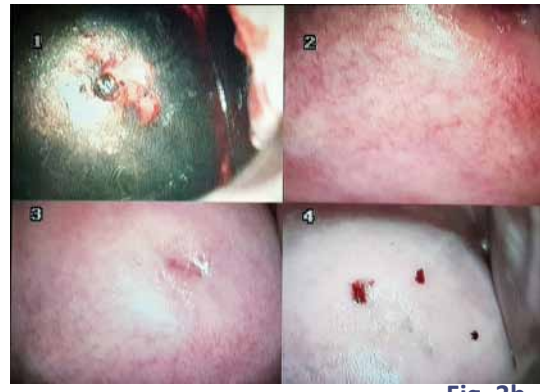

Fig. 3b

Fig. 3a, b - L-SIL and DNA HPV genotype 74

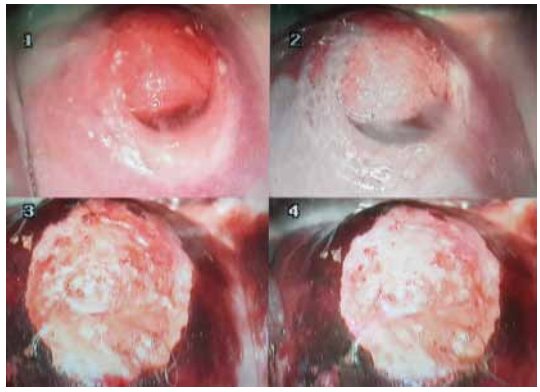

Fig. 4a

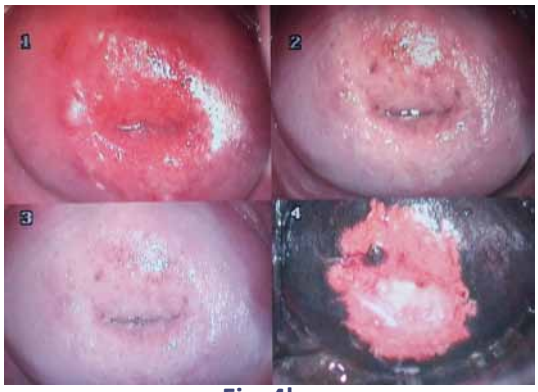

Fig. 4b

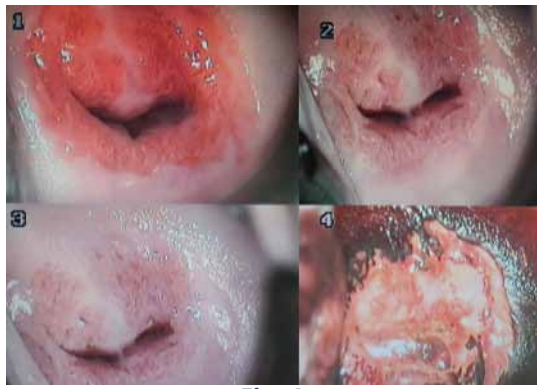

Fig. 4c

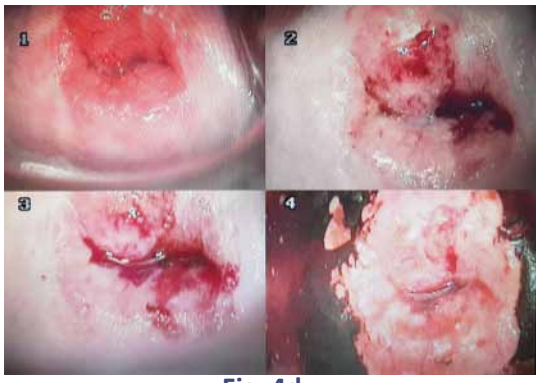

Fig. 4d

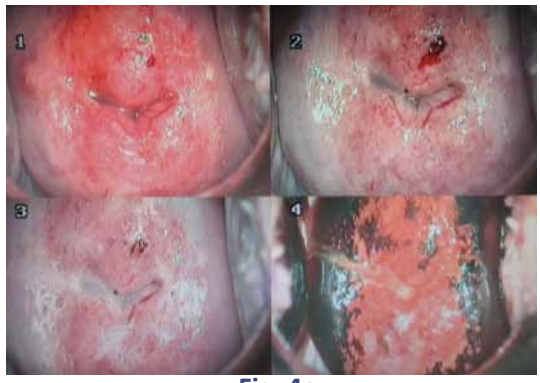

Fig. 4e

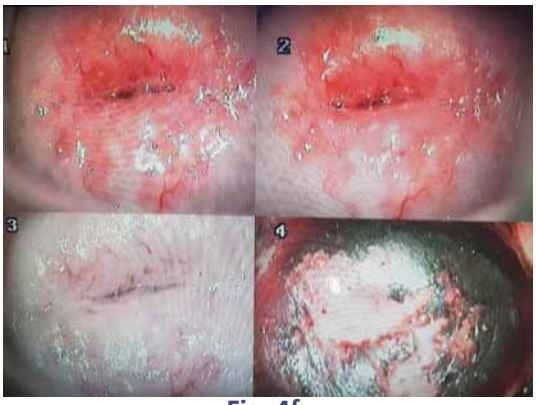

Fig. 4f

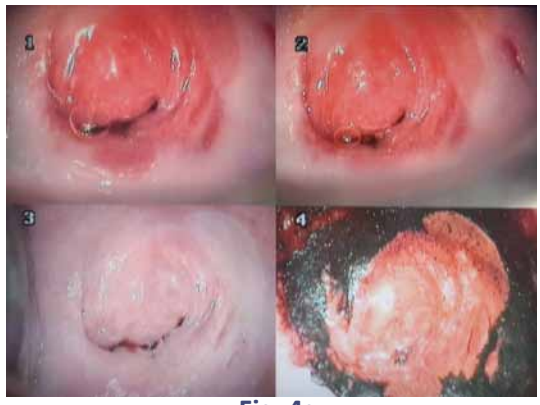

Fig. 4g

Fig. 4a - Colposcopic view for ASC-US and LR-HPV 42 and 82; **b, c-** L-SIL and DNA HPV negative; **d, e-** Colposcopic examination for L-SIL and HR-HPV types 18 and 51; **f, g**-colposcopic view for ASC- US and HR-HPV type 16

mor suppressor protein). The cell removes the genomic lesions caused by the oncogenic virus, before the cancer sets, thus determining the natural healing of the infection and removal of the virus from the cell immune system in 70% of the cases, during the first year and 90% of the cases, 12-36 months later, including in the case of infection with high risk genotypes (Giuliano AR et al. 2002).

RNAm demonstration for oncoproteins E6 and E7, which differentiate the precancerous of the cancerous lesions, indicates the oncogenic risk of the infested cervix, in a progressive manner from CIN II to CIN III or CIS, everything being proven by PCR or real time PCR molecular methods.

Zhou and his collaborators believed that the immune response in the HPV infection appears in only 54-69% of the females infected with genotypes 6, 16 and 18; thus, there is not a 90% healing of the initial infection.

I mention that the organism acts in complex manners for removing the oncogenic genotype. The HPV infection affects the basal layer of the cervical epithelium, especially the keratinocytes in progress of differentiation. The cellular immune response is acknowledging the infected cells and stimulating the Th1 lymphocytes, which activate the T cytotoxic lymphocytes that would then destroy the virus. Other defense manners are physical barrier (cell integrity), chemical barrier (acid pH, mucous, and fatty acids), and humoral factors-interferon, lysozyme (A. Guyton), thus explaining the great healing degree in patients of 90%.

But immunity can increase or decrease in certain situations such as, for example, pregnancy, case when the anal and vulvar condyloma disappear after curettage.

In that situation, the gestation process diminishes the immunity by increasing the immune tolerance with the aim of keeping the product of conception, which is an allograft. After the pregnancy is removed, the patient's immunity is restored and the *vulvar condylomatosis* subsides.

Immunity can drop also in the case of a dysimmune status, such as the existence of a chronic condition, like diabetes mellitus, kidney failure, chronic hepatopathy, chronic extragenital infection, or autoimmune or malignant disease located somewhere else, while the level of the molecular biomarkers can vary.

In the case of oncogenic genotypes persistence, one must consider the coexistence of a precancerous lesion, which might evolve towards malignant pathology, case when the immunocytochemical investigations would provide relevant data of great concern.

Infection with oncogenic viruses is much more severe when it is associated with other local or general infectious diseases, such as HSV type 2, B or C virus hepatitis, HIV, Lues, cytomegalic virus, Chlamydia, the use of oral contraceptives because HPV has structures activated by steroid hormones, particularly progesterone, vaginal or of spermicides that damage the vaginal mucosa and that of the cervix, while favoring the inoculation of the oncogenic viruses. To this the difficult cervical epithelium regeneration adds because the surgery made has not completely eliminated the pathologic tissue (fig. 5 - clinical case).

I must state in that such a context, the lesion cervix, which did not heal following the surgery performed, not even after several months – 1 year, can be a sign of cancer lesion.

On the sexuality and high oncogenic potential of some genotypes, I must emphasize the part played by the early start of sexual activity (12-15 years), too. Right now, in many countries, Romania included, the sexually transmitted disease (STDs) are contracted much easily due to the low immunity background to which the insufficient somatic, neuropsychic and sexual maturity add.

I mention that the oncogenic viruses are easily contracted at that age also because the cervical epithelium of the cervix becomes much more sensitive to the mutagen factors (cancer is usually caused by mutations), such as the oncogenic genotypes of HPV and HSV type 2, due

Clinical case

Patient aged 32, unmarried, first sexual contact at 22. She had 7 sexual partners. She came for a specialty gynecology examination because after 2 LEEP surgeries on the cervix, within 2 years, the initial lesion was not eliminated. As well, lately she had begun experimenting postcoital, pre and postmenstrual bleeding.

The previous investigations indicated that the reason for the surgeries made was Bethesda cytology test (ASC-US) and HPV genotype present (genotype 16).

Valve speculum examination: In the vagina, there was abundant leucorrhea with polymorph appearance and foul smell; cervix indicated no births, with a large erosion area surrounding the opening. Marginal ulceration bled lightly when the cervix and the Douglas pouch were touched by the speculum with the aim of clearly seeing the extent of the lesion.

Biological sample was taken from the vaginal discharge (Candida); cervical canal bacteriological culture (Staphylococci), HPV genotyping (genotypes 51, 53, IS39 present), and immunocytochemical test for markers p16/ki67 (positive) were performed.

Given that the colposcopy showed the presence of atypical terminal capillaries, much dilated, having irregular size and shape, located under the very thin pavement epithelium on the tumor lesion (abnormal vascular system easily seen also macroscopically), the need to perform an emergency was set out histopathology examination(H.E.).

During the surgery performed, the histopathology examination for the sample taken on the spot proved the existence of a carcinoma.

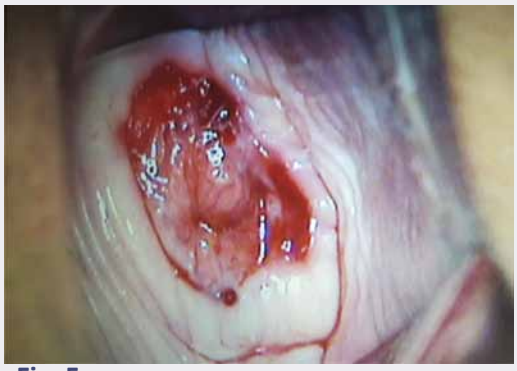

Fig. 5a

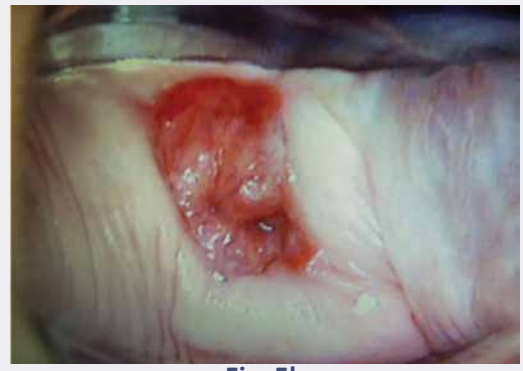

Fig. 5b

Fig. 5 a, b - Cervix following 2 LEEP surgeries, with marginal ulcerations, positive for HPV and molecular test

to the active physiological metaplasia process (active cellular growth) specific to that age.

All those particular situations systematically impose, aside of the Bethesda cytology test, the performing of colposcopy, determination of molecular biomarkers, with the aim of setting out the risk that the disease would evolve into neoplasia and when needed, a histopathology examination. Proteins p16 and Ki-67 show the abnormality of cellular division and an increase in the severity of the lesion infected with HPV oncogene genotype (fig. 7a, b, c, d).

On the high risk HPV genotypes, according to Munoz, the strain 16 is present in 50% of the

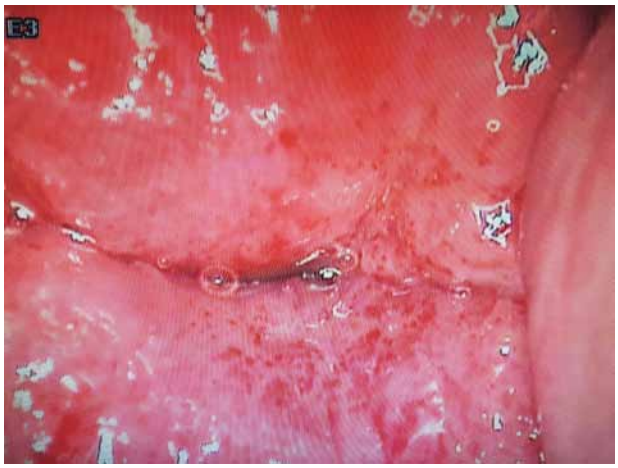

Fig. 6 - Macroscopic aspect of cervix with PAP-ASC-H associated with HPV genotypes 18 and 33

squamous carcinoma cases, and the strain 18 in 89% of the adenocarcinomas in females under 40 years old and 40% in females over 40 years old. The persistence of a high risk oncogene genotype over 3 years can last 10-20 years, thus explaining the appearance of cancer in older females. In this case, we are now investigating, including by molecular screening, many persons aged between 45 and 58 years old, with high risk oncogenic genotypes.

As a rule, the molecular screening and genotyping methods are assessed while considering ASC-US (ASC-US= Genotyping). (fig. 8.)

The therapy adopted based on the Bethesda system classification (fig. 9.):

Koilocytes present → HPV genotyping → HPV present= Molecular screening

If high risk oncogene genotypes are present, that being the immunocytochemical tests for p16 and Ki-67, which are prone to metaplasia, which were performed on the cervix without any macroscopic lesions or with colposcopy-related modifications, deep LEEP, conisation or cervix amputation was performed, thus referring directly to the cervical pathology, particularly that from the squamocolumnar junction outside the cervix, as well as the areas that were changed due to colposcopy.

I mention that the modification of the volume of tissue extracted by conisation (the so-called "small conisation") decreases the value of the diagnosis by 10-25%, according to I. Chiricuță.

In that situation, the post-surgery monitoring indicated that in 97-98% of the cases, the previous oncogenic genotypes disappeared (survey made on 2 experimental batches of females aged 25-55 years old – each batch had 40 persons). During the checkup performed subsequently, sometimes new HPV genotypes were found.

Regarding the contamination manner, it is done by penile-vagina intercourse, by the infected (contaminated) hand that entered in contact with the male's or female's genitalia, during the manual maneuvers on the erectile areas, contaminated underwear of the person, sperm, saliva, blood, the partners' genitalia coming close to each other, towel, etc.

Our research proved that cervical neoplasia is 7 to 10 times more frequent in wives of males with penile cancer, respectively HSV type 2, HPV oncogene genotype 16 and 18, who experienced repeated marriages, repeated viral infections of the cervix, and numerous pregnancies. Over 70% of the persons carrying HPV infection display no symptoms, which determines a very high risk of contamination.

The HPV infection can be transmitted during delivery, from mother to newborn, on a hema-

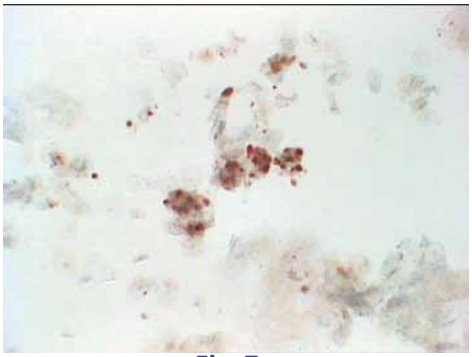

Fig. 7a

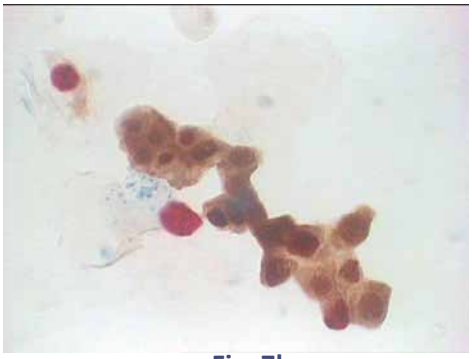

Fig. 7b

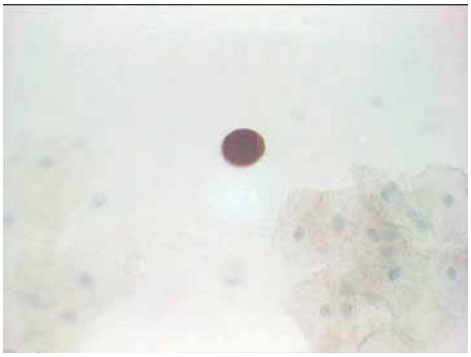

Fig. 7c

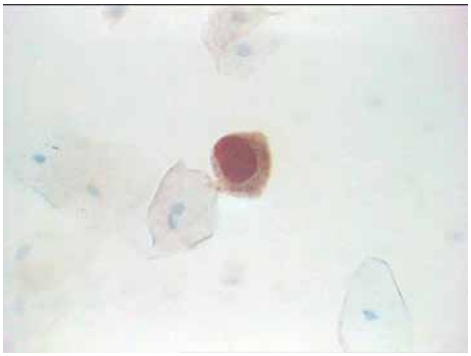

Fig. 7d

Fig. 7a, b, c, d - Immunocytochemical tests for p16 and Ki-67- positive

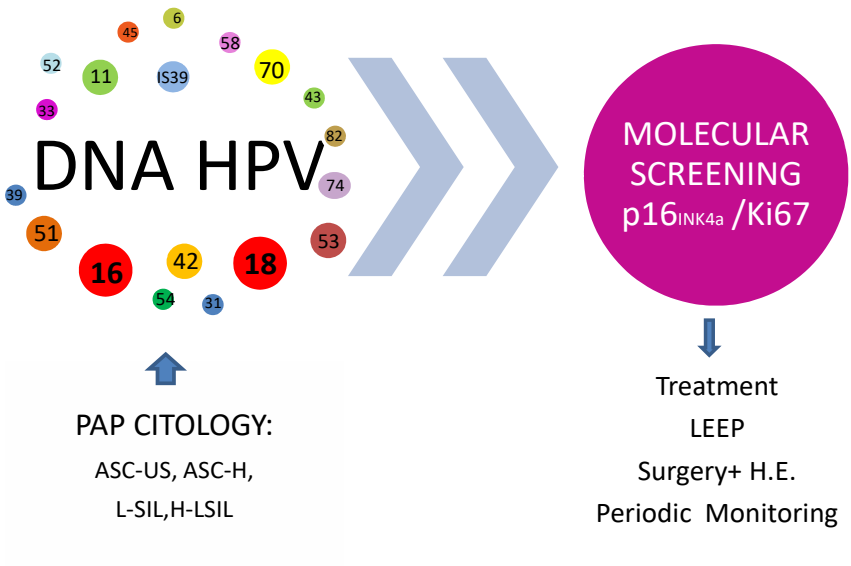

Fig. 8 - Medical conduct in cervical pathology

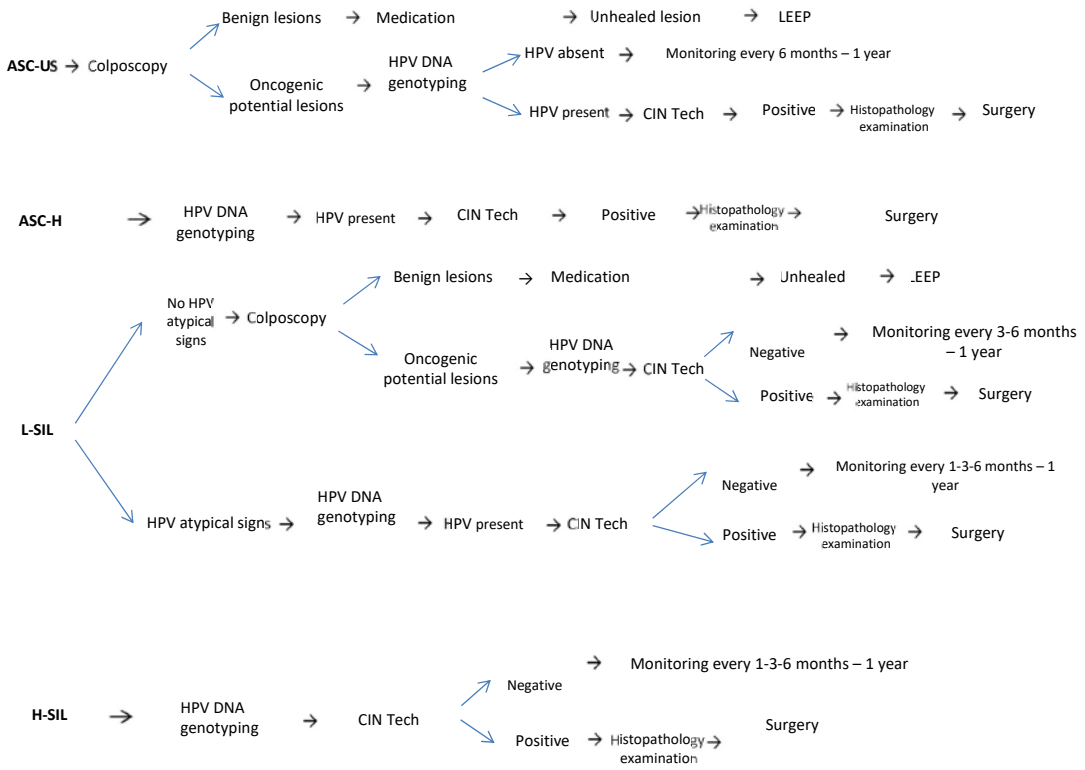

Fig. 9 - Therapy based on the Bethesda system classification

togenic, amniotic fluid, fetal membranes or direct pathway, by the placenta (Rintala M.A. et al. 2005). Mother is responsible for the HPV respiratory infection of the newborn. Wang X. et al explained, in 1998, the possibility of transmitting the HPV infection in the case of a newborn extracted by cesarean section, too. The HPV infection of the product of conception is maximum in young mothers aged 20-26.

Penile cancer

For a long time, it was stated that it was a very rare type of cancer and that it represented 1-2% of all neoplasia in European males and up to 19-20% in men in Africa and Asia, around 50-60 years old. In fact, obviously, it is more frequent in Africa, Asia and the Middle East, which explains in a way, among others, the practice of circumcision in those regions, which decreases the prevalence of HPV infection. The penile cancer is an epidermoid carcinoma. The primary form, usually the epithelioma, is the most frequent by comparison to the secondary penile neoplasia, which occurs as complication or metastasis, next to the rectum, testicular, bladder, kidney and liver neoplasm. Intestinal metastases are very rare.

The tumor affects the glans, balanopreputial sulcus, foreskin, and body of the penis.

HPV was found (according to Rintala M et al. -2000) in the urethra epithelium, vas deferens, seminal vesicles, semen and sperms coming from asymptomatic males, as well as in the glans, neck or body of the penis. The HPV infection is more frequent in homosexual males. In general, the HPV infection has a subclinical manifestation, with asymptomatic carriers, while the infection with HSV type 2, with oncogenic potential, is very painful and macroscopically visible (fig. 2a, b, c).

Risk factors

They are scars or phimosis, which shrinks the *balano-preputial separation* while favoring the infection and inflammations at the level of the glans and neck of the penis in connection with precarious local hygiene. I mention also the transformation of precancerous lesions, such as leucoplakia, caused by HPV genotype 6, 11, showed by HPV DNA methods, anogenital Bowen papulosis connected to HPV 16, 18, 33, Queyrat erythroplasia occurring on the glans, foreskin, and the body of the penis.

In fact, the oncogenic genotypes of HPV and HSV type 2 provide without a doubt a high potential of the precancerous lesions progressing into cancer.

The HPV infection is easily transmitted from males to females, a fact explained by the different anatomical structure of the genitalia, as well as by the manual maneuvers performed on the female's genitalia by the male, while using an infected hand. I underline the fact that the male hand carries more infection than that of the female.

The infection of the penis with HPV is connected to anal cancer, according to some authors in 90% of the cases, and to oral cancer.

Although the penile-anal intercourse is much more frequent in females, by the vulva coming close to the anal orifice, the rectum penetration is much easier also due to the pelvic floor structure. In theory, the possibility of developing anal cancer is much faster; practically, the anal cancer is much more frequent in homosexual males.

However, the HPV infection is present in 70-80% of the females, while in males, in 20-30%.

The patient goes to the sexologist for varied symptoms, in general complaining of "pain during intercourse and pain of the penis, with a slightly increased tone". He is experimenting libido, but at a low level. The present symptomatology does not allow him to complete an intercourse.

The clinical situation and particularly, the superficial inguinal lymphadenopathy, which can ulcerate, respectively the adenopathy of retrocrural lymph nodes and sometimes of the external iliac lymph nodes, display early onset, being a consequence of the infection.

The biological sample taken from the existing tumor leads to setting out the diagnosis and to establishing adequate treatment.

As in all cancer forms, early setting the diagnosis and the treatment can lead or not to the total healing.

The immunocytochemistry tests, with antigen detection (e.g. oncogenic proteins) in the cross section of a cell or by genotyping have a low significance because the histopathology examination is performed directly, often before the immunocytochemical tests.

The penile cancer due to HPV infection is rarer (HPV genotype 16 in 55.65% of the cases, HPV genotype 18 in 13% of the cases, HPV 6 and 11 in 8-8.5%).

The anogenital HPV DNA in males is most frequently found in the *balano-preputial separation* and then in the urethra. The rate of infection decreases by age due to rarer intercourses, as well as the experience of those involved in them.

Oral-pharyngeal-laryngeal lesions

The mucosa, lips, tongue, gums, mouth cavity, soft palate, pharynx, and larynx are contaminated especially in persons with a low immune system, by fellatio and cunnilingus, sexual deviations present in 15-75%, while considering the age (V. Nițescu).

In general, the viruses enter the skin or mucosa lesions and appear in persons with weaken immune systems given diseases such as HIV/AIDS, radiotherapy, cytostatic, or immunosuppression treatment.

The oropharyngeal cancer develops in any area, especially on the lip (fig. 10 - Cases of University Professor Dr. Alexandru Bucur- a, b, c, d, e, f), tongue or floor of the mouth, being caused in 15-25% of the cases by fellatio and cunnilingus, particularly by the oncogenic HPV

genotypes (Mark D. DeLacure – New York University) proven by immunofluorescence methods. HPV acts on the intact or damaged tegument or mucosa.

The frequency of fellatio (by penis – mouth contact in females, homosexual males, and bisexual males) is present in 62-78% of those practicing it while considering their age. The females practicing it leads to orgasm in 72-75% of the cases. Cunnilingus, as orogenital maneuver practiced by males to females and by females within homosexual intercourses, is 50-58% by considering their age.

The infection with oncogenic HPV is transmitted by saliva (kissing), blood, sperm, mother's milk, vulvovaginal discharge, penile discharge, urethral discharge, hand, infected items or self-inoculation.

Sperm stored in the oral cavity has mutagen properties given its DNA content and has a direct action on the epithelium, thus determining the disease.

Right now, the oral sexual practice with partners infected with HPV is gradually more frequent in young females, under 18-20 years, who do not engage in penile-vaginal intercourse but in penile-oral and/or penile-rectum intercourse given their desire to maintain their virginity before their family and the society.

Adopting abnormal sexual practice determines infection with other STDs, too, particularly B and C hepatitis virus, syphilis, gonococcus,

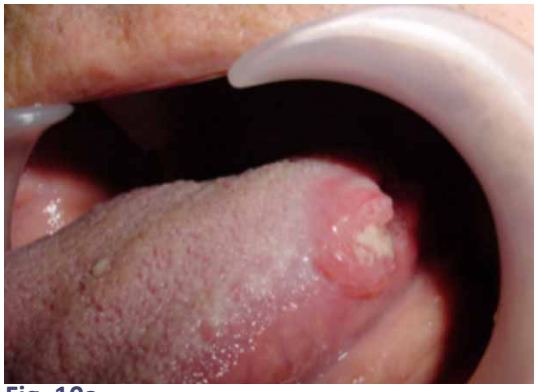

Fig. 10a

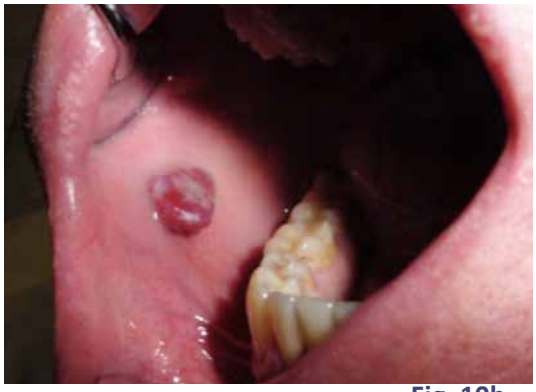

Fig. 10b

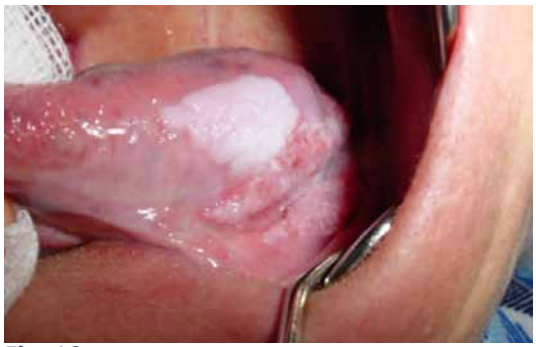

Fig. 10c

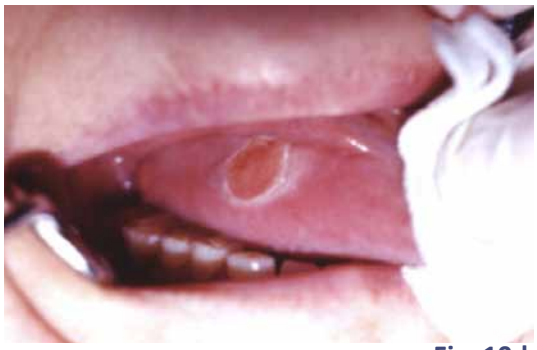

Fig. 10d

Fig. 10a,-Onset forms, tongue tumors; **b** - Jugal mucosa, vegetating form – onset tumors; **c, d** - Malignant tumors of the tongue and floor of the mouth, **e** - Malignant tumors of the lip - onset form, **f** - Malignant tumors of the lip- ulcero-destructive form

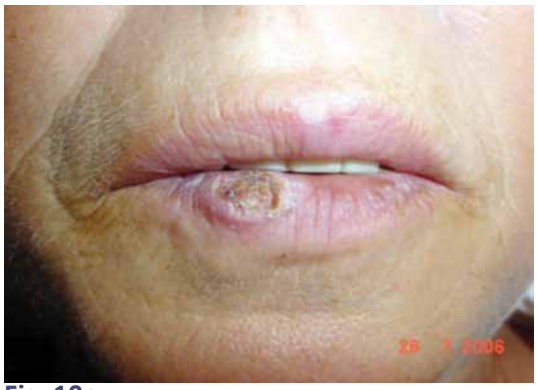

Fig. 10e

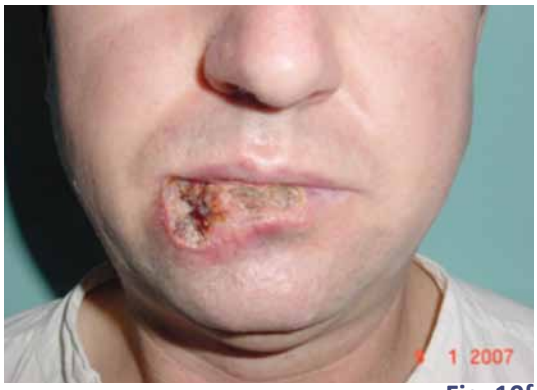

Fig. 10f

and HIV. Given the infection transmitted, they lead to a very severe pathology, which is sometimes lethal (case discussed in 2017- female, aged 27).

The HPV infection is easier transmitted from males to females and the males' hands are more infected with HPV than those of females, although the persistence of infection is 18-20% in females as against 5-6% in males, in the case of oropharyngeal, airway, esophageal and laryngeal lesions.

Cancer develops in any area, particularly on the lip, tongue or floor of the mouth, those areas being prone to HPV infection.

Some researchers have set out that 15-20% of the oropharyngeal cancer forms display infections with HPV oncogenic genotypes.

By applying the immunocytochemical methods, particularly PCR, p16 –Ki 67, the HPV infection in laryngopharyngeal carcinoma was identified in 75-80% of the cases. Right now, according to some authors, the oropharyngeal tumors connected to the HPV oncogenic genotypes are over 70-80%.

A major immune-histamine marker in the appraisal of pharyngeal cancer progress is the absence of the E-cadherin protein (Pascuale H.et.al -quoted by Cernescu), which facilitates the link between "keratinocytes and dendritic cells or Langerhans cells which trigger the immune response."

The oropharyngeal cancer determined by the infection with HPV oncogenic genotypes, particularly 16 and 18, is very frequent in 40-50 year-old persons, mainly those that had over 6-8 oral sexual partners and who contacted the virus at 16-22 years of age.

I emphasize that the HPV infection is the result of the unprotected penile-oral intercourse, in theory, because infection can occur even when the person engages in a protected penile-vaginal intercourse.

One must notice the fact that in the persons displaying oro-pharyngo-laryngeal lesions, the histopathology examination is made directly by using the sample taken by surgery, while classifying the lesion at once, from a histology standpoint. Usually, the pre-histology genotyping misses because its significance in that case is low (no importance) for the surgeon.

Genotypes with oncogenic potential can also affect the squamous epithelium of the oropharyngeal, esophageal, and laryngeal areas.

Although the body has complex forms of fighting the HPV and HSV2, the number of oro-pharyngo-laryngeal infections by STDs has increased enormously, a fact demonstrated by researchers all over the world.

Annually, in Romania die approximately 3,500-4,000 persons due to cervical cancer, 1,000-1,200 persons due to anal cancer, 1,200-1,400 persons due to vulvar-vagina cancer, 200-300 persons due to penile cancer, and approximately 5,000 persons due to oral cavity, pharynx, or larynx cancer (*C. Cernescu, modified)*.

Conclusions

The HPV virus has non-enveloped icosahedral capsids, which multiply their genome in the nuclei of the infected host cells (Longworth MS and Laimins LA-2004).

The virus is removed by the cellular immune system in 70% of the cases, in the first year and in 90% of the cases after 12-36 months, situation in which the surgery will be performed based on the solving manner, after the immune and medication treatment.

The occurrence of precancerous lesions following the infection can last 1-10 years, and some other 10 years would pass until the invasive cancer develops, which might explain the occurrence of the cervical cancer in females over 40-50 years old. That fact imposes a careful monitoring by the use of Bethesda cytology screening annually and when needed, more often, while considering the ASC-US value, genotyping, molecular tests, and colposcopy.

The molecular screening (histochemical witnesses that accumulate in the cells infected with HPV -10% in LSIL, 98% in HSIL, lack of E-cadherin, which appears in laryngeal cancers or increase of TERN-RNA expression – telomerase) and the histopathology test bring real benefits for setting the diagnosis.

The surgical methods of removing oncogenic genotypes performed as earlier as possible and when the tests call for them decrease the number of persons with cancer risk given the oncogenic HPV genotype.

In 99% of the cases, the cause for cervical cancer is given by the infection with oncogenic HPV genotypes (Harold Zur Hansen), and the nucleus and cytoplasm modification of proliferative cells can be shown, in theory, by molecular screening methods, such as the simultaneous detection of proteins p16 and Ki-67.

Early detection of E6 and E7 viral oncoproteins, by the molecular methods of setting the diagnosis allows one to establish the cellular oncogenic risk, provides the fastness of setting the diagnosis, differentiating the precancerous lesions of the cancerous ones and applying the adequate treatment.

Anal cancer is most frequent in homosexual males, although the penile-anal intercourse is most frequent in females.

The cytology screening tests by ASC-US, genotyping and molecular tests allow to set a correct diagnosis and treatment, in this case of 98 %.

Removing the **E6** and **E7** oncoproteins by the person's immune system, by the **p53** gene (tumor suppressor protein), eliminates the genomic lesions caused by viruses posing oncogenic potential and explains the 90% healing in females after 3 years.

The mandatory cell screening, the molecular screening and HPV genotyping have decreased the number of deaths in some countries, such as Finland by 0.5% for every 100,000 females of 20-44 years old, Sweden by 0.9%, Bulgaria and Romania by 13.2%.

The molecular tests can emphasize the presence of malignant cells but they are unable to precisely replace the histopathology examination. The immunohistochemical test is not fail proof; sometimes, there are false positive results, both p16 and Ki-67 proteins can be present in cells that are no dysplastic, such as the mature metaplasia cells. This calls for a correlation of the immunohistochemical test to other investigations, particularly the histopathology examination. The molecular methods are useful for accurately assessing the lesions prior to performing the treatment meant to restore the local morphology.

In the case of oro-pharyngo-laryngeal lesions, when tumors are present, in general genotyping or molecular tests are rarely performed. The histopathology examination for samples taken from that tumor is able to correctly set the diagnosis of the disease (cancer type, differentiation degree, etc.), which iden-

tifies the cancer cell, the stage of the disease and the specific treatment.

Genital diseases are detected early, by comparison to the rectal and oropharyngeal ones, when the patient goes to see the doctor late, usually.

The abnormal colposcopy modifications impose the performing of genotyping and molecular tests, and when suggestive macroscopic modifications are present, we also perform the histopathology examination.

Protein p16 is directly connected to the oncogenic activity of the HPV oncogenic genotypes, its presence proving that the infection became malignant.

After surgery, the patient must be examined every 1-3-6 months, and a cytology test and HPV genotyping must be performed after 6 months, for finding whether the initial HPV genotype is present. In the case of normal evolutions and tests, the patient will be examined every year, when the normal tests will be performed.

I underline that the negative cytology has a 40% sensitivity (Danforth) and it does not exclude, for example, a HPV infection, situation in which the HPV genotyping will be performed for any suspicion, and if the HPV is present, the molecular tests will be performed, too.

The molecular biomarkers are always useful when the cytology test does not provide satisfying results.

By genotyping, one can set the presence of viral, oncogenic and non-oncogenic genotypes. Even if the non-oncogenic viruses are not as significant as the oncogenic genotypes, as indicated before, they too have a negative influence on the progression of the disease.

The result of the cytology test in Bethesda system, which indicates high degree lesions, "which cannot be excluded" (ASC-H, which is approximately 10% and a major risk potential by oncogenic HPV genotypes of over 80%) imposes the performing of molecular tests for p16-Ki67, which are particularly useful. In this case, the presence of E6 and E7 viral oncoproteins allows the differentiation of precancerous and cancerous lesions, the oncogenic risk, as well as assessing the severity of lesions. There is a direct relation between oncoproteins and precancerous lesions.

The diagnosis set by immunocytotest emphasizes the existence of the cancer cell by a specific coloration, while emphasizing the severity of the case and the emergency of the specific treatment.

The deeper alteration of cell layers has determined the 98-99% disappearance of the HPV oncogenic genotypes.

The diagnosis set by immunocytotest cannot replace the histopathology examination, which sets out the certain diagnosis of the disease. Such a diagnosis provides a different future to the patient, if the disease is found in a curable phase.

The cell modifications, such as the vascular and local ones, determine the ulceration and allow an easy entrance of the oncogenic HPV genotype and a faster development of the neoplasia, and the wound fails to heal.

The histopathology examination and the immunocytotest for diagnosis setting purposes decrease the number of investigations.

References

1. Álvarez-Argüelles ME, Melón S, Junquera ML, Boga JA, Villa L, Pérez-Castro S, de Oña M. Human Papillomavirus Infection in a Male Population Attending a Sexually Transmitted Infection Service. PloS One. 2013;8(1):e54375.
2. Castle P.E., Wacholder S., Lorincz A.T.,et al., A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J Natl Cancer Inst 2002, 94,1406-14.
3. Castellsague X.,Diaz M.,de Sanjose S.,Munoz N., Herreo R. et al,Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implication for screening and prevention. J Natl Cancer Inst 2002,98,303-315.

4. Castellsague X., Munoz N., Cofactors in human papillomavirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003,31,20-8.
5. Cernescu C: Vaccinarea în cancerul de col uterin (Vaccination in the cervical cancer), Ed. Academiei Române, 2009;48.
6. Danforth D. Obstetrics and Gynecology; fourth edition ,1982,44-45;83.
7. Dillner J., Lehtinen M.,Björge T.,et al., Prospective seroepidemiological study of human papillomavirus infection as a risk factor for invasive cervical cancer. J Natl Cancer Inst.1997,89,1293-9.
8. Flores R, Abalos AT, Nielson CM, Abrahamsen M, Harris RB, Giuliano AR. Reliability of sample collection and laboratory testing for HPV detection in men. J Virol Methods.2008 Apr;149(1):136-43.
9. Giuliano A.R.,Harris R. et al,:Incidence, prevalence and clearance of type- specific human papillomavirus infections: The Young Women's Health Study, J.Infect. Dis.2002,186,462-469
10. Green J., Berrington D.G.,Sweetland S.,et al.,Risk factors for adenocarcinoma and squamous cell carcinoma of the cervix in women aged 20-44 years: the UK National Case-Control Study of Cervical Cancer. Br J Cancer 2003,89,2078-86.
11. Guyton Arthur C, Hall John E.:Tratat de fiziologie a omului (Treaty of human physiology) , Ed.11 a, Ed.Med.Callisto, pag. 40-41, ISBN 978-976-872-61-4-7;2007.
12. zur Hausen H.,Papillomaviruses and cancer: From basic studies to clinical application. Nat.Rev.cancer 2002,2,342-350.
13. Kjaer S., Høgdall E.,et al:The absolute risk of cervical abnormalities in high-risk human papillomavirus positive, cytologically normal women over a 10-year period, Cancer Res 2006,66,10630-10636.
14. Kjaer S.K., Svare E.I.,Worm A.M., Walboomers JM, Meijer CJ, van den Brule AJ., Human papillomavirus infection in Danish female sex workers. Decreasing prevalence with age despite continuously high sexually activity. Sex transm dis 2000,27,438-45.
15. 15. Longworth MS, Laimins LA., Pathogenesis of human papillomaviruses in differentiating epithelia, Microbiol Mol Biol Rev. 2004 Jun;68(2):362-72.
16. Levi F., Lucchiani F.,et al., Cervical cancer mortality in young women in Europe: patterns and trends. Eur J Cancer 2000,36,2266-71.
17. Meisels A, Fortin R, Roy M., Condylomatous lesions of cervix and vagina: : I. Cytologic patterns. 1976,Acta Cytol 20: 505–509.
18. Mitchell H.,Hocking J.,Saville M., Improvement in protection against adenocarcinoma of the cervix resulting from participation in cervical screening, Cancer 2003,99,336-41.
19. Muñoz N, Bosch FX, de Sanjosé S.,et al., Epidemiologic classification of human papillomavirus types associated with cervical cancer. N.Engl J Med 2003,348,518-27.
20. Munoz N., Bosch F.X., Castellsague X., et al: Against which human papillomavirus types shall we vaccinate and screen? The International Perspective. International Journal Of Cancer 2004,111,278-285.
21. Nielson C.M.,Schiaffino M.K., Dunne E.F.,Salami J.L., Giuliano A.R., Associations between male anogenital human papillomavirus infection and circumcision by anatomic site sample and lifetime number of female sex partners. J Infect Dis 2009,199,7-13.
22. Nițescu Vasile, Tratat de Sexologie Clinică (Treaty of clinical sexology), Editura Didactică și Pedagogică,2015, pag247-248, ISBN-978-973-30-38-06-1.
23. Nițescu Vasile, Treaty of Clinical Sexology, The Publishing House of the Romanian Academy,2018, pag 261-262.
24. Prigenzi KCK, Heinke T, Salim RC, Focchi GRA, et al. Dual p16 and Ki-67 Expression in Liquid-Based Cervical Cytological Samples Compared to Pap Cytology Findings, Biopsies, and HPV Testing in Cervical Cancer Screening: A Diagnostic Accuracy Study. Acta Cytol 2018 Mar 22. pii: 000487504. doi: 10.1159/000487504.
25. Rintala MA, Grénman SE, Puranen MH, Isolauri E, Ekblad U, Kero PO, Syrjänen SM.,Transmission of high-risk human papillomavirus (HPV) between parents and infant: a prospective study of HPV in families in Finland.
26. Schiffman M.,Castle P.E., Jeronimo J, Rodriguez AC, Wacholder S.:Human papillomavirus and cervical cancer. The Lancet 2007,370,890-907.
27. Schlecht N.F., Platt R.W., Duarte-Franco E., et al.,Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. J Natl Cancer Inst 2003,95,1336-43.
28. Zielinski G.D.,Snijders P.J.,Rozeendal L.,et al.,The presence ot the high-risk HPV combined with specific p 53 and p16 ^{INK4a} expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma *in situ* and adenocarcinoma of the cervix. J Pathol 2003;201:535-43.
29. Wheeler C.M., Hunt W.C., Schiffmann M., Castle P.E., Human papillomavirus genotypes and the cumulative 2-year risk of cervical precancer. J infect dis 2006,194,1291-9.