QUANTITATIVE ANALYSIS OF HUMAN PAPILLOMA VIRUSES AND HERPES VIRUSES IN MEN WITH INFERTILITY

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КОЛИЧЕСТВЕННЫЙ АНАЛИЗ ВИРУСОВ ПАПИЛЛОМЫ ЧЕЛОВЕКА И ГЕРПЕСВИРУСОВ У МУЖЧИН С БЕСПЛОДИЕМ

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Annotation. Infertility occurs in 12-15% of couples of reproductive age and is a pressing medical and social problem, despite the advances in reproductive technologies. In almost 50% of cases, the cause of infertility is associated with a male factor; in more than 50% of cases of male infertility, its etiology remains unknown. The presence of markers of HSV and cytomegalovirus (CMV) in the organs and tissues of the male reproductive system, as well as data on the increased frequency of detection of these viruses in the ejaculate of infertile men indicate a possible connection of herpesvirus infection with impaired fertility [1, 6].

Key words: human papilloma virus, herpes virus, infertility, spermogram, polymerase chain reaction.

Аннотация. Бесплодие наблюдается у 12-15% пар репродуктивного возраста и является актуальной медицинской и социальной проблемой, несмотря на успехи репродуктивных технологий. Почти в 50% случаев причина бесплодия связана с мужским фактором, более чем в 50% случаев мужского бесплодия его этиология остается неизвестной. Присутствие маркеров ВПГ и цитомегаловируса (ЦМВ) в органах и тканях мужской репродуктивной системы, а также данные о повышенной частоте обнаружения этих вирусов в эякуляте бесплодных мужчин указывают на возможную связь герпесвирусной инфекции с нарушением фертильности [1, 6].

Ключевые слова: вирус папиломы человека, вирус герпеса, бесплодие, спермограмма, полимеразно-цепная реакция.

Introduction. Human papillomaviruses (HPV), like herpesviruses, are widespread in the human population. Other human herpesviruses (HHVs) are also found in the sperm of men from infertile couples - human herpes virus type 6 (HHV-6), Epstein-Barr virus (EBV). However, the opinions of researchers regarding the role of herpes viruses in the etiology of male infertility diverge from a complete denial of their influence on the process of maturation of male germ cells to the establishment of a direct correlation between viral infection of the ejaculate and male infertility [3, 14].

Based on data from epidemiological and molecular biological studies, HPV types are divided into clides and classified as clides of low, intermediate or high oncogenic risk. High carcinogenic risk types (HCR) are clades A9 (types 16, 31, 33, 35, 52, 58, 67), A7 (types 18, 39, 45, 59, 68, 70, 85), A5 (types 26, 51, 69, 82), A6 (types 30, 53, 56, 66) and A11 (types 34, 73). Separation into clades is important for dynamic monitoring of viral infection and more accurate prediction of its development, since different types of HPV have different oncogenic potential, as well as the ability to persist [1, 8]. At the same time, generally accepted criteria for assessing the risk of progression of human papillomavirus infection (PVI) in men have not yet been developed.

In addition to the oncogenic aspect, the role of HPV in the formation of male infertility and its effect on the course of pregnancy are currently being actively studied.

Purpose of the study: to study the distribution and quantitative analysis of HPV and HPV HCR in men with idiopathic infertility and patients whose regular partners had cases of spontaneous abortion, as well as to assess the impact of viral infections on sperm parameters.

Material and methods: 196 men were examined. Patients were included in one of 3 groups. Group 1 (mean age 32.8±6.8 years; n = 112) included men with infertility

of unknown etiology. The diagnosis using standard procedures was established by excluding urogenital disorders and malignant tumors, infections of the urogenital tract, varicocele, endocrine disorders and immunological factors as possible causes of infertility [2, 11, 17]. Patients were registered as infertile if, after 12 months of regular sexual activity without contraception, the woman did not become pregnant. The majority of examined individuals in this group (57%) had sperm parameters that corresponded to WHO standards; some (43%) had infertility with pathozoospermia of unknown origin.

Group 2 (mean age 36.1±8.1 years; n = 63) included patients whose partners had a history of 1 or more cases of spontaneous termination of pregnancy of unknown etiology in the first trimester (hereinafter referred to as miscarriage).

Group 3 (comparison) (average age 32.5±9.3 years; n = 21) consisted of practically healthy men who applied for a preventive examination.

We studied ejaculate samples obtained by patients through masturbation after 3 days of sexual abstinence. Semen volume ranged from 1.5 to 3 ml. The samples were immediately divided into 2 equal parts: the first was used for spermiological analysis, the second was used for DNA extraction with subsequent detection of viral DNA.

Results. The ejaculates of patients of groups 1-3 were studied. In total, HPV and HCR HPV DNA was detected by PCR in the ejaculates of 42 (21.4%) of 196 patients examined. Among 42 patients with infected ejaculates, 31 (73.8%) had idiopathic infertility, 11 (26.2%) had a history of miscarriage in their partners; the difference between the 1st and 2nd groups is statistically insignificant (p = 0.3).

The data showed that in the sperm of men of the 2nd group, DNA of HHF and HCR VCR was detected in a total of 17.5% of cases, in the sperm of men of the 1st group - in 27.7% of cases. Despite the fact that the frequency of detection of viruses in men with idiopathic infertility was more than 1.5 times higher than in men whose partners suffered from miscarriage, the difference between the 1st and 2nd groups did not reach statistical significance (p = 0.053).

In the structure of the identified viruses, all 3 HHVs accounted for a total of 57.1% (24/42), while in the group of men with idiopathic infertility their DNA was isolated in 17/24 (70.8%), in the 2nd group - in 7/ 24 (29.2%); p > 0.05. HPV HCR DNA was detected in a total of 42.9% (18/42) of patients: in 14/18 (77.8%) in group 1 and in 4/18 (22.2%) in group 2, with no significant difference between these groups (p > 0.05).

An analysis of the frequency of occurrence of each of the three studied HHVs showed that in none of the semen samples from patients of the 2nd group did CMV occur as a monoinfection, but in 2 samples it was found in combination with HHV-6, while in patients with idiopathic infertility, CMV as a monoinfection was detected in 5/112 (4.5%) patients and was not detected in combination with other HHVs. EBV

occurred in 2.7% (3/112) of patients in group 1 as a monoinfection and in 1 (0.9%) patient in combination with HHV-6 (0.9%). In group 2, EBV was detected only in 2 (3.2%) ejaculates. HHV-6 was most often found in ejaculates: in 8 (7.1%) patients of group 1 as a monoinfection and in 1 (1/112; 0.9%) in combination with EBV. In group 2, HHV-6 was detected in 3 (4.8%) patients as a monoinfection and in 2 (3.2%) in combination with CMV. In total, in the ejaculates of 175 infertile men of both groups, HHV-6 was detected in 14 (8%), EBV in 6 (3.4%) and CMV in 7 (4%). Statistical processing of the data showed that the differences in the incidence of HHF between the compared groups were insignificant. The detection frequency of the 3 studied HHFs also did not differ significantly ($p > 0.05$).

When analyzing the frequency of occurrence of different types of HPV HPV in semen, it was found that in the structure of the identified HPV genotypes, the most common genotypes were those belonging to clade A9 (14/175; 8.0%), which were defined as monoinfection in 12/175 (6, 8%) patients in both groups and additionally in 2 (1%) patients in combination with A5/6 and A7.

In a comparative analysis of the incidence of HPV HCR of all types in patients of the 1st (14/112; 12.5%) and 2nd groups (4/63; 6.3%), the differences turned out to be statistically significant ($p = 0.02$) . The most common genotypes among HCR HPV in ejaculates were clyde A9 (14/18; 77.8%), the difference with other clides was statistically significant ($p = 0.04$). It should be noted that Clyde A5/6 HPV was not detected in patients of group 2. At the same time, in patients of group 1, HPV of all studied clides (A5/6, A7 and A9) were detected not only as a monoinfection, but also in combinations.

Quantitative analysis of the DNA of the viruses studied revealed a wide variation between the samples: the minimum value was 4 DNA copies per 100 thousand cells, the maximum was 112,201 copies per 100 thousand cells. The median value for all positive samples turned out to be relatively low - 217 copies per 100 thousand cells (2.34 ^ 10). Since the number of samples containing HPV HPV A5/6 and A7 was small (2 and 4, respectively), the median value of HPV HPV DNA concentration was calculated for all ($n = 20$) HPV-positive samples and amounted to 687 copies per 100 thousand cells (2.84^10). Comparison of HVV DNA concentrations showed that the median value in samples containing CMV was 6489 copies per 100 thousand cells (3.81 ^10), which significantly exceeded the viral load in the ejaculates of patients infected with EBV and HHV-6, as well as HPV VKR. It is important to note that in some patients (13/42, 30.9%), the concentration of viral DNA in the ejaculate exceeded the average values by more than 100 times and reached 4-5^10 per 100 thousand cells.

Discussion. In this work, the DNA of the studied viruses was found in the ejaculate of every 5th of 196 men examined. Herpes viruses CMV, HHV-6, EBV were detected in 12.2%, HPV HCR - in 9.2% of patients without clinical signs of viral

diseases. HCR HPV was not detected in the ejaculate in any of the practically healthy fertile men.

One of the actively studied issues regarding asymptomatic infection in men is the effect of viruses on sperm quality. A number of studies have shown that the presence of HPV in the ejaculate is associated with a decrease in sperm motility or ejaculate pH, as well as a decrease in the number of morphologically normal germ cells [2, 16]. Other authors do not confirm the negative impact of HPV on the main parameters of spermogram [1, 10]. Equally significant is the difference in opinion regarding changes in sperm quality in HHF-infected patients. Some authors report a decrease in the concentration and motility of sperm in CMV-infected ejaculates [2, 12]. Other researchers have not found an effect of CMV, EBV, or HHV-6 on sperm parameters [1, 7, 18].

In this study, when comparing the main parameters of sperm in virus-infected samples, it was found that sperm motility, as well as the number of morphologically normal germ cells in the ejaculates of virus-infected patients, are significantly reduced compared to healthy men without fertility problems. This indicates the possibility of a negative impact of viral infections on the reproductive function of men.

A separate issue is finding out the cause of infertility in married couples caused by the impossibility of pregnancy or loss of pregnancy. The answer to this question is very difficult due to the need to assess the role of both male and female factors. The data obtained showed that in the ejaculates of men from 63 infertile couples with miscarriage, HCR HPV DNA was found significantly less frequently (6.3%) than in men with idiopathic infertility (12.5%). The data from this work suggest that in spontaneous abortions, the negative role of HPV infection is associated with the female factor rather than the male factor. In support of this assumption, we can refer to the results showing that in HPV-positive women, pregnancy occurs 2.4 times less often than in HPV-negative women ($p < 0.02$) [2, 9]. In cases of herpetic infection of sperm, no statistically significant differences were detected between the 1st and 2nd groups, while other authors noted the association of HHF in ejaculates with spontaneous abortion, as well as with failures when using reproductive technologies [1, 13, 15].

Currently, many researchers recognize that screening for herpes viruses and HPV is necessary. To assess the risk of developing infectious and oncogenic diseases in women, indicators of the viral load of HPV HCR in the organs of the urogenital tract (UGT) are widely used. For men, similar indicators have not yet been established. High viral loads in ejaculates in a significant proportion of asymptomatic patients (30.9%) indicate the advisability of developing criteria for assessing the risk of clinically significant diseases. Considering that with a high viral load, the persistence of viruses in the UGT of men is observed statistically significantly more often than with a low viral load, it can be assumed that asymptomatic patients with a high viral load are at

risk for disease progression and the possible development of oncopathology [1, 9]. However, to determine the reliability of using quantitative determination of viral DNA as a predictor of unfavorable development of infection in men, further dynamic studies are needed to analyze the course of the disease and its outcome.

Conclusions. In conclusion, it should be noted that viral DNA was not detected in any of the apparently healthy men, but was found in the ejaculates of 24% of patients with fertility problems, and HPV and HPV HCR occurred with approximately the same frequency. Quantitative DNA analysis made it possible to determine high concentrations of HPV and HCR HPV DNA in some patients with infertility. This means that determination of the copy number of DNA viruses can be used as a useful indicator for monitoring the development of viral infections and making timely decisions about initiating therapy. Further research will help determine the role of viral infections in reproductive dysfunction in men and develop effective approaches for their treatment.

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