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L.H. Musayeva, S.M. Ismayilova
Azerbaijan Medical University, Baku, Azerbaijan

EVALUATION OF LOCAL IMMUNE RESPONSE IN PREGNANT WOMEN WITH HUMAN PAPILLOMAVIRUS INFECTION

e-mail: med_avtor@mail.ru

This study investigated the impact of Human Papillomavirus infection on the local immune system during pregnancy. For this purpose, local immune responses – immunoglobulins and functional activity of neutrophils – in cervical secretions, were comparatively examined in three groups. Group I – 25 pregnant women with transient Human Papillomavirus infection, Group II – 24 pregnant women with persistent Human Papillomavirus infection, and a control group of 25 pregnant women without detected Human Papillomavirus infection. The results showed that, especially in cases of persistent Human Papillomavirus infection, although an increase in the total number of neutrophils in cervical mucus was observed, their phagocytic activity and functional capacity were significantly reduced. This is explained by the depletion of functional reserves of neutrophil cells due to the chronic course of the infection. At the same time, in persistent infections, the humoral immune response was found to be enhanced, with a statistically significant increase in IgG levels. This is interpreted as an indicator of the adaptive immune response developed against the prolonged presence of the virus in the body. The findings of this study demonstrate the potential diagnostic significance of local immune parameters – neutrophil activity and IgG levels – for predicting the clinical course and outcomes of Human Papillomavirus infection during pregnancy.

Key words: papillomavirus infection, pregnant women, immunoglobulins, neutrophils.

Л.Х. Мусаєва, С.М. Ісмаїлова

ОЦІНКА МІСЦЕВОЇ ІМУННОЇ ВІДПОВІДІ У ВАГІТНИХ ЖІНОК З ІНФЕКЦІЄЮ ВІРУСУ ПАПІЛОМИ ЛЮДИНИ

У даному дослідженні нами вивчався вплив інфекції вірусу папіломи людини на місцеву імунну систему під час вагітності. З цією метою були проведені порівняльні дослідження місцевої імунної відповіді – рівня імуноглобулінів і функціональної активності нейтрофілів – у цервікальних виділеннях у трьох груп жінок. Група I – 25 вагітних жінок із транзиторною інфекцією вірусу папіломи людини, Група II – 24 вагітні жінки з персистувальною інфекцією вірусу папіломи людини, контрольна група – 25 вагітних жінок, у яких вірусу папіломи людини не було виявлено. Результати показали, що особливо за персистуючої інфекції вірусу папіломи людини, незважаючи на збільшення загальної кількості нейтрофілів у цервікальному слизу, їх фагоцитарна активність і функціональна здатність були значно знижені. Це пояснюється виснаженням функціональних резервів нейтрофільних клітин унаслідок хронічного перебігу інфекції. Одночасно при персистуючих інфекціях спостерігалося посилення гуморальної імунної відповіді, що супроводжувалося статистично значущим підвищенням рівня IgG. Це розцінюється як показник адаптивної імунної відповіді, що розвивається у відповідь на тривалу присутність вірусу в організмі. Отримані дані свідчать про потенційну діагностичну значущість параметрів місцевої імунної відповіді – активності нейтрофілів і рівня IgG – для прогнозування клінічного перебігу та результатів інфекції вірусу папіломи людини під час вагітності.

Ключові слова: інфекція вірусу папіломи, вагітні жінки, імуноглобуліни, нейтрофіли.

The prevalence of Human Papillomavirus infection (HPV) in pregnant women has been investigated in several studies, and overall results indicate that the risk of HPV among pregnant women is higher compared to non-pregnant women. In a systematic review compiling studies on the prevalence of HPV in pregnant women, Liu et al. found the prevalence to be 16.82 % in pregnant women and 12.25 % in non-pregnant women [3]. In a study comparing pregnant and non-pregnant women of similar age, also observed a higher prevalence in pregnant women—24.2 % vs. 14.8 %, respectively [2]. Most studies on pregnant women have examined the infection in the cervix [3, 4, 10]; however, HPV DNA has also been detected in the placenta [6], amniotic fluid [7, 8], and umbilical cord [8], indicating the possibility of vertical transmission to the fetus [9]. However, this trend is mostly observed in the first two trimesters of pregnancy, while the postpartum period is characterized by increased viral clearance. This later “recovery process” is considered sufficiently effective to compensate for the first two trimesters, as studies have observed that the overall clearance rate of HPV over a 12-month period is similar between pregnant and non-pregnant women [11]. Some authors found reported various adverse pregnancy outcomes, including preterm birth [9], spontaneous abortion [2], premature rupture of membranes [13], intrauterine growth restriction [8], low birth weight [5], and fetal death [7]. Cervical cancer and other HPV-related diseases represent a significant global public health issue. For this reason, the WHO has reiterated its recommendation to include HPV vaccines in national immunization programs [12]. Studying the immune response to HPV during pregnancy can help determine the range of complications caused by the virus and further emphasize the need for and effectiveness of immunization.

The purpose of the study was to investigate the local immune response in pregnant women with human papillomavirus infection.

Materials and methods. The study was conducted between 2013 and 2015 at Maternity Hospital No. 1 and Women's Consultation Clinic No. 6 under the Ministry of Health of the Republic of Azerbaijan. A total of 77 women of reproductive age were examined and women were divided into three groups: Group I – 28 pregnant women with initial HPV-positive results who tested negative 6 months later (transient HPV); Group II – 24 pregnant women who remained HPV-positive 6 months after initial testing (persistent HPV); Group III (control) – 25 pregnant women with no detected HPV.

Anamnesis data and infection-related clinical symptoms were collected; colposcopy and cytological examination of cervical secretions were performed. Additionally, to detect the virus via polymerase chain reaction (PCR), a universal swab was used to obtain a sample from the cervical surface. The swab's material-carrying tip was cut off, placed in a transport medium inside an Eppendorf tube, and delivered to the laboratory. After DNA extraction from the sample, electrophoretic analysis of PCR products was conducted on an agarose gel. Cytological examination of the swab was performed. Microscopy was carried out using the oil immersion technique, and neutrophil leukocytes, fibroblasts, macrophages, and eosinophils in the prepared slides were counted. Furthermore, immunoglobulin levels in vaginal secretions were measured using the enzyme-linked immunosorbent assay (ELISA) method. The obtained data were statistically analyzed using the Statistica 6.0 software package and Microsoft Excel spreadsheet editor. The arithmetic mean and standard error were calculated. Group comparisons were made using Student's t-test, and qualitative comparisons were based on the Van der Waerden X-criterion. A difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. The age range of the pregnant women was between 17 and 40 years. The average age was 27.2±6 years in Groups I and II, and 28 ± 5 years in the control group. In addition to the life cycle of HPV, local immune factors of the host organism also play an important role in the progression of the disease. To evaluate the local immune response in women, the quantity and functional activity of neutrophils in cervical mucus were investigated. The results are presented in Table 1.

Table 1

Comparative characteristics of local immune parameters in cervical mucus by groups

Indicators	Control group n=25	Group I Transient HPV n=28	Group II Persistent HPV (6 months), n=24
Total leukocyte count, 10^9/l	7.45±0.89	18.23±2.54 p<0.001	11.42±1.93
Viable neutrophils, %	39.61±2.82	40.60±2.8	38.25±2.27
Viable neutrophils, 10^9/l	2.64±0.34	7.74±1.11 p<0.001	3.51±0.59 p<0.002
Neutrophil lysosomal activity, AU	29.22±3.37	218.61±18.92 p<0.001	139.30±24.77 p<0.001
NBT-spontaneous test, %	23.63±2.08	26.55±2.55	23.23±3.72
NBT-spontaneous test, AU	0.25±0.03	0.34±0.03	0.27±0.04
NBT-stimulated test, %	39.36±2.96	37.77±2.44	41.98±3.50
NBT-stimulated test, AU	0.51±0.04	0.44±0.04	0.57±0.03
Neutrophil functional reserve, AU	2.02±0.13	2.31±0.28	2.45±0.31
Neutrophil phagocytic activity, %	61.35±2.76	51.12±1.85 p<0.001	44.64±3.82 p<0.001
Neutrophil phagocytic activity, AU	2.52±0.20	1.68±0.11 p<0.001	1.32±0.14 p<0.001
Phagocytic index, AU	3.81±0.21	3.19±0.14 p<0.001	2.80±0.17 p<0.01

Note: AU – Arbitrary Units, NBT – Nitroblue tetrazolium test (measures oxidative burst in neutrophils), $p < 0.05$ indicates statistically significant difference.

When studying the immunological parameters of cervical mucus in transient and persistent courses of HPV (papillomavirus infection), it is observed that the functional activity of neutrophils and their phagocytic index are aligned. This is characterized by significant differences in neutrophil lysosomal activity, phagocytosis intensity, and the neutrophil phagocytic index. Changes in the phagocytic activity response of neutrophils are indicative of disruptions in their ingestion and digestion capabilities, as well as the ineffectiveness of intracellular functions during HPV. Compared to transient HPV, neutrophil functional activity was lower in persistent HPV. This is likely due to a high antigen load in this group,

which in turn depletes the functional granules of neutrophils. The study of humoral factors in cervical mucus showed that in persistent HPV, the humoral components of the immune system are also engaged in the body's response. (Table 2).

Table 2

Comparative levels of immunoglobulins in cervical mucus by groups

Indicators	Control group, n=25	Group I, Transient HPV, n=28	Group II, Persistent HPV (6 months) n=24
sIgA, g/L	0.021±0.006	0.025±0.007	0.036±0.005
IgA, g/L	0.10±0.026	0.09±0.017	0.14±0.018
IgM, g/L	0.012±0.002	0.027±0.008	0.022±0.003
IgG, g/L	0.23±0.06	0.42±0.06 p<0.05	0.64±0.03

Note: p – statistical significance of the difference compared to control group indicators.

It is known that an increase in IgG levels indicates the completion of the immune response, which contributes to the elimination of HPV from the body. When analyzing the levels of IgG class antibodies against HPV, it was found that particularly high titers (1:800) were more commonly observed in women of group II (p<0.001). In contrast, antibody titers against HPV of 1:200 or lower were mostly found in women from group I and the control group. (Fig. 1).

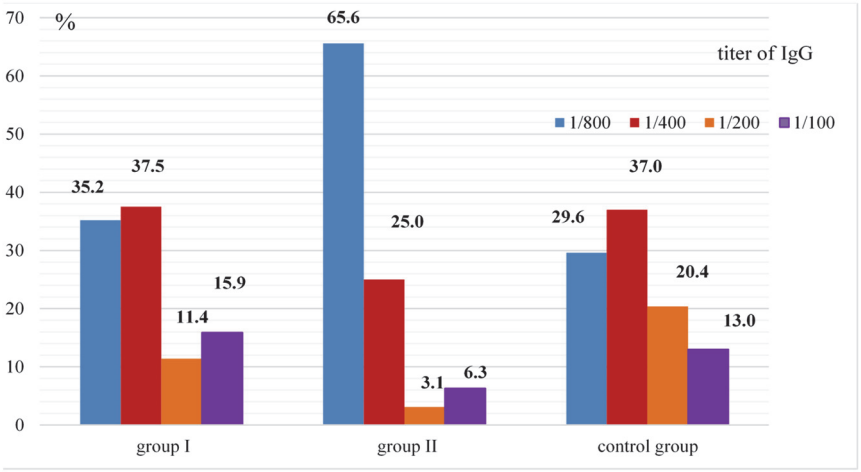

Fig 1. Comparative characteristics of IgG titers in pregnant women with HPV by group.

Based on the obtained results, it can be concluded that in persistent HPV, the increased viral load leads to depletion of the neutrophil defense mechanism in cervical mucus.

Thus, the findings of this study showed that during pregnancy, human papillomavirus infection causes significant changes in both cellular and humoral components of local immunity. Notably, in persistent HPV cases, although an increase in the total number of neutrophils in cervical mucus was observed, a significant decrease in their functional activity and phagocytic capacity was also recorded. These findings indicate that the chronic nature of the infection, due to a high antigen load, depletes the functional reserves of neutrophil cells, which in turn weakens intracellular defense mechanisms.

Additionally, alterations were also detected in humoral immune factors of cervical mucus in persistent infections. The statistically significant increase in IgG levels (p<0.01) can be interpreted as an indicator of an intense immune response developed against the persistent presence of the virus in the body. Higher IgG titers (1/800 and above) were mostly observed in women with persistent HPV, which may be related to the strengthening of the adaptive immune response in chronic infections.

Studies have shown that the clearance of HPV is delayed during pregnancy, and high viral loads additionally support persistence of the virus, further reinforcing the hypothesis that pregnancy is characterized by a modified immune response [3]. It is hypothesized that HPV and/or its persistent presence may be promoted by altered immunological adaptations aimed at maintaining immune tolerance to the semi-allogeneic fetus [10]. The obtained results are consistent with previous studies showing that changes in the immune system during pregnancy may delay the clearance of HPV infection, while postpartum immune recovery may lead to increased elimination of the infection. Similar findings have been reported in other studies as well. Mu Li et al. demonstrated a positive correlation between the levels of sIgA, IgG, and IL-10 with the degree of cervical lesions and the presence of HPV 16 and HPV 18 infections [12]. This study also demonstrated that during persistent HPV, weakening of local and humoral immunity may have a potentially negative impact on the clinical course and outcomes of HPV during pregnancy.

Conclusion

In persistent HPV, a significant decrease in the phagocytic activity and functional reserve of neutrophils in cervical mucus was observed. The phagocytic count and activity of neutrophils were lower in persistent infection compared to transient HPV, indicating functional depletion due to massive antigen load. During persistent HPV, the IgG level in cervical mucus increased significantly, reflecting an active response of the adaptive immune system against the infection. High IgG titers indicate viral persistence in the body and the formation of a strong immune response. Changes in local immune indicators can be used to predict the course and outcomes of HPV infection.

References

1. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal Immunological Adaptation During Normal Pregnancy. Front. Immunol. 2020;11:2627. doi: 10.3389/fimmu.2020.575197.

2. Aldhous MC, Bhatia R, Pollock R, Vragkos D, Cuschieri K, Cubie HA, et al HPV infection and pre-term birth: A data-linkage study using Scottish Health Data. Wellcome Open Res. 2019;4:48. doi: 10.12688/wellcomeopenres.15140.1.

3. Condrat CE, Filip L, Gherghe M, Cretoiu D, Suciu N. Maternal HPV Infection: Effects on Pregnancy Outcome. Viruses. 2021 Dec 7;13(12):2455. doi: 10.3390/v13122455.

4. Carlsen KCL, Granum B, Haugen G, Hedlin G, Hilde K, Nordlund B, et al. BMC Pregnancy Childbirth. 2024 Nov 19;24(1):764. doi: 10.1186/s12884-024-06958-2.

5. Ford JH, Li M, Scheil W, Roder D. Human papillomavirus infection and intrauterine growth restriction: A data-linkage study. J. Matern.-Fetal Neonatal Med. 2019;32:279–285. doi: 10.1080/14767058.2017.1378330.

6. Ismayilova SM. A modern approach to the management of pregnancy and childbirth in women infected with papillomavirus: PhD dissertation abstract in medical sciences. Baku, 2015: 6.

7. Niyibizi J, Zanré N, Mayrand M-H, Trottier H. Association Between Maternal Human Papillomavirus Infection and Adverse Pregnancy Outcomes: Systematic Review and Meta-Analysis. J. Infect. Dis. 2020;221:1925–1937. doi: 10.1093/infdis/jiaa054.

8. Niyibizi J, Zanré N, Mayrand M-H, Trottier H. The association between adverse pregnancy outcomes and maternal human papillomavirus infection: A systematic review protocol. Syst. Rev. 2017;6:53. doi: 10.1186/s13643-017-0443-5.

9. Subramaniam A, Lees BF, Becker DA, Tang Y, Khan MJ, Edwards RK. Evaluation of Human Papillomavirus as a Risk Factor for Preterm Birth or Pregnancy-Related Hypertension. Obstet. Gynecol. 2016;127:233–240. doi: 10.1097/AOG.0000000000001247.

10. Værnesbranden MR, Staff AC, Wiik J, Sjøborg K, Rueegg CS, Sugulle M, et al. Human papillomavirus infections during pregnancy and adverse pregnancy outcomes: a Scandinavian prospective mother-child cohort study. BMC Pregnancy Childbirth. 2024 Nov 19;24(1):764. doi: 10.1186/s12884-024-06958-2.

11. Værnesbranden MR, Wiik J, Sjøborg K, Staff AC, Carlsen KCL, Haugen G, et al. Maternal human papillomavirus infections at mid-pregnancy and delivery in a Scandinavian mother-child cohort study. Int. J. Infect. Dis. 2021;108:574–581. doi: 10.1016/j.ijid.2021.05.064.

12. World Health Organisation Human papillomavirus vaccines: WHO position paper, May 2017—Recommendations. Vaccine. 2017;35:5753–5755. doi: 10.1016/j.vaccine.2017.05.069.

13. Xiong YQ, Mo Y, Luo QM, Huo ST, He WQ, Chen Q. The Risk of Human Papillomavirus Infection for Spontaneous Abortion, Spontaneous Preterm Birth, and Pregnancy Rate of Assisted Reproductive Technologies: A Systematic Review and Meta-Analysis. Gynecol. Obstet. Investig. 2018;83:417–427. doi: 10.1159/000482008.

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