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Investigation of risk factors, PKM2 & Stat3 biomarkers, and psychological impact in high-risk HPV-positive women undergoing cervical cancer screening in outpatient clinics

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Abstract: The aim of the present study was to analyze the influencing factors of cervical lesions with positive human papillomavirus (HPV) infections in women and the significance of combining pyruvate kinase M2 (PKM2) and signal transducer and activator of transcription 3 (Stat3) in cervical cancer screening. The research results are interpreted from a psychosocial perspective to provide new perspectives for the comprehensive management of cervical cancer. The study analyzed data from 149 women who underwent cervical cancer screening at The First People's Hospital of Yuhang District, Hangzhou Hospital, between October and December 2024. Participants were tested for HPV infection using PCR-reverse dot hybridization and underwent liquid-based cytology assessment. Biomarker analysis of PKM2 and Stat3 levels in cervical secretions was performed using chemiluminescence. Statistical analyses, including logistic regression and ROC curve analysis, were conducted to evaluate risk factors for high-risk HPV infection and assess the predictive value of PKM2 and Stat3 in cervical cancer screening. In the univariate analysis, the number of sexual partners, frequency of sexual activity, frequency of condom use, reproductive tract inflammation, number of pregnancies, PKM2, and Stat3 were found to be associated with the occurrence of cervical cancer ($P < 0.05$). Logistic multiple regression analysis indicated that PKM2, Stat3, reproductive tract inflammation, and frequency of sexual activity had a significant impact. From a psychosocial perspective, sexual behavior-related factors are not only biological risk factors but may also have a negative impact on patients' mental state and quality of life, such as anxiety, depression, and other emotional problems. Additionally, in receiver operating characteristic (ROC) analysis, the area under the curve (AUC) for cervical secretion screening for cervical cancer was higher than for any other single indicator ($P < 0.05$), providing a useful reference for clinical screening of high-risk cervical cancer populations and improving patient prognosis. The results of this study also suggest that sexual behavior-related factors may have an impact on patients' psychology and quality of life, further emphasizing the importance of comprehensive consideration of psychosocial factors in cervical cancer screening and management.

Keywords: cervical cancer screening; human papillomavirus (HPV); psychosocial; risk factors; outpatient clinics; pyruvate kinase M2 (PKM2)

1. Introduction

Cervical cancer remains a significant global health issue, primarily caused by persistent infection with high-risk types of human papillomavirus (HPV). Research has consistently shown that HPV infection plays a central role in the development of cervical cancer, with high-risk genotypes such as HPV 16 and 18 being the most strongly associated with disease progression [1]. While HPV infection is common

among sexually active individuals, most cases resolve naturally. However, in some women, the virus persists, leading to cervical dysplasia and, eventually, invasive cervical cancer if left untreated [2].

Despite advancements in HPV awareness, cervical cancer remains a major health challenge in low- and middle-income countries due to inadequate screening and prevention programs. Countries with widespread vaccination and routine screening have seen significant declines in both cervical cancer incidence and mortality. In contrast, regions lacking these essential public health measures continue to experience high disease burdens. The World Health Organization (WHO) reported in 2020 that there were approximately 604,000 new cervical cancer cases and 342,000 deaths globally, with 80% of these cases occurring in lower-income nations [3]. In China, cervical cancer is the second most common malignancy among women, with 115,000 new cases and 59,000 deaths reported in 2020 [4]. This underscores the urgent need for improved prevention, screening, and treatment strategies to mitigate the growing impact of the disease [5].

Proper treatment of cervical cancer requires researchers to understand why high-risk HPV infections persist along with their progression toward malignancy. The infection shows minimal symptoms after HPV transmission, which leads numerous women to stay unaware of their current HPV status. The screening of cervical dysplasia and cancer mostly becomes known through routine examinations [6]. Testing for Pap smears and HPV DNA serves as the primary method to identify pre-invasive cellular conditions so health professionals can intervene before invasive cancer develops. HPV infection starts the disease process that advances from persistent viral infection through abnormal cell development into preclinical lesions that become cancer if untreated [7].

Several risk factors contribute to the persistence of HPV infection and to future development of cervical cancer. Having sex, especially at an early age or with many sexual partners, greatly increases a person's chances of getting and holding on to high-risk HPV infections [8]. Transmission further amplifies the likelihood of transmission in unprotected sexual activity [9]. Immunosuppression, smoking, and concomitant sexually transmitted infections (STIs) also render susceptibility to HPV persistence and accelerate progression to cervical cancer [10].

The development of cervical cancer is also related to reproductive health factors. Although the mechanisms by which multiple pregnancies contribute to an increased risk are not fully understood, studies have suggested that such pregnancies may increase an individual's risk [11]. The chronic inflammation from infections, such as chlamydia or bacterial vaginosis, that may persist is associated with a greater likelihood of persistence of HPV [12], creating a space for dysplasia and progression of cervical cancer. Additionally, women with reduced immune systems, including those who live with HIV or are treated with immunosuppressive drugs, are at an increased risk of cervical lesions or cancer [13].

The HPV infection that is persistent is the most important determinant of cervical cancer among all known risk factors. Providing a history of HPV epidemiology and factors determining sustained infection is necessary for effective prevention [11]. Cervical cancer screening's main purpose is to identify pre-invasive diseases and stop them from becoming invasive. Pap smears and HPV DNA testing are traditional

screening methods that have been found to aid in early detection [10]. Despite this, proper cervical cancer screening is not easily accessed by consumers in many low-resource settings where cervical cancer rates are disproportionately high. HPV screening and cervical cancer screening services need to be promoted and made available to the public, and there is an urgent need for widespread screening programs and public health campaigns to achieve this goal.

The introduction of HPV vaccination programs has provided a promising avenue for primary prevention. Countries that have implemented widespread vaccination efforts targeting HPV 16 and 18 have observed a substantial reduction in HPV prevalence and cervical cancer rates, particularly among women vaccinated before sexual debut [14]. Studies confirm that vaccines are highly effective in preventing HPV-related cancers [15]. However, challenges persist in ensuring equitable vaccine distribution and access to screening services, particularly in resource-limited regions where cervical cancer remains a significant public health concern. Overcoming these obstacles is essential to reducing the global burden of cervical cancer and improving women's health outcomes worldwide.

Research questions

- 1) What are the key risk factors associated with cervical cancer in high-risk HPV-positive women?
- 2) What is the predictive value of PKM2 and Stat3 compared to traditional risk factors in identifying high-risk cervical cancer cases?
- 3) What is the psychosocial impact of HPV infection and cervical cancer screening on patients' mental health and quality of life?

Hypotheses

H1: There are some risk factors associated with cervical cancer in high-risk HPV-positive women.

H2: Increased levels of PKM2 and Stat3 in cervical secretions are significantly associated with a higher risk of cervical cancer.

H3: PKM2 and Stat3 biomarkers have higher predictive value (AUC) in cervical cancer screening compared to traditional risk factors such as reproductive tract inflammation and sexual behavior.

H4: HPV infection and cervical cancer screening have a significant psychosocial impact, contributing to increased anxiety, depression, and reduced quality of life in affected women.

2. Literature review

A literature review on the relationship between infection with HPV and cervical cancer identifies the complexity of this worldwide health issue. The leading cause of cervical cancer is human papillomavirus (HPV) infection, especially with high-risk HPV types including HPV 16 and 18 [13]. More than 99 percent of cervical cancers worldwide and the growth of cervical lesions into invasive cancer are directly associated with HPV, specifically high-risk types. However, transient and self-limiting HPV infection is usually transmitted sexually, though a proportion of women develop persistent infection, which may result in cervical dysplasia and invasive cancer. The

etiology of cervical cancer is based on this persistent infection, especially with high-risk HPV types [15,16].

Globally, HPV infections are common, and studies show that almost 80% of women will be exposed to the virus in their lifetime [17]. Nevertheless, most people's immune response overcomes the infection within one to two years. However, persistent infection can cause abnormal cell changes in the cervix. Generally, these changes are differentiated in high-grade and low-grade lesions; the high-grade lesions are associated with a higher risk of progression to cancer. These steps are a series of well-documented steps from the initial infection with HPV to HPV persisting and integrating its DNA into the human host genome, all of which cause the disruption of normal cell cycle regulation and promote oncogenes [18]. According to various studies, persistent infection with high-risk HPV strains is needed for the development of cervical precancer and cancer, but not all HPV infections progress to cancer; therefore, other factors are involved in the progression of the disease [16,19].

The immune system playing its role in clearing the virus is a critical factor in the persistence of HPV infection and development of cervical cancer. The persistence of HPV infection and the subsequent risk for cervical cancer can be elevated in women with compromised immune systems, such as women who are HIV infected or taking immunosuppressive therapy [20]. In most cases the immune system clears the majority of HPV infections within one to two years, but if the immune system is weakened (immunocompromised), the virus is not cleared, and the virus can pass further on and cause cellular changes that may lead to malignancy. Moreover, an individual's immune response to HPV may be determined by some genetic factors. Persistent HPV infections can be enhanced by certain genetic polymorphisms in immune-related genes in women [21]. These findings highlight the role immune surveillance plays in blocking the development of cervical cancer from HPV infection. Aside from immune system factors, lifestyle and sexual behavior have also been documented to play a role in acquiring and sustaining HPV infection. Early sexual debut, a high number of sexual partners, and lack of condom use have been shown to increase the likelihood of acquiring HPV infection and further developing persistent infection, particularly with high-risk HPV types [22]. Indeed, this is especially true in women who have had multiple sexual partners because they are potentially exposed to different strains of HPV. Additionally, the inconsistent use of condoms has been linked with a higher risk of transmitting HPV because condoms cannot completely block the transmission of the virus during sex [23]. The spread of HPV and its incidence to cause cervical cancer development are contributed to by these behavioral factors, which also predispose people to prolonged infection.

Reproductive tract infections have also been shown to influence the persistence of HPV as well as the progression of cervical cancer. Chlamydia and other chronic infections, especially one that affects the cervix, such as bacterial vaginosis, have been found to persist the HPV infection by creating a chronic inflammatory environment where the viral infection can persist [24]. These infections can cause inflammation, which can impair the local immune response and allow the virus to persist in the cervical epithelium. Also, chronic inflammation can cause actual changes in the cervical cells, which make them susceptible to promoting the development of 'pre-cancerous' lesions. In reality, some studies have shown that women with a history of

genital infection are at greater risk of persistent HPV infection and cervical cancer [22]. This underscores the need to incorporate reproductive tract infections into a complementary strategy for cervical cancer prevention. Smoking is another very important factor linked to the risk of getting cervical cancer. Cigarette smoking has been associated with impaired immune function and appears to be a cofactor in the development of cervical cancer, whereby it may facilitate the persistence of HPV infection [21]. Smoking is associated with increased risk of cervical intraepithelial neoplasia (CIN), a precursor to cervical cancer, and of having persistent high-risk HPV infection. Cigarette smoke contains toxic substances that can harm cervical cells, making them easier to become infected with HPV and prone to the development process of cancer changes. Smoking makes a woman more likely to develop persistent HPV infections and cervical cancer than not smoking [25].

In addition to HPV infection, there is also a complicated role of certain reproductive factors in relation to cervical cancer. Pregnancies a woman has had: more pregnancies mean more risk of cervical cancer. This is believed to be linked to hormonal changes in pregnant women that cause changes to the immune response and cause the increase in vulnerability of the cervix to HPV infection [13]. Also, according to the studies, women with a history of several pregnancies or a change of a cervical epithelium due to childbirth are at greater risk of developing cervical cancer [23]. This is an area that warrants further investigation given the current lack of knowledge about how pregnancy influences HPV infection and therefore risk of cervical cancer. Vaccination to prevent HPV infection is stated to diminish the risk of cervical cancer drastically. With the introduction of vaccines against high-risk HPV types (e.g., HPV 16 and 18), there have been declines in the prevalence of these strains and the associated reduction in cervical cancer incidence, primarily in countries with established vaccination programs [26]. It is known that vaccination against HPV in young women is highly effective in preventing HPV infection, particularly when it is administered before the onset of sexual activity. The use of this primary prevention strategy in conjunction with regular screening is a potent technique in minimizing the risk of cervical cancer.

The study of Xiao et al. [27] made novel contributions to the study of risk stratification of high-risk HPV types and, through a novel risk parameter, introduced a self-defined parameter to assess the intrinsic pathogenicity of these high-risk HPV types beyond mere prevalence rates. Present research, compared to conventional studies that normally examine overall prevalence of HPV and HPV screening based on cytology, demonstrates variation in oncogenic potential of different HPV types after taking infection rate into account. The results highlight the importance of region-specific monitoring for HPV45, HPV16, HPV58, HPV33, and HPV18, with the highest pathogenicity risks in cervical biopsies. This study shows a strong correlation between high-risk HPV typing and diagnostic procedures such as TCT, colposcopy, and biopsy, which can be used to understand such a correlation better and better predict high-grade cervical lesions in order to improve early detection strategies. This represents a novel methodology for future research on personalized risk assessment and targeted screening that has the potential to consume less than the efficient portion of healthcare resources and provide better outcomes for patients.

3. Methodology

3.1. Study design and participants

This study employed a retrospective analysis to collect data from female patients who underwent cervical cancer screening at the gynecology outpatient clinic of The First People's Hospital of Yuhang District, Hangzhou Hospital, between October and December 2024. The inclusion criteria were as follows: (1) age between 20 and 65 years; (2) voluntary participation in cervical cancer screening; (3) no vaginal douching or use of vaginal medications within the last 3 days; (4) no history of severe systemic diseases such as heart, liver, or kidney dysfunction.

The exclusion criteria included (1) pregnancy or being within six weeks postpartum; (2) previous history of hysterectomy or cervical conization; (3) already diagnosed with cervical cancer or any other malignant tumors; and (4) incomplete or unavailable data. A total of 149 women who met the inclusion criteria were included in the study. All participants provided informed consent, and the study was approved by the ethics committee of the hospital. To ensure data accuracy and protect patient privacy, the basic information, clinical data, and laboratory test results of the participants were rigorously controlled for quality and confidentiality.

3.2. Study methods

All study subjects underwent standardized cervical exfoliated cell collection and Human papillomavirus-deoxyribonucleic acid (HPV-DNA) testing. A specialized cervical brush was used to collect exfoliated cells from the cervix, which were then placed in a preservation solution. The HPV DNA detection and genotyping were performed using the polymerase chain reaction (PCR)-reverse dot hybridization method. The test kit used could simultaneously detect 21 HPV genotypes, including 13 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and 8 low-risk types (6, 11, 42, 43, 44, 53, 66, 81) (Solomon et al., 2002) [16]. HPV infection was defined as the detection of any HPV genotype. Liquid-based cytology results were classified based on the 2001 International Agency for Research on Cancer (IARC) recommendations for cervical/vaginal cytology reporting, which include categories such as normal, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma, atypical glandular cells, and adenocarcinoma. If the results indicated ASC-US or higher, the patient was notified to come in for a colposcopy examination and cervical biopsy. Based on the biopsy results, the patients were categorized into five groups: normal, cervical intraepithelial neoplasia (CIN) I, CIN II, CIN III, and cervical cancer.

Biomarker analysis: Selection of PKM2 and Stat3

In addition to HPV testing, the levels of pyruvate kinase M2 (PKM2) and signal transducer and activator of transcription 3 (Stat3) were assessed in cervical secretions using a specialized brush. The samples were placed into a preservation solution and analyzed using a chemiluminescence method with reagents from BD (Becton, Dickinson and Company, USA).

The selection of PKM2 and Stat3 as biomarkers was based on their well-established roles in cervical cancer pathogenesis, particularly in metabolic and inflammatory pathways. PKM2 is an essential regulator of the Warburg effect, a metabolic phenomenon observed in cancer cells where glucose metabolism is reprogrammed to favor proliferation rather than energy production. This metabolic shift is a hallmark of many cancers, including cervical cancer, and has been linked to increased tumor aggressiveness and progression. Stat3, on the other hand, is a crucial transcription factor involved in cell survival, proliferation, and immune evasion, playing a key role in tumor initiation and progression.

Biomarkers such as p16, Ki 67, and HPV E6 E7 oncoproteins have been widely used in cervical cancer research, but PKM2 and Stat3 were selected because they can give more information about the metabolic perturbation and inflammatory signaling pathways, which are rarely illuminated by the conventional biomarkers. Emerging research also indicates that PKM2 and Stat3 could be early disease markers of HPV persistence as well as progression from low- to high-grade lesions and might therefore serve as useful disease markers for risk stratification and early intervention strategies.

3.3. Data collection

Demographic information (such as age, education level, and marital status) as well as lifestyle habits (such as smoking and alcohol consumption) were collected. Reproductive health history, including age at first sexual intercourse, number of sexual partners, and pregnancy history, was recorded, as these factors have been associated with HPV acquisition and persistence. All data collection and laboratory procedures were performed by trained professionals to ensure reliability and consistency in the results.

3.4. Statistical methods

Statistical analyses were conducted with the SPSS version 25.0 software program. The data analysis operated with continuous variables shown as mean $\pm$ standard deviation ($\bar{x} \pm s$) followed by intergroup *t*-test execution. The investigators displayed categorical variables through frequencies and percentages while employing the chi-square (χ^2) test for inter-group assessments. The research employed logistic regression analysis to investigate the risk elements responsible for high-risk HPV infection while calculating odds ratios (OR) along with 95% confidence intervals (CI). Studies performed ROC curve analysis on different variables to determine their predictive value regarding high-risk HPV infection. The results demonstrated clinical significance when the area under the curve reached values above 0.7 with P values less than 0.05. The research established its objective by evaluating the principal factors linked to high-risk HPV infection while assessing the performance of biomarkers PKM2 and Stat3 as advanced cervical cancer screening indicators. Researchers used the gathered data for comparing clinical factors between patients with high-risk HPV infection and those without infection as well as for understanding how demographic, lifestyle, and clinical factors impact HPV infection persistence and development.

4. Results and discussion

Among the 149 women who underwent cervical cancer screening, the pathological results were as follows: 68 cases (52.71%) showed normal findings, 22 cases (17.05%) had cervical intraepithelial neoplasia (CIN) I, 21 cases (16.27%) had CIN II, 19 cases (14.72%) had CIN III, and 19 cases (14.72%) were diagnosed with cervical cancer. These results reflect the distribution of pathological findings among the study population and underscore the varying degrees of cervical lesions observed during screening.

4.1. Comparison of clinical data between the two groups

A comparison was made between the two groups of patients: those with cervical cancer (case group) and those without (control group). There were no statistically significant differences between the groups in terms of body mass index (BMI), education level, age, smoking history, alcohol consumption history, or history of childbirth ($P > 0.05$). However, significant differences were found in several key variables, including the number of sexual partners, frequency of condom use, number of pregnancies, presence of reproductive tract inflammation, and the levels of biomarkers PKM2 and Stat3 ($P < 0.05$). The results are presented in **Table 1**.

Table 1. Comparison of clinical data between groups.

Variable	Non-Cervical Cancer Group 1	Cervical Cancer Group 2
Age (Mean)	1.544118	1.508197
Age (Std)	0.501753	0.504082
Education Level (Mean)	1.764706	1.606557
BMI (Mean)	21.22735	21.26852
BMI (Std)	0.886599	0.835228
Smoking (Mean)	0.617647	0.52459
Alcohol (Mean)	0.441176	0.508197
Childbirth History (Mean)	0.5	0.393443
Number of Partners (Mean)	1.441176	1.57377*
Sexual Frequency (<5/â%¥5) (Mean)	1.441176	1.606557*
Condom Use (Mean)	1.529412	1.409836*
Pregnancy History (Mean)	1.558824	1.459016*
Reproductive Tract Inflammation (Mean)	1.514706	1.47541
PKM2 (Mean)	28.54662	50.82984*
PKM2 (Std)	5.257026	8.968286
Stat3 (Mean)	0.060971	0.096574*
Stat3 (Std)	0.012308	0.017323

(* $P < 0.05$ indicates statistical significance).

Table 1 shows a comparison of clinical data of the non-cervical cancer group (Group 1) and the cervical cancer group (Group 2). By comparison of age, there is a minor difference between the cervical cancer (1.508) and control groups (1.544). However, certain other factors show significant differences. In this group, the cervical

cancer group has a slightly lower education level, a slightly higher average BMI, and lower smoking and alcohol consumption than the non-cervical cancer group. The group with cervical cancer has an average higher number of sexual partners, frequency of sexual intercourse, and pregnancy history, whereas in the non-cervical cancer group, there is a greater level of condom use. Additionally, the score of reproductive tract inflammation is slightly higher in the non-cervical cancers. Additionally, PKM2 and Stat3 are both notably increased (50.83 and 0.097, respectively) in the cervical cancer group compared to the non-cervical cancer group (28.55 and 0.061, respectively), suggesting their involvement as biomarkers of this difference based. Our findings indicate that PKM2 and Stat3 levels apparently have a strong association with cervical cancer as well as certain lifestyle and reproductive history factors that also follow some notable trends.

Figure 1 provides a comparative overview of the clinical data between the Non-Cervical Cancer Group and the Cervical Cancer Group. It highlights the differences in key variables such as age, BMI, smoking habits, reproductive history, and molecular markers (e.g., PKM2 and Stat3).

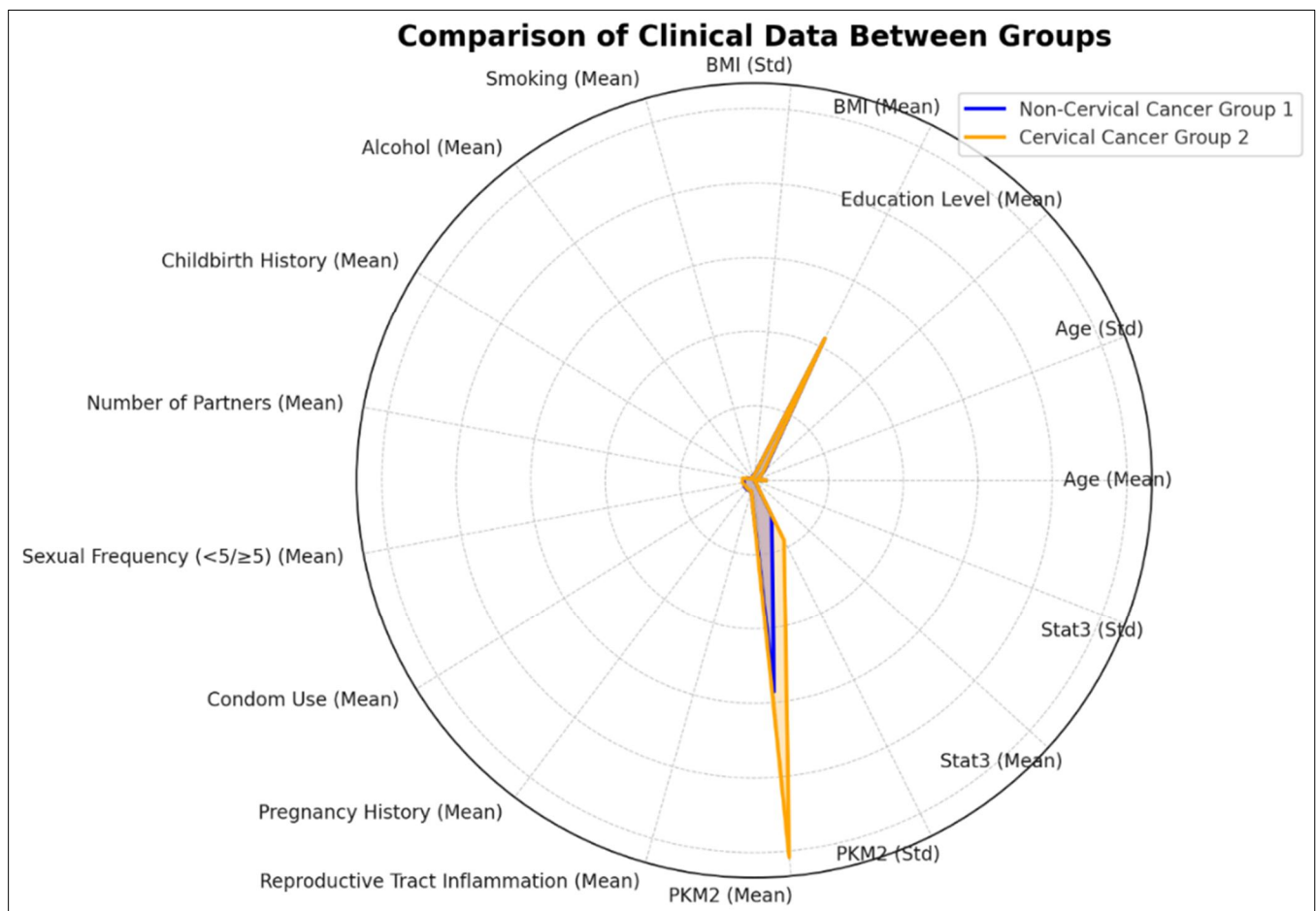


Figure 1. Clinical data comparison: Non-cervical cancer vs. cervical cancer groups.

4.2. Multivariate analysis

A multivariate analysis was then performed to investigate the associated factors of high-risk HPV infection. A logistic regression analysis was also done, where the

dependent variable was group membership (cervical cancer vs. non-cervical cancer). Although the results were inconclusive, the analysis suggested that about 80% of the variability in group membership can be explained by the number of sexual partners, sexual activity frequency, condom use, number of pregnancies, reproductive tract inflammation, and PKM2 (U/mL) and Stat3 (ng/mL). The analysis showed that all the sexual frequency, reproductive tract inflammation, PKM2, and Stat3 have a statistically significant positive impact on the likelihood of belonging to the cervical cancer group, with the model equation as Still, the group membership was not significantly influenced by the number of sexual partners, condom use, or number of pregnancies. Unfortunately, these findings indicate that though some lifestyle and reproductive factors are tied to cervical cancer, the markers found in this study (PKM2 and Stat3) are the key components in determining whether one is likely to develop cervical cancer.

Table 2 shows results of binary logistic regression analysis explaining factors that contribute to cervical cancer development. In terms of the above factors tested, there was a statistically significant positive relation between cervical cancer and increased sexual frequency, reproductive tract inflammation, PKM2 levels, and Stat3 levels. In particular, sexual frequency was positively associated with cervical cancer risk, with a high odds ratio (OR = 14.604), meaning that frequency of sexual activity was associated with a significantly increased risk of cervical cancer. There also was a significant association with reproductive tract inflammation, odds ratio 8.9, and reproductive tract polymorphisms among the genomic sequencing polymorphisms, which was suggestive of an increased risk of cervical cancer. PKM2 and Stat3 concomitantly appeared as two of the most powerful predictors, with a PKM2 OR of 1.304 and, unsurprisingly, the highest odds ratio of 32.9 for Stat3. The extremely high odds ratio (OR) for Stat3 (4.54-238.0) suggests potential issues such as model overfitting, multicollinearity, or an artifact of the data distribution. This could be due to the small sample size, extreme variability in Stat3 levels, or a near-perfect separation in the dataset, where Stat3 values almost completely differentiate between cervical cancer and non-cervical cancer groups. Such an inflated OR may indicate a strong association, but it should be interpreted with caution and validated with further studies to ensure its biological and clinical significance. Thus reaffirming the likelihood of PKM2 and Stat3 as biomarkers that delineate high-risk individuals. However, number of partners, condom use, and number of pregnancies were not found to meaningfully influence the propensity of the woman to develop cervical cancer in this analysis. Finally, we find that when we control for other factors, the variables do not have a strong independent effect with regression coefficients and *p*-values close to 0. Therefore, condom use was not a significant predictor in this study, in contrast to its protective role in preventing the transmission of HPV. These findings underline the importance of biomarkers such as PKM2 and Stat3 when determining the risk of a woman for developing cervical cancer, although they also suggest that lifestyle factors associated with sexual activity and reproductive health could be important factors in increasing the risk as well, further requiring investigation to assess this.

Table 2. Results of binary logistic regression analysis.

Variable	Regression Coefficient	Standard Error	Z-Value	Wald χ^2	p-value	OR (Odds Ratio)	95% CI
Number of Partners	0.247	1.031	0.24	0.057	0.811	1.28	0.170–9.655
Sexual Frequency	2.681	1.349	1.987	3.95	0.047	14.604	1.038–205.507
Condom Use	−0.547	1.003	−0.545	0.297	0.586	0.579	0.081–4.135
Number of Pregnancies	−0.333	1.109	−0.3	0.09	0.764	0.717	0.082–6.303
Reproductive Tract Inflammation	2.186	1.102	1.983	3.933	0.047	8.9	1.026–77.222
PKM2 (U/mL)	0.265	0.077	3.456	11.941	0.001	1.304	1.122–1.516
Stat3 (ng/mL)	3.491	1.010	2.412	5.818	0.016	32.9	4.54–238.0

Note: $p < 0.05$ indicates statistical significance, and $p < 0.01$ indicates a highly significant result.

4.3. Diagnostic value of high-risk HPV genotyping combined with PKM2 and Stat3 in cervical secretion screening

Receiver operating characteristic (ROC) curves are also constructed for different indicators in order to assess the diagnostic value of predicting cervical cancer, with the cervical cancer group as the positive sample and the non-cervical cancer group as the negative sample. Consequently, they examined the following factors: sexual frequency, inflammation of the reproductive tract, and levels of the biomarkers PKM2 and Stat3. The analysis obtained the following results, as is depicted in **Table 3**.

Table 3. Summary of ROC results (AUC).

Indicator	AUC	Standard Error	p-value	95% CI	Optimal Cut-off	Sensitivity	Specificity
Sexual frequency	0.661	0.048	0.002	0.567–0.756	0.323	0.705	0.618
Reproductive Tract Inflammation	0.668	0.048	0.001	0.574–0.763	0.337	0.557	0.779
PKM2 (U/ml)	0.963	0.015	0	0.933–0.994	0.861	0.934	0.926
Stat3 (ng/mL)	0.949	0.019	0	0.912–0.987	0.768	0.885	0.882

Note: * $p < 0.05$ indicates statistical significance, ** $p < 0.01$ indicates a highly significant result.

The diagnostic performance of four factors—sexual frequency, inflammation of the reproductive tract, PKM2, and Stat3—in diagnosing cervical cancer is compared using ROC curve, as shown in **Figure 2**. Each scatter plot shows how well the respective factor differentiates the cervical cancer and non-cancer groups. The result shows that PKM2 and Stat3 are the proteins with the highest diagnostic value, since their curves are closer to the top left corner, which means that they have the highest sensitivity and specificity. Accordingly, AUC values of the sexual frequency and inflammation of the reproductive tract are lower, indicating that these factors are less reliable per se to diagnose cervical cancer than PKM2 and Stat3. It shows that the model is making predictions no better than random guessing.

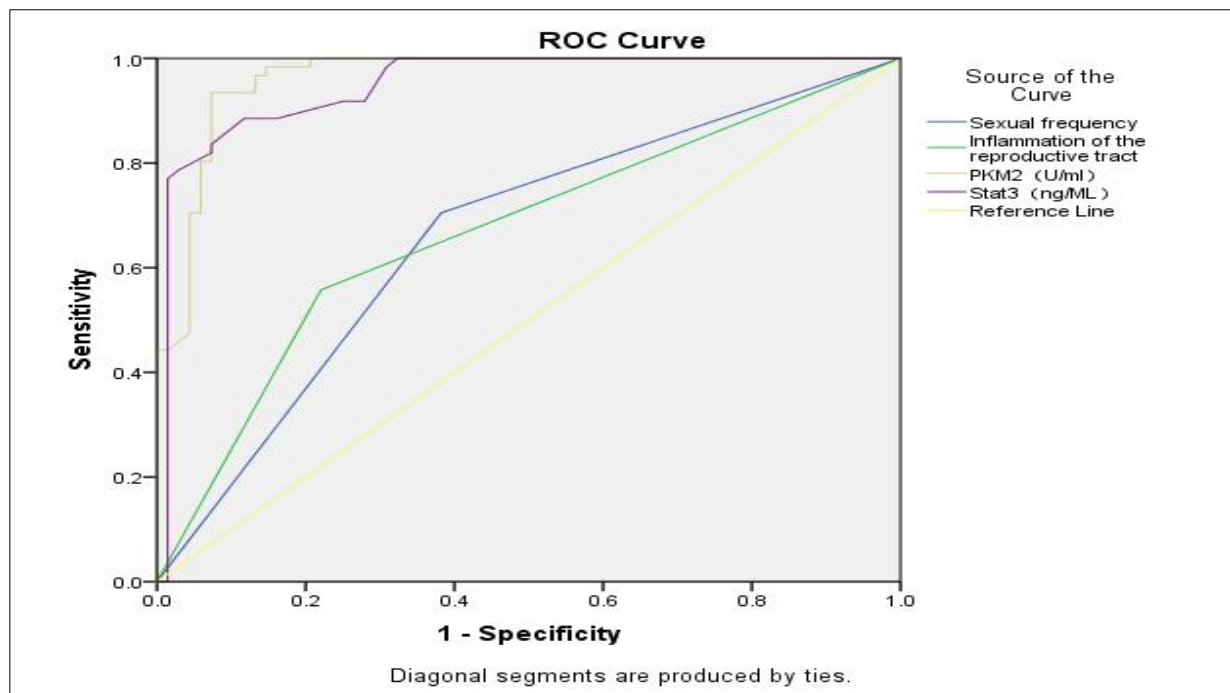


Figure 2. Diagnostic value of sexual frequency, reproductive tract inflammation, PKM2, and Stat3 in cervical cancer screening: ROC curve analysis.

The analysis shows that among the biomarkers for differentiation between the cervical cancer and non-cervical cancer groups, PKM2 and Stat3 are the best markers. Additionally, both markers showed very good diagnostic performance with high sensitivity and specificity for cervical cancer screening. However, sexual frequency and reproductive tract inflammation were less valuable diagnostically and may not be as reliable when used alone in the clinical situation.

Table 3 summarizes the ROC curve analysis results for diagnosing cervical cancer by predicting various factors. The optimal cut-off is chosen based on the balance between sensitivity (true positive rate) and specificity (true negative rate) to maximize diagnostic accuracy. It is typically selected using statistical methods such as the Youden Index, which finds the point that maximizes the difference between sensitivity and specificity ($\text{sensitivity} + \text{specificity} - 1$). Stat3 and PKM2 both had AUC values greater than 0.9, which represents very good diagnostic value for the detection of cervical cancer. With an optimal cutoff value of 0.861, PKM2 showed a sensitivity of 93.4% and a specificity of 92.6%, while Stat3, with an optimal cutoff of 0.768, showed a sensitivity of 88.5% and a specificity of 88.2%. On the other hand, AUC values were lower for sexual frequency (0.661) and reproductive tract inflammation (0.668), which indicates that these are of less diagnostic value for cervical cancer, though they still have a statistical significance. Sensitivity of sexual frequency was 70.5%, and specificity was 61.8%; for reproductive tract inflammation, sensitivity was 55.7%, and specificity was 77.9%. Thus, sexual frequency and inflammatory status of the reproductive tract as diagnostic tools for cervical cancer are less reliable than the two protein biomarkers PKM2 and Stat3.

Figure 3 summarizes the ROC analysis for four clinical indicators: sexual frequency, reproductive tract inflammation, PKM2 (U/mL), and Stat3 (ng/mL). The

results include AUC values, sensitivity, and specificity, with PKM2 and Stat3 demonstrating the highest AUCs of 0.963 and 0.949, respectively, indicating their strong diagnostic performance.

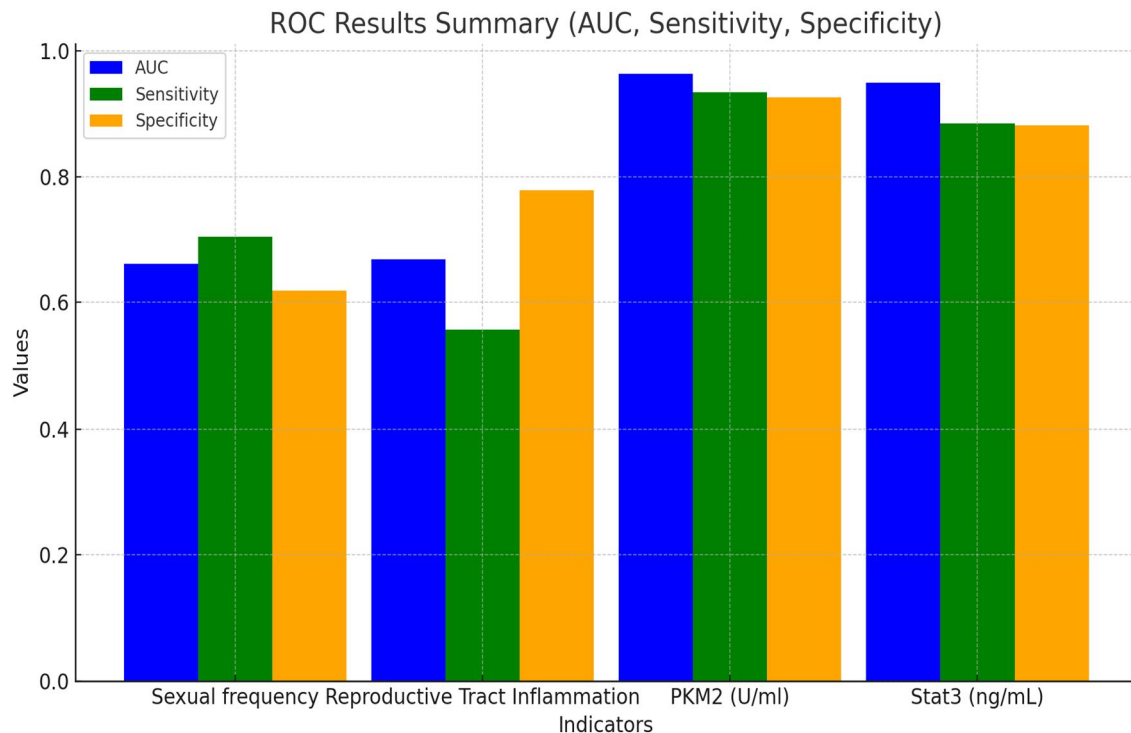


Figure 3. Comparison of ROC metrics for various clinical indicators.

5. Discussion

Univariate analysis showed a correlation between the occurrence of cervical cancer and number of sexual partners, frequency of sexual activity, condom use, presence of reproductive tract inflammation, number of pregnancies, PKM2, and Stat3 ($P < 0.05$). Independent risk factors for HPV-positive cervical lesions included PKM2, Stat3, reproductive tract inflammation, and sexual frequency. It shows that women who had sexual intercourse more often were more likely to contract HPV and, as a result, increase their risk of developing cervical cancer. This may be because in frequent sexual activity the cervical epithelial cells are damaged, and the virus is thus able to invade the cells more easily and to trigger pathological changes. And, obviously, so that does help to control the occurrence of cervical cancer. It is suggested that women lead healthy lifestyles, not exert more physical strain and mental stress, and maintain regular, good personal hygiene, including safe sex measures, in order to reduce the chance of contracting HPV. Reproductive tract inflammation also can confer the risk of HPV infection and the progress of cervical lesions. Prevention and treatment of these conditions are important to reduce the rates of cervical cancer, due to the fact that chronic reproductive tract inflammation results in abnormal epithelial cell proliferation and malignant transformation. Reproductive health education should be reinforced, and females' awareness of reproductive tract inflammation as well as self-care practices should be enhanced. Furthermore, medical institutions have to strengthen the screening and treatment of reproductive tract inflammation.

PKM2, which represents the rate-limiting enzyme within the glycolytic pathway, is localized partly in the cytoplasm and also is able to enter the nucleus, where the molecule acts as a protein kinase and is involved in transcriptional regulation and protein modification. Previous research has demonstrated that PKM2 levels rise gradually in normal cervix, cervicitis, and cervical cancer in accordance with the development of cervical cancer. In line with their findings, this study concurs that PKM2 performs an important role in facilitating cancer progression. PKM2 is an effective enzyme at using glucose to produce lactate and energy, and as glycolytic intermediates are accumulated in the cell, they can interact with nucleic acids and proteins, which support rapid growth and proliferation of cancer cells. In addition, after being translocated into the nucleus, PKM2 is able to promote cell proliferation and inhibit apoptosis by histone H1 phosphorylation and independent pathways from the known cysteine protease apoptosis pathway and acts as an oncogenic factor. The findings of clinically significant reference values are that when the level of PKM2 is more than 38.670 U/mL, the sensitivity of cervical cancer screening is 93.4%, and specificity is 92.6%.

The locus of Stat3 is on chromosome 17 (q21.1–q21.2) and contains a conserved amino acid domain, a domain in which the DNA binds, and a C-terminal transcription activation domain. It can be activated by many growth factors and cytokines and is active in critical intracellular signaling and target gene transcription. It can be concluded from the results of this study that levels of Stat3 in cervical cancer patients are greater when compared to non-cervical cancer patients and are an independent risk factor of cervical cancer. HPV infection and cervical hyperplasia activate Stat3, which transduces extracellular signals for the nucleus to result in transcription of anti-apoptotic genes such as B-cell lymphoma 2 (Bcl-2) and of pro-proliferative genes such as cyclin D1, as well as antigenic factors such as vascular endothelial growth factor (VEGF), and so extends the cell survival cycle and also enhances proliferation, having the role of a cancer promoter. In the condition of Stat3 levels greater than 0.076 ng/mL, the sensitivity for cervical cancer screening is 88.5%, and the specificity for cervical cancer screening is 88.2%, which shows that Stat3 is a good clinical reference for cervical cancer screening.

This study evaluates in detail HPV infection in women screened for cervical cancer in outpatient clinics and determines the most important risk factors for high-risk HPV persistent infection: number of sexual partners, sexual frequency, consummation of condoms, reproductive tract inflammation, and number of pregnancies. The findings can be used for the development of scientific evidence towards developing targeted prevention strategies. These factors are considered when assessing HPV risk by the study. Furthermore, detection of the levels of PKM2 and Stat3 in cervical secretions can provide a new way to enhance the accuracy of cervical cancer screening. Further research should include more precise and efficient screening strategies and the investigation of strategies involving individual HPV prevention and treatment to further impact cervical cancer incidence and mortality. In the future, efforts to reduce cervical cancer, strengthening public health education, and raising HPV vaccination rates will also play an important role.

Practical implications

Therefore, this study has practical significance to suggest that combining biomarkers PKM2 and Stat3 with cervical cancer screening and prevention strategies can be used to improve them. Thus, these biomarkers have a very high diagnostic value and could be used to predict accurately high-risk HPV infections and the early cervical lesions in order to improve the early identification of cervical cancer. Other lifestyle factors like degree of sexual frequency, inflammatory status of the reproductive tract, or safe sexual practices can also reduce the risk for getting infected or the progression to cancer by HPV. This work also highlights the importance of improving public health programs such as reproductive health education and HPV vaccination campaigns to decrease the incidence and mortality of cervical cancer in areas without access to screening. The findings are integrated into routine clinical care for healthcare providers to be able to offer more personalized and relevant prevention strategies that can decrease the responsibility of cervical cancer and the affected outcome of patients globally.

6. Conclusion

Finally, this study suggests that infection with high-risk HPV and biomarkers PKM2 and Stat3 play a key role in the development of cervical cancer. These findings stress the need to integrate these biomarkers into clinical assessment for higher sensitivity and better early detection. Moreover, lifestyle factors such as sexual frequency and reproductive tract inflammation contribute toward HPV persistence and cervical lesions, which likewise emphasizes the need to develop a comprehensive HPV prevention strategy including education and immunization. This research offers important information on improving cervical cancer prevention and treatment through the use of molecular biomarkers and lifestyle interventions aimed towards decreasing incidence and mortality.

Limitations and future research

A limitation of this study is that it is partially retrospective and thus may introduce biases in associations relating to the data collection and recall biases, especially for self-reported factors such as sexual behavior and vital reproductive history. In addition, data were collected from one healthcare center, which reduces the extent to which the findings are applicable to different populations or regions, potentially with different demographic and healthcare characteristics. However, the relatively small sample size also serves as a limitation since it may not represent all the different types of cervical cancer. The findings need further prospective research with larger, multi-center studies. In addition, although PKM2 and Stat3 were shown to be potential biomarkers for cervical cancers, they still need to be investigated as to the underlying biological mechanisms that define their roles in the course of progression of cervical cancers. Prognostic data associated with these biomarkers are currently limited and would best be obtained from the longitudinal study of the progression of HPV infection and its progression to cervical cancer. There is also an opportunity to investigate the possibility of using PKM2, Stat3, and other molecular markers in combination with more advanced imaging techniques to create more

effective, noninvasive screening strategies. Finally, future studies should assess the impact of personalized prevention approaches such as targeted HPV vaccination and lifestyle interventions to lower the cervical cancer burden globally.

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References

1. Gao B, Liou YL, Yu Y, et al. The characteristics and risk factors of human papillomavirus infection: an outpatient population-based study in Changsha, Hunan. *Scientific Reports*. 2021; 11(1). doi: 10.1038/s41598-021-94635-1
2. Yang D, Zhang J, Cui X, et al. Risk Factors Associated With Human Papillomavirus Infection, Cervical Cancer, and Precancerous Lesions in Large-Scale Population Screening. *Frontiers in Microbiology*. 2022; 13. doi: 10.3389/fmicb.2022.914516
3. Babi A, Issa T, Issanov A, et al. Prevalence of high-risk human papillomavirus infection among Kazakhstani women attending gynecological outpatient clinics. *International Journal of Infectious Diseases*. 2021; 109: 8-16. doi: 10.1016/j.ijid.2021.06.006
4. Chinese Society of Pathology, Women's Reproductive System Disease Group. Guidelines for the pathological diagnosis of cervical cancer and precancerous lesions. *Chinese Journal of Pathology*. 2019; 48(4): 265-269.
5. Shamaun S, Jaleel R, Gull Y, et al. Knowledge and Attitude of Cervical Cancer Screening and Vaccination in Patients Attending Gynecology Outpatient Clinic at a Tertiary Care Hospital in Pakistan. *South Asian Journal of Cancer*. 2022; 12(01): 017-022. doi: 10.1055/s-0042-1751093
6. Wang H, Nie K, Liu Z, et al. Analysis of risk factors associated with cervical HPV infection and their effects on female sexual function and anxiety: a multicenter cross-sectional study based on Chinese women. *Frontiers in Oncology*. 2024; 14. doi: 10.3389/fonc.2024.1468160
7. Phaiphichit J, Paboriboune P, Kunnavong S, et al. Factors associated with cervical cancer screening among women aged 25 – 60 years in Lao People's Democratic Republic. Yi S, ed. *PLOS ONE*. 2022; 17(4): e0266592. doi: 10.1371/journal.pone.0266592
8. Yan X, Shen L, Xiao Y, et al. Prevalence, characteristics, and distribution of HPV genotypes in women from Zhejiang Province, 2016 – 2020. *Virology Journal*. 2021; 18(1). doi: 10.1186/s12985-021-01676-z
9. Wright JD, Chen L, Tergas AI, et al. Overuse of Cervical Cancer Screening Tests Among Women With Average Risk in the United States From 2013 to 2014. *JAMA Network Open*. 2021; 4(4): e218373. doi: 10.1001/jamanetworkopen.2021.8373

10. Abdi M, Tamiru A, Tilahun T, et al. Factors associated with human papillomavirus infections among women living with HIV in public health facilities in Western Oromia, Ethiopia. *BMC Women's Health*. 2024; 24(1). doi: 10.1186/s12905-024-03249-y
11. Erdogan M, Cihan F, Kutlu R, et al. Awareness of cervical cancer, human papilloma virus (HPV) and HPV vaccination among women in a cancer screening, early diagnosis and education center. *Annals of Medical Research*. 2021; 28(9): 1735. doi: 10.5455/annalsmedres.2020.10.1041
12. Rai R, Sehgal R, Singhal S, et al. Cervical Cancer Screening Coverage at Tertiary Care Institutes Across India. *Asian Pacific Journal of Cancer Prevention*. 2023; 24(12): 4269-4275. doi: 10.31557/apjcp.2023.24.12.4269
13. Chen J, Zhang R, Xu W, et al. Knowledge and Practices Regarding Human Papillomavirus and Cervical Cancer Screening Among Women in Low-Income Areas of China: A Cross-Sectional Study. *Cureus*. 2024. doi: 10.7759/cureus.55930
14. Vaidya JS, Bulsara M, Wenz F, Sperk E, Massarut S, Alvarado M, Williams NR, Brew-Graves C, Bernstein M, Holmes D, Vinante L, Pigorsch S, Lundgren S, Uhl V, Joseph D, Tobias JS; TARGIT-A authors. The TARGIT-A Randomized Trial: TARGIT-IORT Versus Whole Breast Radiation Therapy: Long-Term Local Control and Survival. *Int J Radiat Oncol Biol Phys*. 2023 Jan 1;115(1):77-82. doi: 10.1016/j.ijrobp.2022.08.029. Epub 2022 Aug 23. PMID: 35998867.
15. Lv Z, He X, Li Z, et al. Outcomes and associated factors of cervical human papillomavirus infection among 608 women in Shenzhen, China, 2018 – 2023. *Frontiers in Public Health*. 2025; 12. doi: 10.3389/fpubh.2024.1523839
16. Li J, Lai H, Qin H, et al. Current status of high-risk HPV infection and correlation with multiple infections in cervical lesions in Western Guangzhou. *Frontiers in Medicine*. 2024; 11. doi: 10.3389/fmed.2024.1252073
17. Nang DW, Tukirinawe H, Okello M, et al. Prevalence of high-risk human papillomavirus infection and associated factors among women of reproductive age attending a rural teaching hospital in western Uganda. *BMC Women's Health*. 2023; 23(1). doi: 10.1186/s12905-023-02342-y
18. Hirani S, Khan S, Akram S, et al. Knowledge, awareness, and practices of cervical cancer, its risk factors, screening, and prevention among women in Karachi, Pakistan. *European Journal of Cancer Prevention*. 2020; 30(1): 97-102. doi: 10.1097/cej.0000000000000590
19. Oneko O, J Mahande M, Amour C, et al. Willingness to HPV self-sampling for cervical cancer screening and its predictors among women attending outpatient clinics in Meru District, Arusha Region, Northern Tanzania. *African Health Sciences*. 2022; 22(2): 97-106. doi: 10.4314/ahs.v22i2.12
20. Macios A, Didkowska J, Wojciechowska U, et al. Risk factors of cervical cancer after a negative cytological diagnosis in Polish cervical cancer screening programme. *Cancer Medicine*. 2021; 10(10): 3449-3460. doi: 10.1002/cam4.3857
21. Plisko O, Zodzika J, Jermakova I, et al. Aerobic Vaginitis—Underestimated Risk Factor for Cervical Intraepithelial Neoplasia. *Diagnostics*. 2021; 11(1): 97. doi: 10.3390/diagnostics11010097
22. Ndateba I, Kabatsinda A, Ndabarora E. Uptake of Cervical Cancer Screening and Associated Factors Among Women Attending Outpatient Services in Rwamagana Hospital, Rwanda. *Rwanda Journal of Medicine and Health Sciences*. 2021; 4(3): 387-397. doi: 10.4314/rjmhs.v4i3.8
23. Worku E, Yigizaw G, Admassu R, et al. Prevalence and risk factors associated with precancerous and cancerous cervical lesions among HIV-infected women in University of Gondar specialized comprehensive referral hospital, Northwest Ethiopia: cross-sectional study design. *BMC Women's Health*. 2024; 24(1). doi: 10.1186/s12905-024-03174-0
24. Zhang H, Zhang S. Prevalence and genotype distribution of human papillomavirus infection among female outpatients in Northeast China: a population-based survey of 110,927 women. *Archives of Gynecology and Obstetrics*. 2022; 308(1): 35-41. doi: 10.1007/s00404-022-06653-7
25. Taye BT, Mihret MS, Muche HA. Risk factors of precancerous cervical lesions: The role of women's socio-demographic, sexual behavior and body mass index in Amhara region referral hospitals; case-control study. Spradley FT, ed. *PLOS ONE*. 2021; 16(3): e0249218. doi: 10.1371/journal.pone.0249218
26. Bhattacharya A, Chakraborty K, Saha P, et al. Cervical cytology abnormalities and associated genotype patterns of High-Risk HPV Infection in Women of Tripura, North-East India: A Hospital-Based Study. *American Journal of Epidemiology*. 2024. doi: 10.1093/aje/kwae364
27. Xiao Y, Liu R, Wang S, et al. Predicting the risk of high-grade precancerous cervical lesions based on high-risk HPV typing in Changsha China. *BMC Women's Health*. 2025; 25(1). doi: 10.1186/s12905-025-03562-0