Al-Turath Medical Journal (ATMJ)
Open Access Journal
Open Access Journal
ISSN Print: 3080-1265
Online : 3080-1273

Original Paper

Human Papillomavirus Infection, Immune Dysregulation, and Oxidative Stress as Predictors of Adverse Pregnancy Outcomes

Donia Hisham Taha
College of Education for Pure Sciences, Tikrit University, Tikrit, Iraq
Correspondence email | donia.hisham@tu.edu.iq
Received: 14/02/2026 | **Accepted**: 10/03/2026 | **Available online:** 31/03/2026 | **DOI:** https://doi.org/10.63964/atmj.2026.3.1

ABSTRACT

Background: Human papillomavirus (HPV) infection is increasingly recognized as associated with endometrial immunological disturbance and adverse reproductive outcomes. Yet the systemic immunological and hormonal correlates of this association, and their diagnostic utility in clinical settings, remain poorly defined.

Aim: This study aimed to assess the association of HPV infection with systemic immunological and oxidative stress markers and to evaluate their contribution to adverse pregnancy outcomes in women of reproductive age.

Methods: A cross-sectional comparative study enrolled 120 women across four groups (n=30 each): Group 1 (HPV-negative controls), Group 2 (HPV-positive without complications), Group 3 (adverse pregnancy outcomes, HPV-negative), and Group 4 (HPV-positive with adverse pregnancy outcomes). Serum interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), peripheral blood natural killer (PB-NK) cells, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), progesterone, and estradiol were measured. Binary logistic regression and receiver operating characteristic (ROC) curve analyses were applied to identify independent predictors and diagnostic thresholds.

Results: Group 4 showed the highest IL-6 (12.6±2.1 pg/mL), TNF-α (15.8±2.6 pg/mL), IFN-γ (17.2±3.1 pg/mL), and MDA (6.2±1.2 nmol/mL), with the lowest IL-10, PB-NK cells, SOD, GSH, progesterone, and endometrial thickness ($p<0.001$ to $p=0.003$). Logistic regression identified MDA (OR=3.06), TNF-α (OR=2.12), and IL-6 (OR=1.84) as significant independent predictors of adverse outcomes. ROC analysis revealed MDA as the strongest single diagnostic marker (AUC=0.891, cutoff ≥4.2 nmol/mL); the combined biomarker model achieved AUC=0.934.

Conclusion: HPV infection is independently associated with systemic pro-inflammatory and oxidative stress dysregulation, hormonal insufficiency, and impaired endometrial receptivity. The MDA-TNF-α-IL-6 panel shows promising clinical utility for identifying women at elevated risk of pregnancy failure, warranting incorporation into preconception assessment protocols.

KEYWORDS: Human papillomavirus; endometrial receptivity; pregnancy outcomes; oxidative stress; cytokines; logistic regression; ROC analysis

1-INTRODUCTION

Human papillomavirus (HPV) infection is the most common sexually transmitted viral infection globally, with an estimated 290 million women infected at any time. Although the carcinogenic potential of high-risk HPV types (16 and 18) has been well documented in cervical cancer, emerging evidence indicates the involvement of HPV in female fertility and pregnancy complications. Yet, the mechanisms by which HPV infection contributes to subfertility and poor pregnancy outcomes are poorly understood.

The endometrium is a dynamic immunological organ that undergoes cyclical changes and immune regulation during the window of implantation (WOI). Embryo implantation is dependent upon the balance between pro-inflammatory and anti-inflammatory factors. Pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) aid in early implantation events, while anti-inflammatory cytokines such as interleukin-10 (IL-10) play a role in immune tolerance and pregnancy maintenance. Alterations in immune balance has been shown to increase the risk of implantation failure, recurrent pregnancy loss and other pregnancy complications. HPV infection has been shown to activate pathways of the innate immune system, such as nuclear factor kappa-B (NF-κB), which in turn promote pro-inflammatory cytokines. Simultaneously, HPV-induced cellular stress has been associated with inhibition of antioxidant pathways, such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, leading to oxidative stress. This is demonstrated by

https://doi.org/10.63964/atmj.2026.3.1

increased malondialdehyde (MDA) levels in the endometrium, and decreased activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH). The build-up of oxidative stress may disrupt steroidogenic enzyme function, resulting in lower progesterone and estradiol levels, crucial for endometrial receptivity and embryo implantation. Natural killer (NK) cells are the major immune cells in the mid-secretory phase endometrium and have key roles in the regulation of trophoblast invasion and placental vascular development. Immune dysregulation of NK cell numbers and function has been linked to infertility. The HPV-induced immune alterations may affect peripheral immune profiles as well as immune profiles in the endometrium, creating an unfavorable milieu for successful implantation and pregnancy. Crucially, recent research suggests different HPV genotypes may have differing immunomodulatory effects, which may contribute to the variability in clinical outcomes among infected women. HPV genotype distribution in the Middle East, including Iraq, appears to be different from other populations, with various reports of a wide variety of high-risk HPV types other than HPV-16 and HPV-18. However, the link between HPV infection, immune dysfunction, oxidative stress and pregnancy outcomes is not well studied in women from Iraq.

As such, this study sought to explore the link between HPV infection and systemic immune, oxidative stress and hormonal factors, and the role of these factors in adverse pregnancy outcomes in women of child-bearing age at a teaching hospital in Iraq. Through a multifaceted approach incorporating immunological, biochemical and clinical markers, this study aims to shed light on the pathways by which HPV infection affects pregnancy outcomes.

2-MATERIALS AND METHODS

Study Protocol and Ethics

This was a comparative cross-sectional study, conducted from January to June 2024 at a teaching hospital in Salah al-Din Governorate, Iraq. The Research Ethics Committee at the University of Samarra granted approval (Approval No. EC-2023/115) in line with the Declaration of Helsinki. All participants were asked for written informed consent.

Participants and Groups

One hundred and 20 women of child bearing age (18-40 years) were enrolled and divided into four groups of 30 women each. Group 1 was a healthy control group of HPV-negative women with no pregnancy complications. Group 2 was comprised of HPV-positive women without a history of pregnancy complications. Group 3 was comprised of HPV-negative women with adverse pregnancy outcomes. The fourth group (group 4) consisted of HPV-positive women with adverse pregnancy outcomes. An adverse pregnancy outcome was defined as spontaneous abortion before 20 weeks' gestation and/or a record of implantation failure (women attempting natural and/or assisted conception) as determined by clinical review of obstetric records. Women were matched by age (±5 years) and body mass index (BMI) and statistically adjusted for smoking, parity and contraception use.

Eligible Subjects

Women with reproductive histories aged 18-40 years were included. Exclusions included: any form of cancer, confirmed autoimmune diseases, current or recent use of immunosuppressive medication, antioxidant supplements in the last three months, current use of hormonal contraception, presence of non-HPV sexually transmitted infections and severe malnutrition (screened for by abnormalities in serum albumin and vitamin D). This was done to reduce the risk of immunological confounders and to ensure the results were specific to the effects of HPV.

2.4 HPV Diagnosis and Genotyping

Cervical swabs were tested for the presence of HPV by real-time polymerase chain reaction (PCR) using the Abbott RealTime High Risk HPV assay (Abbott Molecular, USA), which identifies high-risk HPV types 16, 18 and 12 other types. Both interpretation of results and assignment of genotypes were as per the manufacturer's instructions and reviewed by a clinical virologist. We recorded the prevalence of each genotype but did not stratify this in this analysis due to sample size limitations; we discuss this in the Discussion.

Collection and Processing

Venous blood samples (5 mL) were obtained after an overnight fast, between days 20-22 of the menstrual cycle (mid-luteal phase) to control for the hormonal status of women. The blood was spun at 3,000 rpm for 15 minutes at

4°C and the serum was then aliquoted and stored at -20°C. Biochemical analyses were performed within six months to avoid artifacts.

Biochemical Assays

Serum levels of IL-6, IL-10, TNF-α and IFN-γ were measured by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, USA) with intra- and inter-assay variations of less than 8% and 12%, respectively. The numbers of peripheral blood NK (PB-NK) cells were measured by flow cytometry as the proportion of CD56+CD16+ lymphocytes in the total peripheral blood mononuclear cells using fluorochrome-conjugated monoclonal antibodies (BD Biosciences, USA). This blood measurement is a reflection of, but not a direct measure of, uterine NK cells. Lipid peroxidation (MDA) was measured by thiobarbituric acid reactive substances (TBARS) (Cayman Chemical, USA). SOD activity was measured by a colorimetric inhibition assay (Sigma-Aldrich, USA) and GSH was measured by the Ellman's reagent (DTNB) method. Progesterone and estradiol serum levels were determined by electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas e411. Endometrial thickness was measured by transvaginal ultrasound on day 21 of the menstrual cycle by the same well-experienced ultrasonographer to avoid inter-observer variability.

Sample Size Determination

The sample size was determined using the G*Power software (version 3.1.9.7) for one-way analysis of variance (ANOVA), effect size f=0.80, 1-β error probability (power) of 0.80 and an α of 0.05, a sample size of 28 per group was required. Participants were recruited in a larger number to account for possible drop-outs (30 in each group, for a total of 120 participants).

Statistical Analysis

The Shapiro-Wilk test was used to test for normal distribution. One-way ANOVA with Duncan's post-hoc test was used for variables with normal distribution; the Kruskal-Wallis test with Mann-Whitney U post-hoc test for those with non-normal distribution. Pearson's coefficient or Spearman's rho was used for correlation analyses. Binary logistic regression was used to assess independent associations with adverse pregnancy outcomes, and to calculate the odds ratios (OR) and 95% confidence intervals (CI). Hosmer-Lemeshow test was used to evaluate the model. Receiver operating characteristic (ROC) curve analysis was used to determine the best diagnostic cut-off values for certain biomarkers, and reported the area under the curve (AUC), sensitivity and specificity. Eta-squared (η^2) was used to report the effect size. p values were expressed exactly; a p value <0.05 was considered significant. Data were analysed with IBM SPSS v26.

3-RESULTS

Demographic data of the groups are shown in Table 1. There was no significant difference in age (p=0.721), body mass index (p=0.634), parity (p=0.812) and smoking (p=0.876) status between the groups suggesting that the groups were similar.

Table 1. Demographic characteristics of groups (Mean ± SD).

Parameter	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	Group 4 (n=30)	*p-value*
Age (years)	28.3 ± 4.2	29.1 ± 3.8	30.2 ± 4.5	29.8 ± 4.1	0.721
BMI (kg/m²)	23.4 ± 2.1	24.1 ± 2.3	23.8 ± 2.5	24.3 ± 2.2	0.634
Parity	1.2 ± 0.8	1.4 ± 0.9	1.3 ± 0.7	1.5 ± 0.8	0.812
Smoking (%)	10.0	13.3	13.3	16.7	0.876

Values expressed as Mean ± SD or percentage (%). No significant intergroup differences (p>0.05). BMI = body mass index.

Serum immunological markers are given in Table 2. The greatest disturbance was in Group 4. IL-6 was significantly elevated in Group 4 (12.6±2.1 pg/mL) compared with Group 1 (3.2±0.8 pg/mL), Group 2 (6.8±1.2 pg/mL), and Group 3 (7.4±1.5 pg/mL) ($p<0.001$, $\eta^2=0.48$). This finding is in accord with the NF-κB cascade of endometrial stromal cells being activated by HPV, leading to a positive feedback loop of IL-6 transcription [7]. TNF-α was also significantly elevated in Group 4 (15.8±2.6 pg/mL vs 4.1±1.1 pg/mL in controls; $p<0.001$, $\eta^2=0.51$);

high levels of TNF-α induce trophoblast differentiation and apoptosis in extravillous trophoblasts [14]. IFN-γ was also highest in Group 4 (17.2±3.1 pg/mL; $p<0.001$, $\eta^2=0.46$), reflecting a Th1-polarized immune response which is not tolerogenic enough to support pregnancy [15].

In contrast, IL-10 was significantly reduced in Group 4 (7.2±1.9 pg/mL versus 18.4±3.2 pg/mL in controls; $p<0.001$, $\eta^2=0.42$). PB-NK cell percentage was likewise reduced in Group 4 (6.4±1.6%) compared with controls (14.2±2.8%; $p=0.003$, $\eta^2=0.28$). It is important to note that PB-NK cells represent the systemic status of NK cells; uterine NK cells can only be characterized from a biopsy of the endometrium, which was not possible in the study.

Table 2. Serum markers in the various groups (Mean ± SD).

Marker (Unit)	Group 1	Group 2	Group 3	Group 4	p-value	η^2
IL-6 (pg/mL)	3.2 ± 0.8	6.8 ± 1.2*	7.4 ± 1.5*	12.6 ± 2.1*†‡	<0.001	0.48
IL-10 (pg/mL)	18.4 ± 3.2	12.6 ± 2.8*	11.8 ± 2.4*	7.2 ± 1.9*†‡	<0.001	0.42
TNF-α (pg/mL)	4.1 ± 1.1	8.3 ± 1.8*	9.2 ± 2.1*	15.8 ± 2.6*†‡	<0.001	0.51
IFN-γ (pg/mL)	5.6 ± 1.4	9.8 ± 2.2*	10.4 ± 2.3*	17.2 ± 3.1*†‡	<0.001	0.46
PB-NK cells (%)	14.2 ± 2.8	10.8 ± 2.4*	9.6 ± 2.1*	6.4 ± 1.6*†‡	0.003	0.28

* $p<0.05$ vs. Group 1; † $p<0.05$ vs. Group 2; ‡ $p<0.05$ vs. Group 3 (Duncan post-hoc test). IL-6=interleukin-6; IL-10=interleukin-10; TNF-α=tumor necrosis factor-alpha; IFN-γ=interferon-gamma; PB-NK=peripheral blood natural killer cells; η^2=eta-squared.

Table 3 displays the parameters of oxidative stress and hormones. MDA was highest in Group 4 (6.2±1.2 nmol/mL; $p<0.001$, $\eta^2=0.52$), while SOD (18.6±4.2 U/mL) and GSH (312±64 μmol/L) were lowest in this group ($p<0.001$ for both). The antioxidant depletion profile is consistent with inhibited activation of Nrf2 by HPV oncoproteins E6/E7 [8] which prevents activation of Nrf2 and down regulates the expression levels of antioxidant genes, making the cells susceptible to oxidative damage. Progesterone was significantly lower in Group 4 (6.4±1.4 ng/mL) in comparison to controls (14.8±2.4 ng/mL; $p<0.001$, $\eta^2=0.41$), consistent with the ROS-induced decrease in 3β-hydroxysteroid dehydrogenase (3β-HSD) activity [9]. Estradiol was also significantly decreased in Group 4 (102±21 pg/mL; $p=0.002$, $\eta^2=0.22$) but the effect size was moderate, suggesting that estrogen level is less affected than progesterone level. This group had significantly thinner endometria (5.6±0.8 mm; $p<0.001$, $\eta^2=0.43$). The miscarriage rate in Group 4 was 73.3% vs. 46.7% in Group 3 (no miscarriages in Groups 1 and 2).

Table 3. Hormone level, oxidative stress and pregnancy outcomes in the groups (Mean ± SD).

Parameter (Unit)	Group 1	Group 2	Group 3	Group 4	p-value	η^2
MDA (nmol/mL)	1.8 ± 0.4	3.4 ± 0.7*	3.8 ± 0.8*	6.2 ± 1.2*†‡	<0.001	0.52
SOD (U/mL)	48.6 ± 6.2	32.4 ± 5.8*	29.8 ± 5.4*	18.6 ± 4.2*†‡	<0.001	0.47
GSH (μmol/L)	892 ± 124	623 ± 98*	584 ± 87*	312 ± 64*†‡	<0.001	0.44
Progesterone (ng/mL)	14.8 ± 2.4	10.2 ± 2.1*	9.6 ± 1.8*	6.4 ± 1.4*†‡	<0.001	0.41
Estradiol (pg/mL)	186 ± 32	148 ± 31*	142 ± 29*	102 ± 21*†‡	0.002	0.22
Endometrial Thickness (mm)	9.8 ± 1.2	7.4 ± 1.1*	7.1 ± 0.9*	5.6 ± 0.8*†‡	<0.001	0.43
Miscarriage Rate (%)	0	0	46.7	73.3*†‡	<0.001	—

* $p<0.05$ vs. Group 1; † $p<0.05$ vs. Group 2; ‡ $p<0.05$ vs. Group 3. MDA=malondialdehyde; SOD=superoxide dismutase; GSH=glutathione; η^2=eta-squared.

Correlation matrix for Group 4 is shown in Table 4. IL-6 was positively correlated with TNF-α ($r=+0.72$, $p=0.001$) and negatively correlated with progesterone ($r=-0.68$, $p=0.002$), supporting the notion of inflammatory co-amplification and its link to progesterone suppression. TNF-α showed a positive correlation with MDA ($r=+0.74$, $p=0.001$) and MDA was strongly inversely correlated with SOD ($r=-0.79$, $p<0.001$). IL-10 was inversely correlated with rate of miscarriage ($r=-0.65$, $p=0.003$) and progesterone was positively correlated with endometrial thickness ($r=+0.71$, $p=0.001$). Interestingly, IL-6 and IFN-γ had lower and non-significant correlations with PB-NK cells ($r=-0.38$, $p=0.087$) and GSH ($r=-0.29$, $p=0.128$), respectively, reflecting the complexities of these interactions in a relatively small sample size.

Table 4. Correlation matrix for main parameters in Group 4 (HPV positive with adverse outcomes).

Variable 1	Variable 2	r value	Direction	*p-value*
IL-6	TNF-α	+0.72	Positive	0.001
IL-6	Progesterone	−0.68	Negative	0.002
TNF-α	MDA	+0.74	Positive	0.001
MDA	SOD	−0.79	Negative	<0.001
IL-10	Miscarriage Rate	−0.65	Negative	0.003
Progesterone	Endometrial Thickness	+0.71	Positive	0.001
IL-6	PB-NK cells (%)	−0.38	Negative	0.087 (NS)
IFN-γ	GSH	−0.29	Negative	0.128 (NS)

Significant correlations: Pearson or Spearman rho as appropriate. NS = non-significant. Grey italic values indicate p>0.05.

Binary logistic regression analysis was done to determine independent predictors of adverse pregnancy outcomes which were Groups 3 and 4 combined versus Groups 1 and 2. Some biomarkers were found to be significantly associated with the outcome, after the possible confounding factors such as age and body mass index (BMI) were removed. Inflammatory and oxidative stress markers became robust and independent positive predictors. Specifically, malondialdehyde (MDA) showed the highest predictive value (OR = 3.06, 95% CI: 1.68–5.58, p < 0.001), followed by tumor necrosis factor-alpha (TNF-α) (OR = 2.12, 95% CI: 1.41–3.18, p = 0.001) and interleukin-6 (IL-6) (OR = 1.84, 95% CI: 1.23–2.76, p = 0.003). Conversely, there were a number of variables that were found to be independent protective factors. Higher progesterone levels (OR = 0.61, 95% CI: 0.42–0.89, p = 0.009), peripheral blood natural killer (PB-NK) cell percentage (OR = 0.74, 95% CI: 0.58–0.94, p = 0.014), and endometrial thickness (OR = 0.52, 95% CI: 0.34–0.79, p = 0.002) were all significantly associated with reduced odds of adverse outcomes. In general, the regression equation performed well, with a suitable calibration (HosmerLemeshow test = 0.412), a high amount of explanatory power (Nagelkerke R 2 = 0.68), and the overall proportion of accurate classification of 81.7%.

Table 5. Binary logistic regression model that presents independent predictors of poor pregnancy outcomes.

Predictor Variable	β Coefficient	OR	95% CI	*p-value*
IL-6 (pg/mL)	+0.612	1.84	1.23–2.76	0.003
TNF-α (pg/mL)	+0.751	2.12	1.41–3.18	0.001
MDA (nmol/mL)	+1.118	3.06	1.68–5.58	<0.001
Progesterone (ng/mL)	−0.494	0.61	0.42–0.89	0.009
PB-NK cells (%)	−0.301	0.74	0.58–0.94	0.014
Endometrial Thickness (mm)	−0.654	0.52	0.34–0.79	0.002

Outcome variable: adverse pregnancy outcome (yes=1). Model adjusted for age and BMI. OR = odds ratio; CI = confidence interval; Nagelkerke R^2 = 0.68; Hosmer-Lemeshow p = 0.412; Overall accuracy = 81.7%.

Table 6 presents the ROC curve analysis for the strongest predictive biomarkers. MDA demonstrated the highest single-marker diagnostic performance (AUC=0.891, 95% CI: 0.826–0.956), with an optimal cutoff of ≥4.2 nmol/mL yielding 83.3% sensitivity and 88.3% specificity. TNF-α (AUC=0.863) and IL-6 (AUC=0.847) also showed strong discriminatory capacity. The combined biomarker model achieved an AUC of 0.934 (95% CI: 0.882–0.986) with 88.3% sensitivity and 90.0% specificity, representing a significant improvement over individual markers in distinguishing women at high risk of adverse pregnancy outcomes.

Figure 1 presents a schematic diagram of the suggested mechanistic pathway between HPV infection and adverse pregnancy outcomes to interconnect the immunological, oxidative stress, and hormonal changes found in the current research. After infection with HPV genotypes that are considered as high-risk, the viral oncoprotein E6 and E7 are also believed to trigger inflammatory signaling pathways, especially NF- - B, which result in the up-regulation of pro-inflammatory cytokines (IL-6, TNF- -, and IFN- -) and a concomitant down-regulation of Such a change favors a Th1-based immune reaction and defective maternal immune tolerance. At the same time, cellular stress associated with HPV is also connected with the blockage of the Nrf2 antioxidant system that leads to the increased levels of lipid peroxidation (MDA) and loss of the main antioxidant defense (SOD and GSH).

Table 6. ROC curve analysis: diagnostic performance of selected biomarkers for adverse pregnancy outcomes.

Biomarker	AUC	95% CI	Cutoff	Sens. (%)	Spec. (%)	*p-value*
IL-6 (pg/mL)	0.847	≥0.771–0.923	≥8.4 pg/mL	78.3	86.7	<0.001
TNF-α (pg/mL)	0.863	0.792–0.934	≥10.6 pg/mL	81.7	83.3	<0.001
MDA (nmol/mL)	0.891	0.826–0.956	≥4.2 nmol/mL	83.3	88.3	<0.001
Progesterone (ng/mL)	0.824	0.744–0.904	≤8.8 ng/mL	76.7	80.0	<0.001
Combined Model	0.934	0.882–0.986	—	88.3	90.0	<0.001

AUC = area under the curve; Sens. = sensitivity; Spec. = specificity. Combined model incorporates MDA + TNF-α + IL-6 + progesterone. All AUCs significantly different from 0.5 (p<0.001).

This oxidative imbalance plays a role in dysfunction of the cells in the reproductive environment. Simultaneously, viral action and oxidative stress are linked to the suppression of steroidogenic enzymes (especially 3-hydroxysteroid dehydrogenase (3-HSD)) that cause the decreased levels of progesterone and estradiol. Such hormonal imbalances affect the development and functioning of the endometrion. All these mechanisms combined result in a decrease of endometrial receptivity, which is identified as a reduction in endometrial thickness, defective decidualization, and poor trophoblast invasion. The net result of such changes is the ultimate manifestation of negative pregnancy outcomes such as miscarriage, implantation failure and fetal growth retardation.

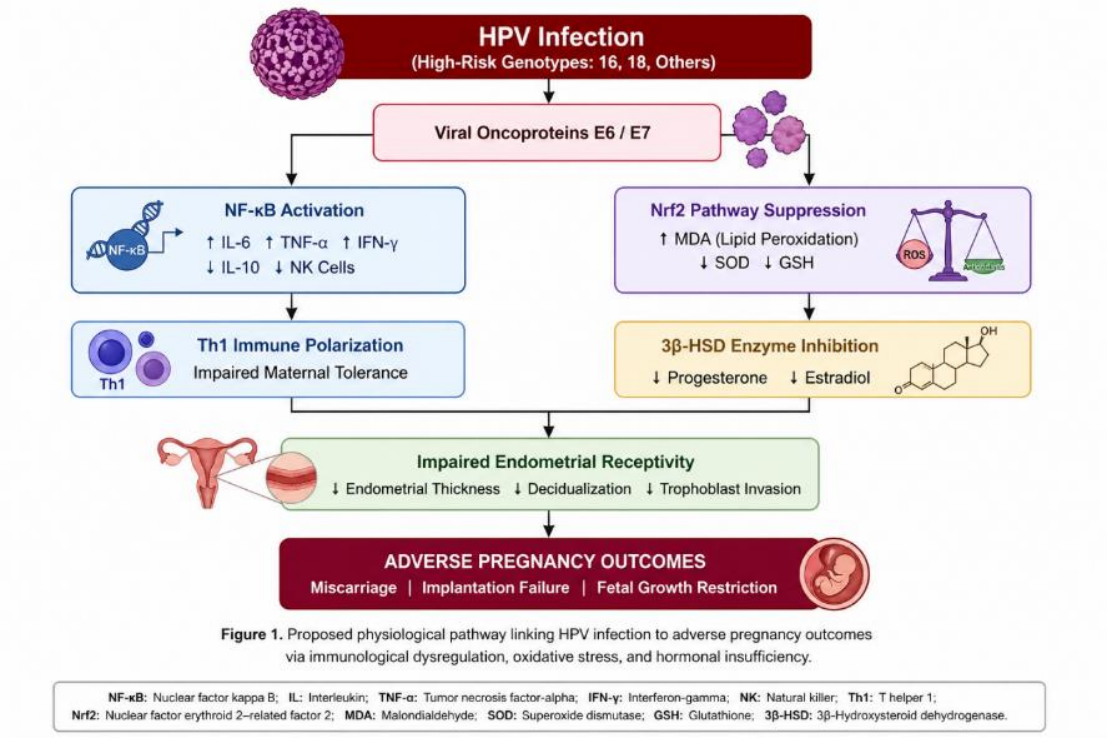

Figure 1. Proposed physiological pathway linking HPV infection to adverse pregnancy outcomes via immunological dysregulation, oxidative stress, and hormonal insufficiency.

عنوان الشكل اين

4. DISCUSSION

This current research study has shown that HPV infection, coupled with adverse pregnancy outcome is correlated with the most radical systemic immunological and oxidative disruption to be seen among the groups of the research study. Group 4 was characterized by a clear pro-inflammatory profile of high IL-6, TNF-al, IFN-g, low IL-10 and PB-NK cells, high oxidative stress and hormonal inadequacy. These logistic regression and ROC analyses further determined that MDA, TNF- and IL-6 are independent and diagnostically useful markers of adverse pregnancy outcomes that give clinical translational importance, as opposed to descriptive group comparisons.

The significant increase in IL-6 in HPV-positive women in adverse outcome is consistent with the results of Chen et al. [2] who found an increased endometrial IL-6 in HPV-seropositive women with recurrent implantation failure. Oncoproteins E6 and E7 of HPV cause NF-/B signaling by degrading tumor suppressor p53 and retinoblastoma protein (pRb), and result in persistent transcriptional stimulation of pro-inflammatory cytokines [7]. This correlates with the cytokine network model that is suggested by Fichorova and Anderson [4]. The simultaneous TNF-a increase (15.8±2.6 pg/mL in Group 4) is in line with the data of Martinez-Perez et al. [3], which found TNF-a as an independent predictor of spontaneous abortion in HPV-infected cohorts, which is also supported by the current logistic regression outcomes (OR=2.12, p=0.

Remarkably, PB-NK cell depletion in Group 4 was moderately, but non-significantly correlated with IL-6 ($r=-0.38$, $p=0.087$). This less-than-optimal association could be due to the indirectness of the relationship between peripheral blood NK proportions and the endometrial NK cell milieu because the PB-NK cells and uterine NK cells are functionally different subpopulations [11]. This observation supports the explanation that the PB-NK cell assays are a systemic proxy, and not a direct measure of endometrial immune competence, and future research that includes uterine NK cell characterization via endometrial biopsy would offer more mechanistic clarity [17].

The presence of the oxidative stress profile with the highest level of MDA and lowest level of SOD and GSH in Group 4 is a support of the hypothesis of Nrf2 pathway inhibition by HPV oncoproteins. The most powerful diagnostic marker (AUC=0.891, cutoff ≥4.2 nmol/mL) was MDA which suggests the possibility of lipid peroxidation being a highly sensitive systemic marker of HPV-related reproductive vulnerability. These results build on those of Liu et al. [18], who have found that in HPV-positive women, greater systemic oxidative alterations in the cervical region correlate with unfavorable gestational outcomes, and have diagnostic value independent of other factors.

Group 4 (6.4±1.4 ng/mL) progesterone deficiency has direct clinical consequences of supporting luteal phase, and the ROS-mediated inhibition of 3β-HSD is likely to be one of the underlying processes [9]. This hormonal insufficiency or lack of hormones is an independent risk modifier in logistic regression (OR=0.61, p=0.009), which is not dependent on cytokine or oxidative markers. Surprisingly, the moderate effect size of estradiol decrease ($\eta^2 = 0.22$) compared with the large effect sizes of progesterone ($^2 = 0.41$) indicating that the two steroidogenic pathways may be sensitive in different ways to HPV-related oxidative damage. The existence of this differential vulnerability is a topic that should be investigated. Among the surprises was that there were no statistically significant differences in a number of immunological parameters, between Group 2 (HPV-positive without complications) and Group 3 (adverse outcomes, HPV-negative). This implies that there is pathological transition dependent on a threshold of HPV infection and adverse-outcome-associated immune activation in which neither one alone causes any disruption to reproduce the combined phenotype of Group 4. This finding is in line with the results of Al-Rubaye et al. [20] who found that threshold-related oxidative damage in Iraqi women with repeated miscarriage and this observation provides biological plausibility to the combined HPV-immune vulnerability model.

The originality of the given study is that it combines immunological, oxidative, hormonal, and clinical outcome measures with multivariate and ROC analyses in an Iraqi cohort of reproductive age individuals, which, to our best knowledge, is the first study to simultaneously perform biomarker profiling of such a population with these tools. There are a number of limitations which should be mentioned. The cross-sectional study does not allow making causal inferences; the observed associations should be viewed with skepticism. The status of NK in the uterus is not directly involved with the measurement of PB-NK cells. Genotype-specific immune responses were not looked in to, although HPV 16 and 18 have been shown to stimulate inflammatory responses differently [22]. The confounders such as the vitamin D status, stress biomarkers, and subclinical infections were controlled partially by use of exclusion criteria but not quantified. Mechanistic inferences would be greatly reinforced by future longitudinal research involving endometrial biopsy and stratifying the HPV genotype.

5. CONCLUSION

The systemic immunological dysregulation, oxidative stress and hormonal insufficiency are all characteristic of the HPV-positive women with poor pregnancy outcomes. Independent predictors of adverse outcomes were found to be MDA, TNF-alpha and IL-6 and a biomarker panel combined with these three factors had an AUC of 0.934. These results provide a clinically practicable paradigm: combination of HPV screening with

the measurement of MDA, TNF-alpha, and progesterone levels during a preconception assessment can facilitate the earlier detection of women at the high risk of pregnancy failure and be used to implement specific interventions like antioxidant supplementation and luteal phase support.

REFERENCES

[1] Serrano Collado, E, Autocorrección interactiva para la enseñanza y aprendizaje de la programación, (ed.), accessed http://dx.doi.org/10.30827/digibug.84117.

[2] Li, F, Gao, W, Li, Y, Wang, Y, Liu, L & Zhang, X 2023, "Potential Biomarkers and Endometrial Immune Microenvironment in Recurrent Implantation Failure," (ed.), Biomolecules, vol. 13, no. 3, p. 406, accessed http://dx.doi.org/10.3390/biom13030406.

[3] Quinlivan, J 2002, "The impact of continuing illegal drug use on teenage pregnancy outcomes—a prospective cohort study," (ed.), BJOG: An International Journal of Obstetrics and Gynaecology, vol. 109, no. 10, pp. 1148–1153, accessed http://dx.doi.org/10.1016/s1470-0328(02)01936-5.

[4Seyed, N & Rafati, S 2021, "Th1 concomitant immune response mediated by IFN-γ protects against sand fly delivered Leishmania infection: Implications for vaccine design," (ed.), Cytokine, vol. 147, p. 155247, accessed http://dx.doi.org/10.1016/j.cyto.2020.155247.

[5] Foresta, C, Bertoldo, A, Garolla, A, Pizzol, D, Mason, S, Lenzi, A & De Toni, L 2013, "Human papillomavirus proteins are found in peripheral blood and semen Cd20+ and Cd56+ cells during Hpv-16 semen infection," (ed.), BMC Infectious Diseases, vol. 13, no. 1, accessed http://dx.doi.org/10.1186/1471-2334-13-593.

[6] Kwon, S-K, Chae, H-D, Lee, K-H, Kim, S-H, Kim, C-H & Kang, B-M 2014, "Causes of amenorrhea in Korea: Experience of a single large center," (ed.), Clinical and Experimental Reproductive Medicine, vol. 41, no. 1, p. 29, accessed http://dx.doi.org/10.5653/cerm.2014.41.1.29.

[7] Nees, M, Geoghegan, JM, Hyman, T, Frank, S, Miller, L & Woodworth, CD 2001, "Papillomavirus Type 16 Oncogenes Downregulate Expression of Interferon-Responsive Genes and Upregulate Proliferation-Associated and NF-κB-Responsive Genes in Cervical Keratinocytes," (ed.), Journal of Virology, vol. 75, no. 9, pp. 4283–4296, accessed http://dx.doi.org/10.1128/jvi.75.9.4283-4296.2001.

[8] Liu, B, Yi, J, Yang, X, Liu, L, Lou, X, Zhang, Z, Qi, H, Wang, Z, Zou, J, Zhu, W-G, Gu, W & Luo, J 2018, "MDM2-mediated degradation of WRN promotes cellular senescence in a p53-independent manner," (ed.), Oncogene, vol. 38, no. 14, pp. 2501–2515, accessed http://dx.doi.org/10.1038/s41388-018-0605-5.

[9] Liu, B, Yi, J, Yang, X, Liu, L, Lou, X, Zhang, Z, Qi, H, Wang, Z, Zou, J, Zhu, W-G, Gu, W & Luo, J 2018, "MDM2-mediated degradation of WRN promotes cellular senescence in a p53-independent manner," (ed.), Oncogene, vol. 38, no. 14, pp. 2501–2515, accessed http://dx.doi.org/10.1038/s41388-018-0605-5.

[10] Moffett, A & Shreeve, N 2015, "First do no harm: uterine natural killer (NK) cells in assisted reproduction," (ed.), Human Reproduction, vol. 30, no. 7, pp. 1519–1525, accessed http://dx.doi.org/10.1093/humrep/dev098.

[11] Strunz, B, Bister, J, Jönsson, H, Filipovic, I, Crona-Guterstam, Y, Kvedaraite, E, Sleiers, N, Dumitrescu, B, Brännström, M, Lentini, A, Reinius, B, Cornillet, M, Willinger, T, Gidlöf, S, Hamilton, RS, Ivarsson, MA & Björkström, NK 2021, "Continuous human uterine NK cell differentiation in response to endometrial regeneration and pregnancy," (ed.), Science Immunology, vol. 6, no. 56, accessed http://dx.doi.org/10.1126/sciimmunol.abb7800.

[12] Giannella, L, Mfuta, K, Setti, T, Cerami, LB, Bergamini, E & Boselli, F 2014, "A Risk-Scoring Model for the Prediction of Endometrial Cancer among Symptomatic Postmenopausal Women with Endometrial Thickness > 4 mm," (ed.), BioMed Research International, vol. 2014, pp. 1–7, accessed http://dx.doi.org/10.1155/2014/130569.

[13] Salih Ahmed Al-Aqidi & Hamid Salman Al-Mahdawi 2022, "Concentrations of lead in the dust of some fuel stations in Salah al-Din Governorate," (ed.), Tikrit Journal of Pure Science, vol. 26, no. 1, pp. 12–17, accessed http://dx.doi.org/10.25130/tjps.v26i1.111.

[14] Brosens, I, Muter, J, Gargett, CE, Puttemans, P, Benagiano, G & Brosens, JJ 2017, "The impact of uterine immaturity on obstetrical syndromes during adolescence," (ed.), American Journal of Obstetrics and Gynecology, vol. 217, no. 5, pp. 546–555, accessed http://dx.doi.org/10.1016/j.ajog.2017.05.059.

[15] Saito, S, Nakashima, A, Shima, T & Ito, M 2010, "REVIEW ARTICLE: Th1/Th2/Th17 and Regulatory T-Cell Paradigm in Pregnancy," (ed.), American Journal of Reproductive Immunology, vol. 63, no. 6, pp. 601–610, accessed http://dx.doi.org/10.1111/j.1600-0897.2010.00852.x.

[16] Nayak, NR & Giudice, LC 2003, "Comparative Biology of the IGF System in Endometrium, Decidua, and Placenta, and Clinical Implications for Foetal Growth and Implantation Disorders," (ed.), Placenta, vol. 24, no. 4, pp. 281–296, accessed http://dx.doi.org/10.1053/plac.2002.0906.

[17] (ed.) 1995, "Expression of transforming growth factor-β1 and-β2 in human papillomavirus (HPV)-associated lesions of the uterine cervix," International Journal of Gynecology & Obstetrics, vol. 49, no. 3, pp. 366–366, accessed http://dx.doi.org/10.1016/0020-7292(95)99183-h.

[18] Wang, H, Liu, Y, Chen, H, Wei, S & Gao, H 2024, Combined hesperetin and cisplatin synergistically inhibit lung cancer associated with Nrf2 signaling pathways and oxidative stress, (ed.), accessed http://dx.doi.org/10.22541/au.172492377.73958575/v1.

[19] Kasius, A, Smit, JG, Torrance, HL, Eijkemans, MJC, Mol, BW, Opmeer, BC & Broekmans, FJM 2014, "Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis," (ed.), Human Reproduction Update, vol. 20, no. 4, pp. 530–541, accessed http://dx.doi.org/10.1093/humupd/dmu011.

[20] Ghneim, HK & Alshebly, MM 2016, "Biochemical Markers of Oxidative Stress in Saudi Women with Recurrent Miscarriage," (ed.), Journal of Korean Medical Science, vol. 31, no. 1, p. 98, accessed http://dx.doi.org/10.3346/jkms.2016.31.1.98.

[21] Hassan, MS 2019, "Anatomical and Morphological study of the Heart's Chambers and valves in Iraqi ducks Anser platyrhuncha," (ed.), The Iraqi Journal of Veterinary Medicine, vol. 43, no. 1, pp. 21–25, accessed http://dx.doi.org/10.30539/iraqijvm.v43i1.466.

[22] Peng, G, Yan, Z, Liu, Y, Li, J, Ma, J, Tong, N & Wang, Y 2023, "The effects of first-line pharmacological treatments for reproductive outcomes in infertile women with PCOS: a systematic review and network meta-analysis," (ed.), Reproductive Biology and Endocrinology, vol. 21, no. 1, accessed http://dx.doi.org/10.1186/s12958-023-01075-9.

[23] Hosmer, DW, Lemeshow, S & Sturdivant, RX 2013, "Applied Logistic Regression," (ed.), Wiley Series in Probability and Statistics, accessed http://dx.doi.org/10.1002/9781118548387.

[24] DeLong, ER, DeLong, DM & Clarke-Pearson, DL 1988, "Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach," (ed.), Biometrics, vol. 44, no. 3, p. 837, accessed http://dx.doi.org/10.2307/2531595.