Research Article

Polymorphism specific Allele Frequencies on Cervical Cancer

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Abstract

Objective: To evaluate the correlation of allele frequencies of IL-6 polymorphisms between healthy women and cervical cancer patients.

Method: The study involved gynecologists diagnosing abnormal cervical tissue in 100 women aged 17-60. Each woman provided two tissue samples: one for pap smear analysis and one for genetic research, along with a blood sample for IL-6 polymorphism analysis. Traditional polymerase chain reaction (PCR) was used for genetic analysis to confirm diagnoses. Allele-specific PCR (AS-PCR) was utilized to identify allelic polymorphisms. Pap smears identified cervical intraepithelial neoplasia (CIN) II and III, characterized by dysplastic cells and mitotic figures.

Results: The diagnostic PCR data demonstrated that 36% of participants were HPV-infected, with the greatest infection rates (50%). The AS-PCR reported that the IL-6 (rs1800795) gene detected at 174 G/C position was presented with GG, GC, and CC genotypes.

Conclusion: The result showed a significant alteration in the IL-6 (rs1800795) gene, strongly correlating with cervical cancer based on human papillomavirus infection.

Keywords: cervix cancer, human papillomavirus, interleukin-6 polymorphism, genetic analysis.

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INTRODUCTION

Papillomaviruses are a large family of viruses that can infect mammals, birds, and reptiles, and they are known to infect various species and tissues in diverse manners. One of the most common sexually transmitted infections (STIs) affecting both men and women is HPV, which infects stratified squamous epithelia. [1] HPV can cause warts and other benign proliferative lesions, with a smaller subset of viruses capable of inducing premalignant and malignant abnormalities. The vaginal area is particularly susceptible to the development of malignant diseases, the most prevalent being cervical cancer.[2]

The initiation of viral genome replication defines the early and late stages of the HPV life cycle, respectively. Additionally, epithelial cell differentiation correlates with the transition from early to late stages in the HPV life cycle. The initial stages involve tropism and entry. Receptor-mediated endocytosis allows HPV virus particles to enter cells after attachment to the extracellular matrix and binding to heparan sulfate proteoglycan receptors.[3] Once inside the cytoplasm, the virus's capsid becomes embedded in endosomes. Acidification of the endosomes (low pH) triggers the release of the L2 capsid protein and subsequent rupture of the endosomal membranes.[4] Each phase of viral genome replication—establishment, maintenance, and amplification—is intricately linked to specific levels of cell differentiation.[5,6].

Human papillomavirus (HPV) is a sexually transmitted virus that primarily affects females but can also infect males. There are over a hundred different types of HPV, but only high-risk (HR-HPV) types are associated with the

progression of cervical intraepithelial neoplasia (CIN) to cancer. However, there are also other types of HPV that cause genital warts, known as low-risk types. HPV-16 infections are responsible for 85-95% of cervical cancer cases. [7-9]

The cytokine interleukin-6 (IL-6) may have a role in the development of cervical cancer. Cervical cancer growth is stimulated by IL-6 through vascular endothelial growth factor (VEGF)-dependent angiogenesis mediated by the signal transducer and activator of transcription 3 (STAT3) pathway.[10,11] Single-nucleotide polymorphisms (SNPs) are only one example of the many potential influences on gene expression. [12, 13] This study aimed to analyze the distribution of allele frequencies of IL-6 polymorphisms in both healthy women and patients with cervical cancer attributed to human papillomavirus (HPV).

METHODS

Patients and samples

The present research used a cross-sectional design and collected data from participants between January 2022 and 2023 to identify HPV, and a case-control study design to identify IL-6 polymorphisms. In this study, gynecologists at Al-Diwaniyah Hospital for Women and Children's Cervix Department and at private clinics in Al-Diwaniyah City, Iraq, detected cervical tissue anomalies in 100 women aged 17 to 60 years old. Women who met the study's criteria were approached for participation and given the opportunity to provide informed permission. Patients' names, ages, menstrual cycles, pregnancies, surgical procedures (hysterectomy, cone biopsy, oophorectomy), treatments (hormonal, radiation, or chemotherapy), and histories of abnormal cervical cytology and other conditions were documented.

Samples were collected from women with normal menstrual cycles during the mid-cycle phase. However, samples from other women were collected at various stages of their menstrual cycles. Participants were instructed to refrain from using tampons, vaginal foams, jellies, or any vaginal creams or medications before sampling. Sampling was not allowed within 48 hours after intercourse. Specimens were collected following protocols established by a pathologist or trained technician, using a sterile, single-use bivalve speculum. Each participant provided two tissue samples: one for pap smear analysis and genetic research, and blood samples were taken for IL-6 polymorphism detection.

Samples were taken from the transformation zone (ecto-endocervical junction) using a thin prep cytobrush that rotated a full 360 degrees for the Papanicolaou technique. The Cytobrushes were quickly placed into methanol-containing ThinPrep transport medium vials.

Pap Smear Test

Pap staining was used to prepare the samples for microscopy, and the slides were put under a microscope [14, 15]. Bethesda criteria were used for the cytological abnormality reporting [14,16, 17].

Molecular Techniques
Extraction of viral DNA

The viral DNA extraction was performed using a G-spinTM Total DNA Extraction Kit (iNtRON, Korea), following the manufacturer's recommended Body fluid protocol. The quantity and quality of the extracted DNA were assessed using a NanoDrop reader. The extracted DNA was stored frozen at -20 degrees Celsius for future use.

Extraction of Human Genomic DNA

The genomic DNA extraction from blood specimens was conducted using the gSYAN DNA Extraction Kit (Geneaid, USA), following the Frozen Blood protocol provided with the kit. The quantity and integrity of the extracted DNA were assessed using a NanoDrop spectrophotometer.

Diagnostic PCR

The primary capsid protein L1 gene served as a molecular marker in the PCR, conducted using the Maxime PCR PreMix Kit (iNtRON, Korea).[18] A total of 20 µl of PCR solution was prepared, comprising 5 µl of DNA (5-50 ng), 1 µl each of forward and reverse primers for the L1 gene (10 pmol/ each), and 13 µl of PCR-grade water. Additional components included DNA polymerase, dNTPs, and MgCl2 in the PCR reaction mixture. The thermocycler was programmed as follows: initial denaturation at 95ºC for 5 mins (1 cycle), followed by denaturation at 95ºC for 30s, annealing at 58ºC for 30s, and extension at 72ºC for 60s for 35 cycles, with a final extension step at 72ºC for 5 mins (1 cycle). The PCR products were analyzed

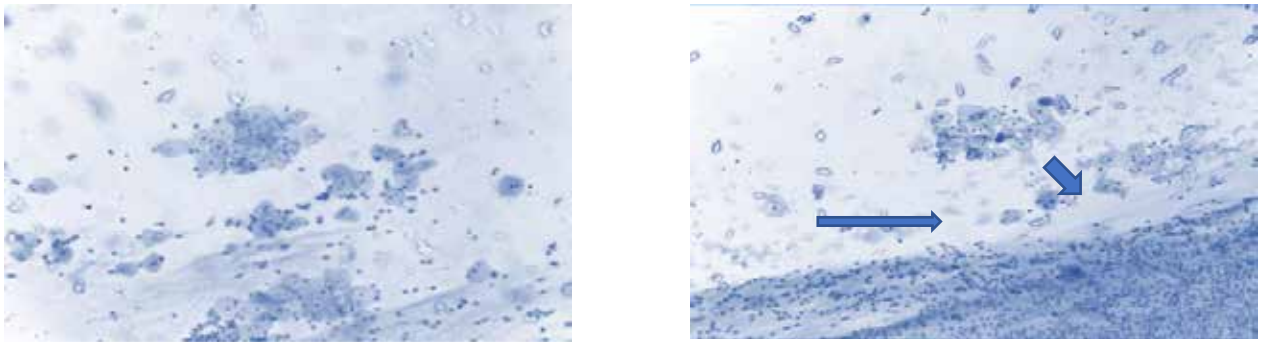
by agarose gel electrophoresis, and the bands were visualized under a UV transilluminator.

PCR of Allele Polymorphism

Patients with HPV infection and healthy controls were subjected to an AS-PCR technique to identify and genotype the IL-6 -174G>C (rs1800795) gene polymorphism. The procedure followed the outline given [8,19]. We used a (GoTaq® G2 Green Master Mix kit) to make the AS master mix, and then used this mix to run two separate reactions on each sample, as recommended by the manufacturer. Each reaction (25µl), 5µl DNA, 2µl (10pmol) for each direction of the primers; forward (wild type or mutant) and common reverse, 12.5µl G2 Green master mix, and 3.5µl PCR water, were utilized. A thermocycler was employed at; (95ºC for 5mins for one-cycle)-initial denaturation, (95ºC for 30s, 55ºC for 30s, and 72ºC for 30s) for 35 cycles of denaturation, annealing, and extension, respectively, and (72ºC for 5mins for one cycle) final extension. An electrophoresis using a 2% Agarose gel was followed with PCR products visualized using a UV-light based screener.

RESULTS

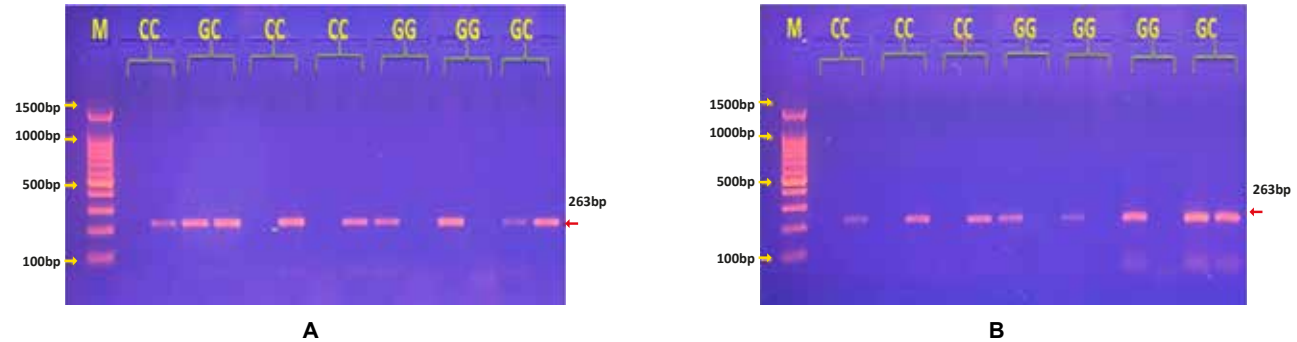
The pap smears revealed cervical intraepithelial neoplasia (CIN); II and III, in which dysplastic cells and mitotic figures were detected (Figure 1).



A B

Figure 1. Cervical intraepithelial neoplasia (CIN). A. CIN II (HSIL). B. CIN III (HSIL). It shows dysplastic cell, mitotic figures (blue arrow). Giemsa stain (X10).

The AS-PCR reported that the IL-6 (rs1800795) gene detected at 174 G/C position was presented with GG, GC, and CC genotypes (Figure 2 and tables 1-4).



A B

Figure 2. Images of agarose gel electrophoresis. It displays IL-6 -174G>C (rs1800795) gene polymorphism in A. HPV-patient and B. Healthy samples. Where M: a (1500-100bp) ladder. The GG: wild (G) homozygote allele, CC: mutant (C) allele, and GC): heterozygote (G and C) allele.

Table 1. Genotypes of IL-6 G/C 174 in Cervical Dysplasia without HPV Women and Healthy Controls

Genotype	G1 No.=50	G2 No.=64	G1 vs G2				
			X^2	*P-value*	OR	Etiological fraction	95% CI
GG	38	32	8.01	0.005*	0.31	0.29	0.1400-0.7122
GC	8	18	2.34	0.126	2.05	0.13	0.8089-5.2171
CC	4	14	4.06	0.044*	3.22	0.1	0.9883-10.4913

Table 2. Genotypes of IL-6 G/C 174 in healthy controls and HPV infected women

Genotype	G1 No.=50	G3 No.=36	G1 vs G3				
			X^2	*P-value*	OR	Etiological fraction	95% CI
GG	38	16	8.92	0.003*	0.25	0.3	0.1003-0.6363
GC	8	10	1.75	0.185	2.01	0.12	0.7062-5.7732
CC	4	10	6.01	0.014*	4.42	0.1	1.2605-15.5204
Allele							
G	84	42	0.4	0.52	0.83	0.29	0.4561-1.4947
C	16	30	0.4	0.52	1.21	0.21	0.6690-2.1927

G1: Negative healthy control; G2: cervical dysplasia without HPV

Table 3. IL-6 G/C 174 allele frequency distribution in healthy control and HPV infected women

Allele	G1 No.=50	G3 No.=36	G1 vs G3				
			X^2	*P-value*	OR	Etiological fraction	95% CI
G	84	42	0.4	0.52	0.83	0.29	0.4561-1.4947
C	16	30	0.4	0.52	1.21	0.21	0.6690-2.1927
Genotype	**G2 No.=64**	**G3 No.=36**	**G2 vs G3**				
			X^2	*P-value*	OR	Etiological fraction	95% CI
GG	32	16	0.28	0.59	0.8	0.24	0.3524-1.8163
GC	18	10	0	0.97	0.98	0.16	0.3955-2.4429
CC	14	10	0.44	0.51	1.37	0.14	0.5367-3.5156

G1: Negative healthy control; G3: Patients with HPV

Table 4. IL-6 G/C 174 allele frequency distribution in HPV infected women and cervical dysplasia without HPV

Allele	G2 No.=64	G3 No.=36	G1 vs G3				
			X^2	*P-value*	OR	Etiological fraction	95% CI
G	78	42	14.08	0*	0.27	0.33	0.1310-0.5429
C	46	30	14.08	0*	3.75	0.15	1.8420-7.6342

G2: cervical dysplasia without HPV; G3: Patients with HPV

DISCUSSION

The current study demonstrated a notable association between IL-6 polymorphisms and high-risk HPV as predictors of cervical cancer incidence in women. This observation is consistent[20], with prior research which documented a significantly higher prevalence of the IL-6-174 GG genotype [OR=3.9; P=0.001]. Moreover, individuals with GG and GC genotypes exhibited elevated IL-6 levels in their serum. Furthermore, untreated patients showed a considerably higher prevalence of IL-6 compared to treated cases.

In another study, the prevalence of the CC genotype was 20.3% among cancer patients and 15.1% among healthy volunteers[21, 22]. A similar study conducted in China reported a lower prevalence among patients (9.4%) compared to controls (4.4%). A meta-analysis suggested that the risk of cervical cancer is associated with the C allele at the -174 G/C locus[23]. Researchers further indicated that individuals with GC+CC genotypes (57.1% in patient cases vs. 41.5% in healthy controls) are at increased risk of cervical cancer. The absence of the CC genotype among cancer cases and its presence in only 1.2% of healthy controls highlights the potential risk of

this cancer in the Brazilian community[24].

The incidence of the GC+CC genotype ranged from 22.5% in group I to 26.4% in group II and peaked at 53.4% in group III. The CC genotype was detected in approximately 2.3% to 3.1% of their respective populations. Similar patterns were observed among South Indian and West Gujarati populations, where the CC genotype frequencies were 2.9% and 1.7%, respectively. Studies conducted in various North Indian communities reported varying frequencies ranging from 0.2% to 6.0% to 6.5%. These findings underscore the relatively low prevalence of the CC allele among Indians, a trend also noted in Gujarati Indians residing in Houston, Texas.[25].

CONCLUSION

The current study highlights a significantly altered IL-6 (rs1800795) gene strongly associated with human papillomavirus (HPV) infection-related cervical cancer in the Iraqi population. Therefore, further investigations into this gene across different regions are crucial for establishing it as a diagnostic marker for papillomaviruses. Additionally, comprehensive genetic studies are recommended to validate these findings and explore their broader implications.

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