Journal of Inflammation Research Dovepress

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Association of Inflammatory Factors with Cervical Cancer: A Bidirectional Mendelian Randomization

Qi Li[1,2,*], Liu Kaidong[3,*], Zhiyu Tian[4], Weihua Diao[1,2], Yuhong Sun[1,2], Ying Bai[1,2], Yueyue Ma[4], Yimiao Wei[1,2], Jiarong Li[4], Weihong Zhao[1,4]

[1]Department of Obstetrics and Gynecology, The Second Hospital of Shanxi Medical University, Taiyuan, People's Republic of China; [2]The Second Clinical Medical College, Shanxi Medical University, Taiyuan, People's Republic of China; [3]Department of Radiation Oncology, Shanxi Provincial Cancer Hospital, Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences, Cancer Hospital Affiliated to Shanxi Medical University, Taiyuan, People's Republic of China; [4]Department of Epidemiology, School of Public Health, Shanxi Medical University, Taiyuan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Weihong Zhao, Department of Obstetrics and Gynecology, The Second Hospital of Shanxi Medical University, No. 182 Wuyi Road, Taiyuan, 030001, People's Republic of China, Email sydeyzwh@sxmu.edu.cn

Purpose: Persistent human papillomavirus infection is thought to be the main cause of the cervical cancer development along with inflammation. However, the potential mechanisms of action of the inflammatory factors in cervical cancer remain unclear. Therefore, this study aimed to assess the relationship between inflammatory factor levels and cervical cancer risk using a two-sample bidirectional Mendelian randomization (MR).

Patients and Methods: MR utilizes single nucleotide polymorphisms as a tool to infer potential causal relationships between exposure factors and outcomes. Datasets for 91 inflammatory factors and cervical cancer were obtained from publicly available pooled data. The inverse variance weighted method was used as the main method and MR-Egger, weighted median, simple mode, and weighted mode were used as auxiliary analyses. Results were tested for robustness using sensitivity tests. In addition, we assessed the possibility of reverse causality between cervical cancer and the derived inflammatory factors by performing a reverse MR analysis. Finally, a preliminary experimental validation was performed.

Results: We found that artemin and monocyte chemoattractant protein-4 levels were significantly correlated with elevated cervical cancer risk (β: 0.0024, P = 0.002 and β: 0.0010, P = 0.016, respectively. In contrast, interleukin-18 and interleukin-22 receptor subunit alpha-1 levels were associated with reduced risk of cervical cancer (β: −0.0010, P = 0.029 and β: −0.0021, P = 0.046, respectively). Sensitivity analyses were more robust as no significant heterogeneity or horizontal pleiotropy was observed.

Conclusion: A significant causal relationship was found between the four inflammatory factors and the risk of cervical cancer, providing new evidence of their clinical implications in cervical cancer diagnosis and treatment.

Keywords: ARTN, CCL13, cervical cancer, human papillomavirus, IL-18, IL-22RA1, inflammatory factors, Mendelian randomization

Introduction

Cervical cancer is a common malignant tumor in women and ranks high in the number of cases and deaths globally, particularly in developing countries. Thus, it poses a serious threat to women's health and quality of life. High-risk human papillomavirus (HR-HPV) infection is an important factor in the development and progression of cervical cancer.[1] When cervical cells are exposed to chronic inflammation caused by HR-HPV over a long period of time, the risk of developing cancer increases.[2] Only less than 1–4% patients with persistent HPV infection eventually develop cervical pre-cancer and cancer; it takes about 10–20 years from infection to cervical cancer.[3] During this period, the host immune system fights to clear the virus. However, when the host's immune response is weak, it fails to clear the virus in a timely manner resulting in recurrent inflammation. Repeated cyclic stimulation of inflammatory factors can lead to malignant transformation of normal cells and cause tumor neovascularization, promoting cervical carcinogenesis.[4–6] Among them,

Received: 20 September 2024
Accepted: 27 November 2024
Published: 30 November 2024

many inflammatory factors play a key role in the development of cervical cancer. For example, Interleukin-2 (IL-2) can suppress the immune response by Treg cells, while promoting cell proliferation in the presence of low levels of IL-2.[7] Interleukin-17A (IL-17A) is highly expressed in cervical cancer, promoting M2 macrophage polarization and enhancing cancer cell proliferation, migration and invasion.[8] Thus, reducing inflammation can effectively treat and prevent the progression of cervical cancer. However, previous studies employing traditional observation designs to investigate the correlation between inflammatory factors and cervical cancer have produced inconclusive results. In particular, case-control studies are insufficient to determine the etiology of cervical cancer, resulting in a lack of clarity regarding the causal relationship between inflammation and cervical cancer.

In contrast, Mendelian randomization (MR) is a method of causal inference that uses the effect of randomly assigned genotypes on phenotypes to infer the effect of biological factors on diseases.[9] Additionally, it is unlikely to be influenced by confounding factors, such as living environment and lifestyle, or reverse causality. Therefore, we aimed to explore the potential causal associations between various inflammatory factors and cervical cancer using MR. Additionally, we aimed to perform a reverse MR analysis to determine whether cervical cancer affects these inflammatory factors, thereby providing a useful theoretical basis for subsequent clinical studies on the diagnosis and treatment of cervical cancer.

Materials and Methods

Study Design

To improve the credibility of the MR analysis results, we hypothesized that: (i) genetic variants are significantly correlated with the levels of inflammatory factors, (ii) genetic variants are associated with the levels of inflammatory factors, but not with any other known or unknown confounders, and (iii) genetic variants affect cervical cancer outcomes only through inflammatory factors.

Data Sources

The exposure factor (91 inflammatory factors) dataset was obtained from the EBI Genome-Wide Association Study (GWAS) catalog (registry numbers: GCST90274758–GCST90274848) and included 14824 participants of European ancestry. The outcome factor (cervical cancer) dataset was obtained from the GWAS database derived from the Medical Research Council Integrated Epidemiology Unit study at the University of Bristol and included 462,933 European pedigree samples. Population selection did not overlap between exposure factors and outcome groups. Both datasets used in the MR analysis were derived from publicly available pooled data (Table 1).

Genetic Instrumental Variable (IV) Selection

We selected $P < 5\times10^{-8}$ as the significance threshold to identify inflammatory factor-associated single nucleotide polymorphisms (SNPs). However, since inflammatory factors had limited or no SNPs at $P < 5\times10^{-8}$ (< 3), we further relaxed the significance threshold ($P < 1\times10^{-6}$) while setting the chain disequilibrium parameter ($r^2 = 0.001$, distance = 10,000 kb) to select the instrumental variables (IVs). Additionally, we set the F value to > 10 to maximize the removal of weak IVs. Ultimately, 2945 SNPs associated with 91 inflammatory factors were identified for use as IVs.

Table 1 Summary of Data from Genome-Wide Related Studies

Phenotype	Consortium or Study	Sample Size	Ancestry	Year	PMID	Accession Numbers	Data source	Reference
Inflammation Factors		14824	European	2023	37563310	GCST90274758 to GCST90274848	https://www.ebi.ac.uk/gwas/studies/GCST90274848	[10]
Cervical Cancer	MRC-IEU	462933	European	2018		GCST009541	https://gwas.mrcieu.ac.uk/datasets/ukb-b-8777/	

Data Analysis

We used inverse variance weighting (IVW) as the main analysis method for MR because it can provide the most accurate estimation. Additionally, MR-Egger, weighted median, simple mode, and weighted mode were used as supplementary analyses to refine the IVW analysis and ensure consistency of the MR results. We concluded that when $p < 0.05$, there was a potential causal association between the results.

Sensitivity analyses were conducted to assess the robustness of this preliminary analysis using various methods, including heterogeneity tests, horizontal multivariate evaluations, and leave-one-out IVW analyses. The MR-Egger regression intercept using the results of the Egger intercept test were used for the horizontal multivariate test. When $p < 0.05$, the result was considered potentially multivalent. Heterogeneity was quantified using Cochran's Q-test, and the resulting P-values were examined in detail; $p > 0.05$ indicated no significant heterogeneity. Finally, a "leave-one-out" test, which requires the gradual removal of individual SNPs from the analysis, was used to demonstrate that the causal effect of exposure on the outcome is not influenced by individual SNPs.

All analyses in this study were performed using "TwoSampleMR", "mendelanrandomization", and "MRPRESSO" in the R software (version 4.3.2) MRPRESSO packages.

Polymerase Chain Reaction (PCR) Experiment

In this study, we collected tissues from eight patients with chronic cervicitis and eight patients with cervical cancer between January and April 2023. The inclusion criteria were HPV16 +, no pregnancy, no hysterectomy, no treatment for cervical or vaginal lesions, no history of other malignancies, and cervical stage ≤ stage IIa1.

Quantitative reverse transcriptase polymerase chain reaction (PCR) was performed as follows: pre-denaturation at 95°C for 30s; followed by PCR reaction for at least 40 cycles, each cycle lasting 3 s; finally, the reaction was performed at 60°C for 30s. Cycle threshold (Ct) values were recorded and calculated by applying the $2^{-\Delta\Delta Ct}$ function. Please refer to Table 1 for information on specific primer sequences (Table 2).

Results

Owing to the limited number of genetically variable SNPs, we relaxed the threshold of the P-value to 1×10^{-5} for the MR analysis. Considering $r^2 < 0.001$, $P < 1 \times 10^{-5}$, 2945 SNPs associated with 91 cytokines were identified as IVs.

Based on the IVW results for the association of 91 cytokines with cervical cancer ($P < 0.05$) (Table 3), four inflammatory factors (artemin [ARTN], interleukin [IL]-18, IL-22RA1, and C-C motif chemokine ligand 13 [CCL13]) showed a causal correlation with cervical cancer risk. ARTN and CCL13 were positively associated with cervical cancer risk (β: 0.0024, P = 0.002 and β: 0.0010, P = 0.016, respectively). Whereas IL-18 and IL-22RA1 were negatively correlated with cervical cancer risk (β: −0.0010, P = 0.029 and β: - 0.0021, P = 0.046, respectively), which supports a causal relationship between the four inflammatory factors and cervical cancer (Table 4) (Figure 1).

Table 2 List of Primers Used in qRT-PCR qRT-PCR, Quantitative Reverse Transcriptase Polymerase Chain Reaction

Name	Primer Sequence
ARTN-F	5′-CGCTCTCCACACGACCTCAG-3′
ARTN-R	5′-GGTTCTCCAGGTGCTGTTGAC-3′
IL-18-F	5′-ATGGCTGCTGAACCAGTAGAAGAC-3′
IL-18-R	5′-AGAGGCCGATTTCCTTGGTCAATG-3′
IL-22RA1-F	5′-TGAGGACGCTGCTGACCATC-3′
IL-22RA1-R	5′-CAAAGTTGCTGGACTGGAATTTCAC-3′
CCL13-F	5′-GTCCCATCTACTTGCTGCTTCAC-3′
CCL13-R	5′-CAGATCTCCTTGCCCAGTTTG-3′

Abbreviations: ARTN, artemin; CCL13, C-C motif chemokine ligand; IL-interleukin; qRT-PCR, Quantitative reverse transcriptase polymerase chain reaction.

Table 3 Effect of 91 Inflammatory Factors on Cervical Cancer in IVW Methods

Exposure	Outcome	Method	nsnp	pval
Eukaryotic translation initiation factor 4E-binding protein 1 levels	Cervical cancer	Inverse variance weighted	9	0.958
Adenosine Deaminase levels	Cervical cancer	Inverse variance weighted	6	0.432
Artemin levels	Cervical cancer	Inverse variance weighted	10	0.002
Axin-1 levels	Cervical cancer	Inverse variance weighted	5	0.86
Beta-nerve growth factor levels	Cervical cancer	Inverse variance weighted	9	0.135
Caspase 8 levels	Cervical cancer	Inverse variance weighted	6	0.14
Eotaxin levels	Cervical cancer	Inverse variance weighted	7	0.842
C-C motif chemokine 19 levels	Cervical cancer	Inverse variance weighted	10	0.242
C-C motif chemokine 20 levels	Cervical cancer	Inverse variance weighted	11	0.538
C-C motif chemokine 23 levels	Cervical cancer	Inverse variance weighted	10	0.803
C-C motif chemokine 25 levels	Cervical cancer	Inverse variance weighted	14	0.52
C-C motif chemokine 28 levels	Cervical cancer	Inverse variance weighted	13	0.053
C-C motif chemokine 4 levels	Cervical cancer	Inverse variance weighted	8	0.878
Natural killer cell receptor 2B4 levels	Cervical cancer	Inverse variance weighted	12	0.37
CD40L receptor levels	Cervical cancer	Inverse variance weighted	9	0.098
T-cell surface glycoprotein CD5 levels	Cervical cancer	Inverse variance weighted	13	0.668
T-cell surface glycoprotein CD6 isoform levels	Cervical cancer	Inverse variance weighted	8	0.693
CUB domain-containing protein 1 levels	Cervical cancer	Inverse variance weighted	12	0.492
Macrophage colony-stimulating factor 1 levels	Cervical cancer	Inverse variance weighted	8	0.536
Cystatin D levels	Cervical cancer	Inverse variance weighted	22	0.646
Fractalkine levels	Cervical cancer	Inverse variance weighted	10	0.322
C-X-C motif chemokine 1 levels	Cervical cancer	Inverse variance weighted	10	0.55
C-X-C motif chemokine 10 levels	Cervical cancer	Inverse variance weighted	14	0.095
C-X-C motif chemokine 11 levels	Cervical cancer	Inverse variance weighted	8	0.274
C-X-C motif chemokine 5 levels	Cervical cancer	Inverse variance weighted	12	0.292
C-X-C motif chemokine 6 levels	Cervical cancer	Inverse variance weighted	8	0.271
C-X-C motif chemokine 9 levels	Cervical cancer	Inverse variance weighted	17	0.021
Delta and Notch-like epidermal growth factor-related receptor levels	Cervical cancer	Inverse variance weighted	14	0.95
Protein S100-A12 levels	Cervical cancer	Inverse variance weighted	7	0.449
Fibroblast growth factor 19 levels	Cervical cancer	Inverse variance weighted	10	0.306
Fibroblast growth factor 21 levels	Cervical cancer	Inverse variance weighted	12	0.769
Fibroblast growth factor 23 levels	Cervical cancer	Inverse variance weighted	10	0.163
Fibroblast growth factor 5 levels	Cervical cancer	Inverse variance weighted	8	0.384
Fms-related tyrosine kinase 3 ligand levels	Cervical cancer	Inverse variance weighted	17	0.105
Glial cell line-derived neurotrophic factor levels	Cervical cancer	Inverse variance weighted	6	0.307
Hepatocyte growth factor levels	Cervical cancer	Inverse variance weighted	8	0.798
Interferon gamma levels	Cervical cancer	Inverse variance weighted	6	0.781
Interleukin-10 levels	Cervical cancer	Inverse variance weighted	9	0.646
Interleukin-10 receptor subunit alpha levels	Cervical cancer	Inverse variance weighted	7	0.09
Interleukin-10 receptor subunit beta levels	Cervical cancer	Inverse variance weighted	10	0.679
Interleukin-12 subunit beta levels	Cervical cancer	Inverse variance weighted	17	0.664
Interleukin-13 levels	Cervical cancer	Inverse variance weighted	12	0.401
Interleukin-15 receptor subunit alpha levels	Cervical cancer	Inverse variance weighted	11	0.944
Interleukin-17A levels	Cervical cancer	Inverse variance weighted	7	0.239
Interleukin-17C levels	Cervical cancer	Inverse variance weighted	10	0.523
Interleukin-18 levels	Cervical cancer	Inverse variance weighted	11	0.029
Interleukin-18 receptor 1 levels	Cervical cancer	Inverse variance weighted	9	0.152
Interleukin-1-alpha levels	Cervical cancer	Inverse variance weighted	6	0.853
Interleukin-2 levels	Cervical cancer	Inverse variance weighted	6	0.87
Interleukin-20 levels	Cervical cancer	Inverse variance weighted	4	0.428
Interleukin-20 receptor subunit alpha levels	Cervical cancer	Inverse variance weighted	3	0.85

(*Continued*)

Table 3 (Continued).

Exposure	Outcome	Method	nsnp	pval
Interleukin-22 receptor subunit alpha-1 levels	Cervical cancer	Inverse variance weighted	4	0.046
Interleukin-24 levels	Cervical cancer	Inverse variance weighted	5	0.458
Interleukin-2 receptor subunit beta levels	Cervical cancer	Inverse variance weighted	7	0.727
Interleukin-33 levels	Cervical cancer	Inverse variance weighted	7	0.579
Interleukin-4 levels	Cervical cancer	Inverse variance weighted	11	0.761
Interleukin-5 levels	Cervical cancer	Inverse variance weighted	8	0.488
Interleukin-6 levels	Cervical cancer	Inverse variance weighted	6	0.948
Interleukin-7 levels	Cervical cancer	Inverse variance weighted	7	0.959
Interleukin-8 levels	Cervical cancer	Inverse variance weighted	9	0.999
Latency-associated peptide transforming growth factor beta 1 levels	Cervical cancer	Inverse variance weighted	8	0.621
Leukemia inhibitory factor levels	Cervical cancer	Inverse variance weighted	10	0.224
Leukemia inhibitory factor receptor levels	Cervical cancer	Inverse variance weighted	11	0.25
Monocyte chemoattractant protein-1 levels	Cervical cancer	Inverse variance weighted	9	0.444
Monocyte chemoattractant protein 2 levels	Cervical cancer	Inverse variance weighted	10	0.53
Monocyte chemoattractant protein-3 levels	Cervical cancer	Inverse variance weighted	7	0.364
Monocyte chemoattractant protein-4 levels	Cervical cancer	Inverse variance weighted	11	0.016
Macrophage inflammatory protein 1a levels	Cervical cancer	Inverse variance weighted	10	0.285
Matrix metalloproteinase-1 levels	Cervical cancer	Inverse variance weighted	11	0.91
Matrix metalloproteinase-10 levels	Cervical cancer	Inverse variance weighted	4	0.616
Neurturin levels	Cervical cancer	Inverse variance weighted	9	0.004
Neurotrophin-3 levels	Cervical cancer	Inverse variance weighted	9	0.495
Osteoprotegerin levels	Cervical cancer	Inverse variance weighted	13	0.622
Oncostatin-M levels	Cervical cancer	Inverse variance weighted	9	0.33
Programmed cell death 1 ligand 1 levels	Cervical cancer	Inverse variance weighted	10	0.335
Stem cell factor levels	Cervical cancer	Inverse variance weighted	24	0.184
SIR2-like protein 2 levels	Cervical cancer	Inverse variance weighted	5	0.325
Signaling lymphocytic activation molecule levels	Cervical cancer	Inverse variance weighted	18	0.318
Sulfotransferase 1A1 levels	Cervical cancer	Inverse variance weighted	10	0.317
STAM binding protein levels	Cervical cancer	Inverse variance weighted	5	0.683
Transforming growth factor-alpha levels	Cervical cancer	Inverse variance weighted	9	0.129
Tumor necrosis factor levels	Cervical cancer	Inverse variance weighted	8	0.596
TNF-beta levels	Cervical cancer	Inverse variance weighted	7	0.225
Tumor necrosis factor receptor superfamily member 9 levels	Cervical cancer	Inverse variance weighted	11	0.419
Tumor necrosis factor ligand superfamily member 14 levels	Cervical cancer	Inverse variance weighted	12	0.37
TNF-related apoptosis-inducing ligand levels	Cervical cancer	Inverse variance weighted	7	0.33
TNF-related activation-induced cytokine levels	Cervical cancer	Inverse variance weighted	14	0.753
Thymic stromal lymphopoietin levels	Cervical cancer	Inverse variance weighted	4	0.725
Tumor necrosis factor ligand superfamily member 12 levels	Cervical cancer	Inverse variance weighted	19	0.088
Urokinase-type plasminogen activator levels	Cervical cancer	Inverse variance weighted	16	0.649
Vascular endothelial growth factor A levels	Cervical cancer	Inverse variance weighted	11	0.739

Abbreviations: IVW, inverse variance weighting.

Subsequently, we performed several sensitivity analyses for each of the four inflammatory factors. Neither Egger's test nor IVW based on Cochran's Q test showed any significant heterogeneity among the IVs ($P < 0.05$). In addition, the results of both MR-Egger regression analyses showed no potential for pleiotropy ($P < 0.05$) (Table 2). The results of the leave-one-out test also showed that none of the causalities was caused by a single SNP (Figure 2).

Moreover, we did not observe a statistically significant association between the four inflammatory factors and cervical cancer in the reverse MR analysis using the IVW method ($P < 0.05$) (Table 5).

Subsequently, the expression levels of the four genes were examined in the tissues of patients with chronic cervicitis and cervical cancer and compared with those of the internal reference, glyceraldehyde 3-phosphate dehydrogenase. The

Table 4 Effect of 4 Inflammatory Factors on Cervical Cancer in MR Analysis

Exposure	Outcome	Method	nsnp	pval	β	OR (95% CI)	P-Intercept	P-Heterogeneity
Artemin levels	Cervical Cancer	MR Egger	10	0.196	0.0130	1.013 (0.995 to 1.031)	0.283	0.222
		Weighted median	10	0.032	0.0020	1.002 (1.000 to 1.004)		
		Inverse variance weighted	10	0.002	0.0024	1.002 (1.001 to 1.004)		0.191
		Simple mode	10	0.506	0.0011	1.001 (0.998 to 1.004)		
		Weighted mode	10	0.317	0.0016	1.002 (0.999 to 1.005)		
Interleukin-18 levels	Cervical Cancer	MR Egger	11	0.016	−0.0025	0.997 (0.996 to 0.999)	0.06	0.796
		Weighted median	11	0.006	−0.0015	0.998 (0.997 to 1.000)		
		Inverse variance weighted	11	0.029	−0.0010	0.999 (0.998 to 1.000)		0.436
		Simple mode	11	0.311	−0.0011	0.999 (0.997 to 1.001)		
		Weighted mode	11	0.017	−0.0015	0.999 (0.997 to 1.000)		
Interleukin-22 receptor subunit alpha-1 levels	Cervical Cancer	MR Egger	4	0.537	−0.0123	0.988 (0.956 to 1.021)	0.602	0.655
		Weighted median	4	0.154	−0.0018	0.998 (0.996 to 1.001)		
		Inverse variance weighted	4	0.046	−0.0021	0.998 (0.996 to 1.000)		0.747
		Simple mode	4	0.439	−0.0016	0.998 (0.995 to 1.002)		
		Weighted mode	4	0.403	−0.0016	0.998 (0.995 to 1.002)		
Monocyte chemoattractant protein-4 levels	Cervical Cancer	MR Egger	11	0.717	0.0004	1.000 (0.998 to 1.002)	0.491	0.967
		Weighted median	11	0.075	0.0009	1.001 (1.000 to 1.002)		
		Inverse variance weighted	11	0.016	0.0010	1.001 (1.000 to 1.002)		0.969
		Simple mode	11	0.188	0.0010	1.001 (1.000 to 1.002)		
		Weighted mode	11	0.125	0.0009	1.001 (1.000 to 1.002)		

Abbreviations: MR, Mendelian randomization.

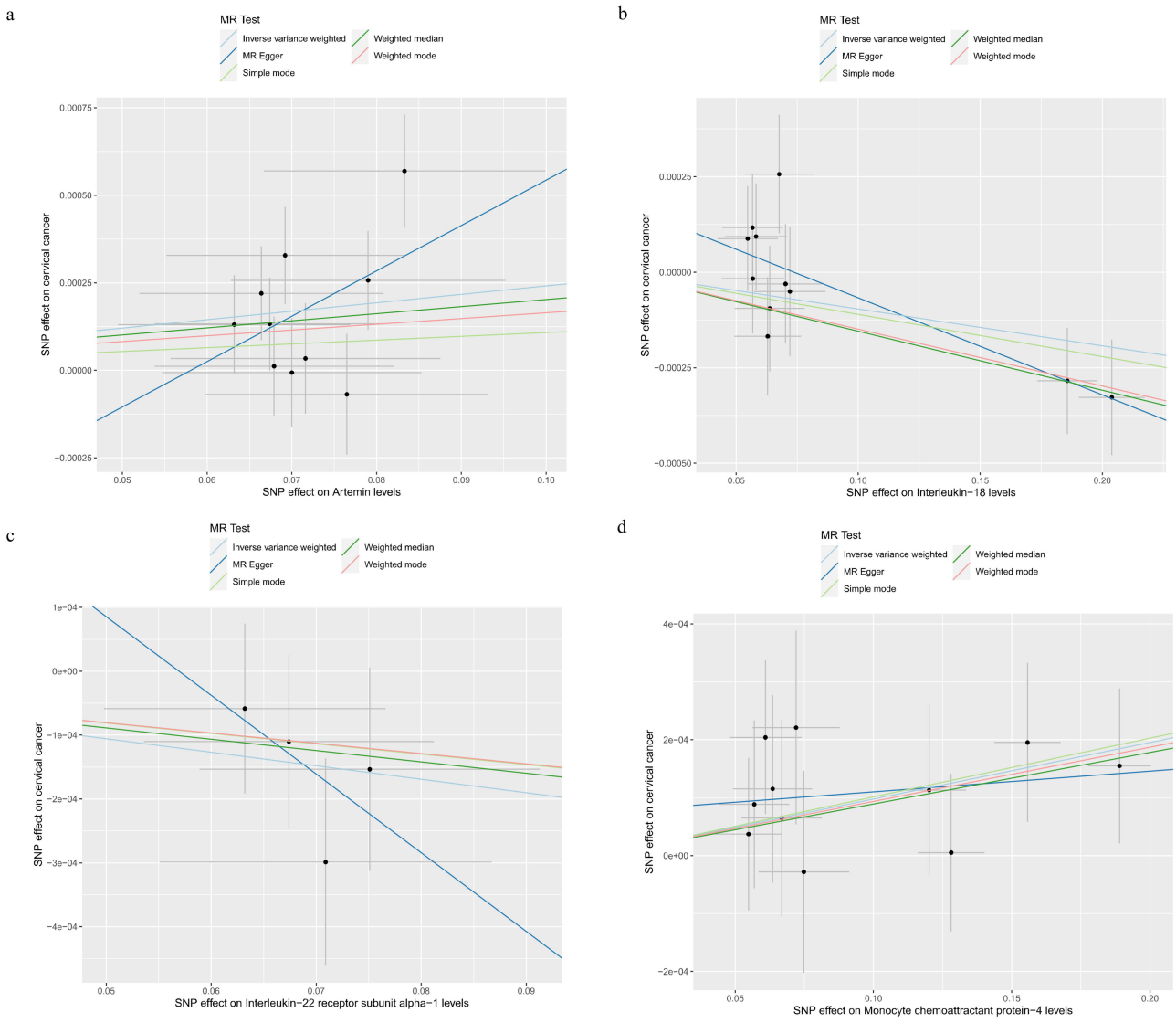

Figure 1 Scatter plots of MR analysis of ARTN (**a**), IL-18 (**b**), IL-22RA1 (**c**), and CCL13 (**d**) in cervical cancer.
Abbreviations: ARTN, artemin; CCL13, C-C motif chemokine ligand; IL, Interleukin; MR, Mendelian randomization.

PCR results showed higher expression of ARTN and CCL13 and lower expression of IL-18 and IL-22RA1 compared with the control group (Figure 3).

Discussion

Although some progress has been made in the prevention and treatment of cervical cancer, including HPV vaccination, surgical excision and chemotherapy, many challenges remain. For example, although HPV vaccination can effectively prevent cervical cancer caused by HPV, the vaccine has no therapeutic role for patients who are already infected with HPV or have developed cervical cancer.[11] Although surgery and chemotherapy can treat cervical cancer, the effects of traditional treatments are often limited in patients with late-stage or recurrent cervical cancer.[12] Therefore, in this study, we used two-way, two-sample MR analysis to explore the causal relationship between 91 inflammatory factors and cervical cancer, with the aim of finding key inflammatory factors and enhancing or inhibiting the function of these factors to prevent and treat the disease and monitor disease recurrence. Although several studies have explored the correlation between cervical cancer and various inflammation-related factors, no causal relationship has been established between cervical cancer and inflammatory factors owing to the limitations of classical epidemiology. Consequently, this is the first

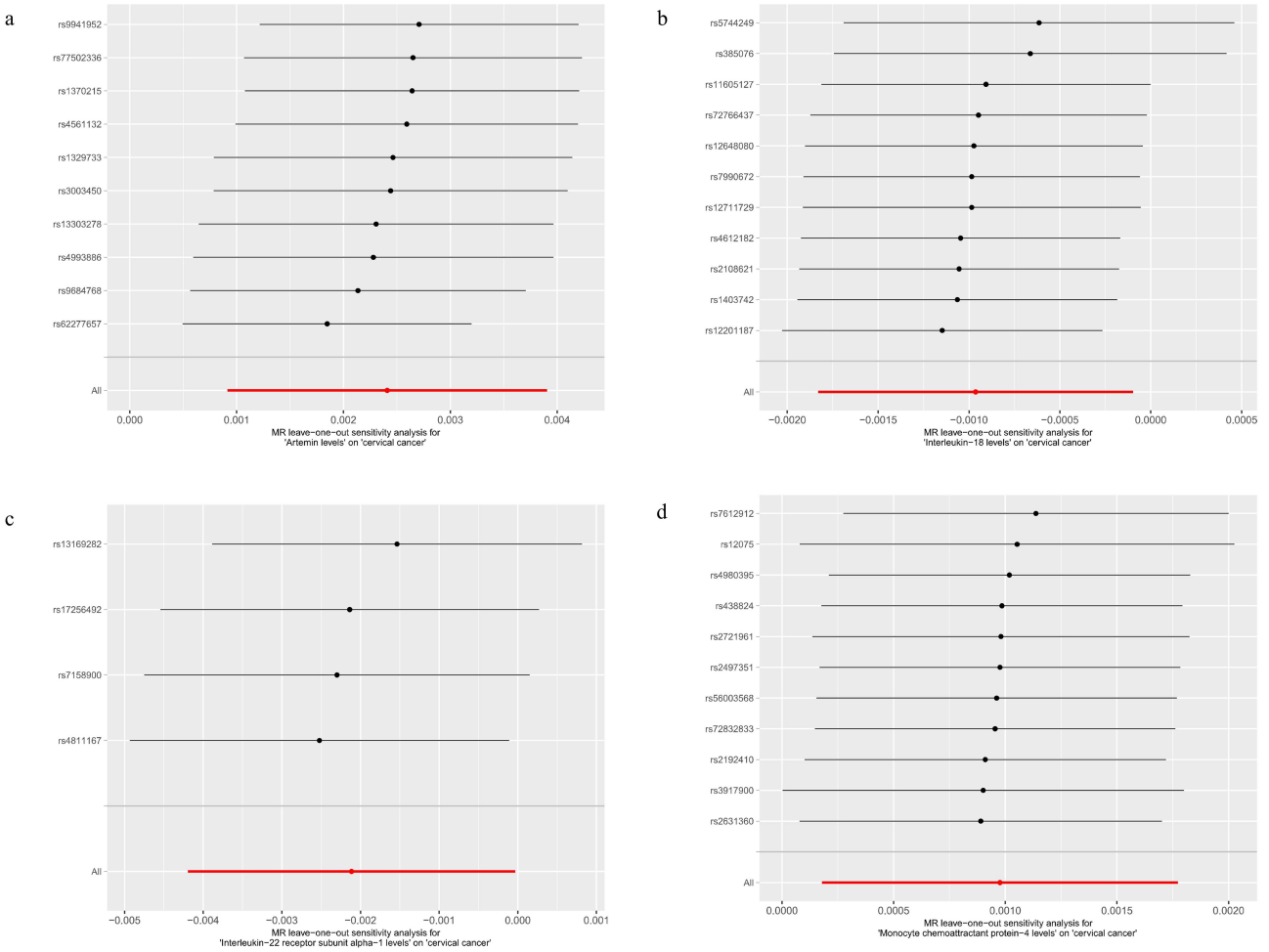

Figure 2 Single nucleotide polymorphisms of ARTN (**a**), IL-18 (**b**), IL-22RA1 (**c**), and CCL13 (**d**) associated with cervical cancer.
Abbreviations: ARTN, artemin; CCL13, C-C motif chemokine ligand; IL, Interleukin.

comprehensive MR study on the association between inflammatory factors and cervical cancer to address the bias introduced by confounding factors and reverse causation. We found that the expression levels of four inflammatory factors (ARTN, IL-18, IL-22RA1, and CCL13) were associated with cervical cancer. Whereas the reverse MR analysis revealed no association between cervical cancer and any of the four inflammatory factors. PCR results using cervical tissues further confirmed the results.

ARTN protein is an isoform of the glial cell-derived neurotrophic factor (GDNF) family of ligands, the fourth member of the GDNF family, which also belongs to the transforming growth factor beta family.[13] ATRN is associated with the activation of immune cells, and its regulatory mechanisms may influence the body's inflammatory and immune responses.[14] Several experimental studies have shown that ATRN protein levels are highly expressed in certain inflammatory diseases (eg, coronavirus disease 2019) and cancer compared with normal tissues. ATRN promotes tumor cell chemotaxis, adhesion, and migration and mediates tumor cell invasion and metastasis.[15,16] High expression of ARTN in several cancers (eg hepatocellular carcinoma, breast cancer) is significantly associated with increased tumor size, rapid recurrence and reduced patient survival. In addition, ARTN promotes resistance to several drugs.[17–19] Some studies have also found that ARTN signaling in tumor cells plays an important role in the antitumor effects of radiotherapy and immunotherapy. However, epidemiological evidence of the relationship between ARTN and cervical cancer is scarce and limited due to small sample sizes and a case-control study design. For example, a previous study evaluating 88 cervical cancer tissues and 30 normal cervical tissues revealed that ARTN levels were higher in both squamous cell carcinoma and adenocarcinoma tissues of the cervix than in normal cervical tissues and that ARTN

Table 5 Effect of Cervical Cancer on 4 Inflammatory Factors in Reverse MR Analysis

Exposure	Outcome	Method	nsnp	pval
Cervical Cancer	Artemin levels	MR Egger	12	0.560
		Weighted median	12	0.899
		Inverse variance weighted	12	0.826
		Simple mode	12	0.876
		Weighted mode	12	0.698
Cervical Cancer	Interleukin-18 levels	MR Egger	12	0.925
		Weighted median	12	0.268
		Inverse variance weighted	12	0.585
		Simple mode	12	0.386
		Weighted mode	12	0.325
Cervical Cancer	Interleukin-22 receptor subunit alpha-1 levels	MR Egger	12	0.606
		Weighted median	12	0.806
		Inverse variance weighted	12	0.519
		Simple mode	12	0.155
		Weighted mode	12	0.444
Cervical Cancer	Monocyte chemoattractant protein-4 levels	MR Egger	12	0.235
		Weighted median	12	0.784
		Inverse variance weighted	12	0.967
		Simple mode	12	0.493
		Weighted mode	12	0.556

Abbreviations: MR, Mendelian randomization.

expression was strongly associated with lymph node metastasis and recurrence in patients with cervical cancer. Another study found that ARTN overexpression significantly enhanced AKT phosphorylation at Ser473 and mTOR phosphorylation at Ser2448 and promoted an epithelial-mesenchymal transition cascade response.[20] Contributing to this information, the present study provides a high level of evidence regarding ARTN as one of the factors contributing to the development

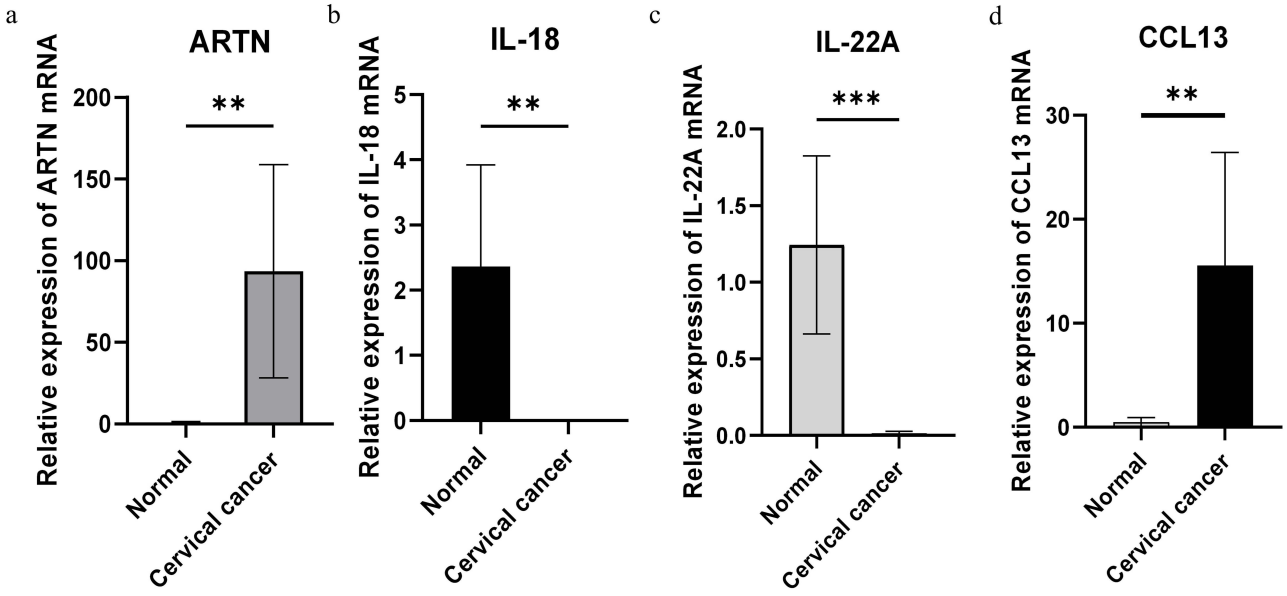

Figure 3 Expression levels of ARTN (**a**), IL-18 (**b**), IL-22RA1 (**c**), and CCL13 (**d**) in normal cervical tissues and cervical cancer. **p<0.01***p< 0.001.
Abbreviations: ARTN, artemin; CCL13, C-C motif chemokine ligand; IL, Interleukin.

of cervical cancer. This confirms the notion that patients with cervical cancer have higher expression levels of ARTN, suggesting its role in the pathogenesis of cervical cancer.

IL-18 is cytokine that induces and mediates inflammatory reactions and autoimmune diseases.[21] It has an important protective role in host defense and responds to cancer and infection by activating various immune cells as it activates CD8+ T cells that act against viral infections.[22,23] Low expression of IL-18 correlates with chronic inflammation and cancer severity and may serve as an anti-cancer immunotherapeutic. IL-18-based therapies have been shown to be effective in controlling tumor growth in preclinical mouse models. Several clinical trials have been conducted to demonstrate the feasibility of IL-18-based cancer therapies, either as monotherapy or in combination with other immunomodulatory factors.[24,25] Additionally, reduced IL-18 expression in cervical carcinogenesis may be one of the mechanisms by which HPV viruses evade the host's immune response and is one of the major contributing factors to chronic inflammation and oncogenesis. Our findings are consistent with those of previous studies. Matamoros et al analyzed 19 normal, 17 LSIL, 29 hSIL, and 9 cervical carcinoma foci using PCR and found that IL-18 expression was significantly lower in HSIL and SCC than in normal controls. The expression of IL-18 was progressively down-regulated as the tumor progressed.[26] Similarly, Shukla et al found a decrease in IL-18 expression in cervical cancer in an experiment comprising 10 CIN I, 10 CIN II, 10 CIN III, 54 cervical carcinomas, and 84 age-matched normal controls.[27] Additionally, the HPV 16 E6 and E7 proteins inhibit IL-18-induced localized interferon (IFN)-γ production in HPV lesions, possibly by inhibiting the binding of IL-18 to its α-chain receptor, evading immunosurveillance, and inhibiting the cascading downstream effects of IL-18 receptor activation.[28,29] This finding suggests that IL-18 may be an efficient biomarker for the diagnosis and treatment of cervical cancer.

IL-22RA1 is a receptor protein expressed on the cell membrane of various cells, including epithelial and pancreatic cells. In recent years, several studies have shown that IL-22RA1 has a protective effect against host infections and can promote the regression of virus-induced inflammation.[30,31] However, the association between IL-22RA1 and cervical cancer has not been widely reported. Our MR analysis revealed a significant causal effect of IL-22RA1 on cervical cancer. Therefore, the mechanism by which IL-22RA1 affects HPV infection and cervical cancer requires further exploration.

CCL13, also referred to as monocyte chemotactic protein, is a small cytokine of the CC chemokine family, and its gene is located on human chromosome 17. It exerts chemotactic effects on various immune cells, including macrophages, eosinophils, basophils, and monocytes. In addition, CCL13 is involved in histamine release from basophils, eosinophil degranulation, adhesion molecule expression, and proinflammatory cytokine production.[32,33] In recent years, studies on the role of CCL13 in human diseases have revealed that CCL13 is upregulated in allergic diseases, such as asthma and allergic rhinitis. High expression of CCL13 is thought to predict poor prognosis in cancer patients (eg, oral cancer, rectal cancer), with significant associations with age, tumor stage, and presence of distant metastases.[34,35] In addition, CCL13 may be involved in the process of estrogen-driven ovarian carcinogenesis by regulating macrophage polarization.[36] We also found that CCL13 is highly expressed in cervical cancer and may contribute to its development. Thus, CCL13 may be a potential diagnostic and therapeutic component for cervical cancer and warrants further research for clinical application.

Taken together, the present study provides clinical support for the theory of inflammatory factors and cervical cancer and offers new clues for predicting the risk of cervical cancer. The four inflammatory factors may have a role in cervical cancer, as preliminarily validated in our experimental study. However, the mechanisms underlying their role in disease initiation and progression require further investigation.

Nevertheless, this study has a few limitations. First, most of the GWAS data were derived from individuals with European ancestry, necessitating further studies to determine the generalizability of the results to other populations. Second, although we used a loose significance threshold of $P < 1\times10^{-5}$ to select IVs, it could produce false-positive variants and consequent bias. However, all IVs had $F > 10$, suggesting a low likelihood of weak instrumental bias. Therefore, these potential associations need to be validated in larger population cohorts and GWAS.

Conclusion

Our study suggests a potential causal relationship between the four inflammatory factors (ARTN, IL-18, IL-22RA1, and CCL13) and risk of cervical cancer. However, further experimental studies are needed to confirm these results, explore the underlying biological mechanisms, and analyze whether the inflammatory factors could serve as future diagnostic and therapeutic targets. The public health sector can increase awareness of cervical cancer screening, especially among high-risk groups. Medical professionals can test the levels of these inflammatory factors in HPV-infected individuals for early detection of cervical cancer in high-risk groups, while cervical cancer patients can be tested for the levels of these inflammatory factors in combination with immunotherapy to improve the prognosis of patients, and in addition to monitoring these indicators to better predict the effectiveness of treatment and prognosis.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This study complies with the Declaration of Helsinki and was approved by the Ethics Review Board (IRB) of the Second Hospital of Shanxi Medical University ([IRB No. (2019) YX (280]). Written informed consent was obtained from all selected patients.

Acknowledgments

We thank Dr. Yanhong Luo from the Department of Health Statistics, School of Public Health, Shanxi Medical University for carefully reviewing the article.

Funding

This work was supported by the National Natural Science Foundation of China (no.81702583), Outstanding Youth Fund Project of Shanxi Province (no. 201901D211506), China Postdoctoral Science Foundation (no. 2019M651072), Shanxi Scholarship Council of China (no. 2022-195), and Scientific research project of Shanxi Provincial Health Commission (2023056).

Disclosure

The authors declare that they have no competing interests.

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