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Molecular Evaluation of TNF-α-308 and TNF-α-238 Polymorphisms and Their Association with HPV Genotypes in Cervical Lesions

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ABSTRACT

Background: Cervical cancer is a multi-stage disease that is contaminated by a DNA virus and is involved in one or more stages in the pathogenesis process. In addition to human papillomaviruses (HPV), the tumor necrosis factor-α gene (TNF-α) is considered a major intermediate mediator of the acute inflammatory response to viruses and a gram-negative bacterium. The pro-region polymorphisms of the TNF-α gene such as -308 and -238 polymorphisms affect the expression of this gene. Therefore, this study aimed to investigate the polymorphisms of the TNF-α gene and its relationship with various genotypes of HPV in cervical lesions.

Methods: In this study, 58 female patients with cervical cancer symptoms were selected, then following histopathologic studies, DNA was extracted from all specimens, and the PCR method was used to determine the types of HPV genotypes and TNF-α gene polymorphisms. Also, an ARMS-PCR reaction was performed to amplify TNF-α -308 and TNF-α -238 polymorphisms. Statistical analysis of the data was carried out by the Epi Info software version 7. 2 and Chi-Square (x2) test using SPSS ver.7.3.1.10. These lesions were categorized into metaplasia groups (93.37%), cervical intraepithelial neoplasia (CIN) I and II (20.68%), CIN III (15.51%) and squamous cell carcinoma (SCC) (25.86%).

Results: In this study 58 lesions were collected and 26 of which were HPV positive. They were categorized as the following: 1 sample (4.53%) metaplasia, 7 samples (33.58%) CIN I and CIN II, 6 samples (66.66%) CIN III and 12 samples (80%) SCC. According to the results of statistical analysis, there was a significant difference between different types of HPV genotypes in different stages. On the other hand, in contrast to the -238 polymorphism of the TNF-α gene, a significant difference was observed between the various types of -308 polymorphism of the TNF-α gene in the three groups of metaplasia, CIN, and SCC. In general, we can conclude that GG genotype testing of the -308 polymorphism of TNF-α gene and HPV types in combination with para-clinical parameters can be effective as risk factors and molecular markers for prognosis and early treatment of cervical cancer.

Discussion: Being compatible with other studies, this study demonstrated that about 80% of cervical cancer lesions hold the most presence of HPV. Subtypes of 18 and 31 of HPV accounted for more frequency along with malignancy progress while, HPV-33 and HPV-35 subtypes didn't have significant association with malignancy progress. However, in other women societies of Iran diverse results were detected. In the current study, it was proven that in TNF-α -308 polymorphism, the A allele accounted for the most frequency (63.63%) in the metaplasia (control) group and GG allele holds its high presence in the CC group. In general, frequency of A allele is various in different countries.

Keywords: Cervical Neoplasm; Human Papilloma Virus; Polymorphism; Tumor Necrosis Factor-Alpha

INTRODUCTION:

Women constitute more than half of human population and their healthiness particularly in their reproductive years can guaranty the next generation health to some extent. Globally, women are most likely to suffer from cervical cancer being the fourth most common cancer, only behind breast cancer (2.1 million cases) and colorectal cancer (0.8 million cases). In 2018, cervical cancer was estimated to have caused around 570,000 cases and 311,000 deaths. The estimated age-related incidence of cervical cancer worldwide was 13.1 per 100,000 women, but there was a significant variation among countries. However, the western developed countries have seen a decrease in cancer incidence, mainly due to lifestyle changes including the cessation of smoking and making improvement in screening and treatment modalities, the low-income and developing countries are experiencing an increase in overall cancer incidence and mortality. Infectious agents, such as human papillomavirus (HPV), H. pylori, and hepatitis B and C viruses, affect developing countries in a disproportionate manner and have a tendency to have fewer structural and financial resources to support robust screening programs [1].

The most prevalent cancer-related cause of death in women in Eastern, Western, Middle, and Southern Africa in 2018 was cervical cancer. Eswatini had the highest estimate for cervical cancer cases, with about 6.5% of women developing it before age 75 years. China and India combined to create over a third of the world's cervical cancer burden, with 106,000 cases and 48,000 deaths in China and 97,000 cases and 60,000 deaths in India. It is estimated that both incidence and prevalence of cancer disease has a growing trend and till 2025 it will increase by 40% in developed countries and three-fold higher than reports in year of 2000 in developing countries [2]. The World Health Organization (WHO) has announced that the invasive cervical cancer can lead to death of about 404000 women a year and 95% of which will occur in developing and low- income countries. In Iran, cervical cancer ranked the second prevalent cancer after breast cancer and the fifth cause of mortality in women affected by cancer. According to the Iranian Ministry of Health, there are 9.56 cases per 100,000 people per year of this cancer (Iran Ministry of Health, 2012) [3].

The main risk factors include HPV infection, HIV prevalence, as well as social and economic determinants such as sex, gender biases, and poverty. HPV, is the main and prevalent sexually transmitted infection that can impact the skin, genital area, and throat. It is common for sexually active individuals to become infected at some point in their lives, usually without any symptoms. HPV is cleared from the body by the immune system in most cases. The development of abnormal cells, which can lead to cancer, can be caused by persistent infection with high-risk HPV. The average time it takes for abnormal cells to become cancer is 15–20 years. But in women with weakened immune systems, such as those with untreated HIV, this process can be faster and take 5–10 years [4].

HPV encircles a double-stranded DNA genome and consists of 72 pentameric capsomeres with a size around 60 nm in diameter particles [5]. The regulatory region of HPV encodes L1 and L2 structural proteins. In current HPV vaccines, the L1 protein is the basis of the vaccine, has self-assembling ability and making virus-like particles. In addition, in high-risk HPVs, the regulatory region encodes some early proteins named E1-E7, playing carcinogenesis role with developing changes in epithelium of cervix [5], [6]. Studies have proven that persistent infection by high-risk HPV types is the prominent cause of cervical cancer and is highly associated with vulva, vagina, mouth/throat, penis and anus cancers [7].

HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are categorized as the carcinogenic ones but HPV-16 is the most dominant type. Genital warts are mostly caused by HPV-6 and HPV-11 [8]. Given that among HPV, the 16th and 18th genotypes are known as the first cause of cervical cancer, screening of these genotypes has considerable importance [9]. The leading countries in the prevalence of cervical HPV among women are sub-Saharan Africa (24%), followed by Latin America and the Caribbean (16%), Eastern Europe (14%), and South-East Asia (14%). This prevalence is truly variable in men due to sexual trends [9].

According to available estimates, every year a number of 1056 women are diagnosed with cervical cancer with 644 mortalities in Iran. Generally, this disease stands as the 14th most frequent cancer among women and as the 10th most frequent cancer among women in ages 15 to 44 [10]. Countries with low and middle income hold the highest rates of cervical cancer incidence since they lack access to HPV vaccination and appropriate screening programs [11].

Formation of the cervical cancer demands a series

of cellular changes over the course of infection to develop a dysplasia to invasive carcinoma. The main etiologic factor in this cancer is constant infection with human papillomavirus (HPV) which is mainly because of transmitted HPV sexually and it is not inherited genetically [12], [13].

Tumor necrosis factor (TNF) is a cytokine which is highly expressed in tumors and has a range of biological activities including two types of TNF-α and TNF-β. TNF-α is mainly made by monocytes and macrophages and plays essential roles in immune response as tumor suppressor and oncogene [14]. TNF-α is also responsible for regulation of tumor cell growth, tumor angiogenesis, and invasion by playing regulatory role in transcription level. It also activates immune-mediated inflammation in the central nervous system and confers formation and progression of cervical lesions [15, 16,17].

Some polymorphisms of TNF-α can prone cervical cells to be affected by HPV infection and lead to development of cancerous cells [18]. In cervical cancer, TNF-α has an inhibitory role in the transcription of E6/E7 keratinocytes affected by HPV-16 and also for HPV-18 oncogene transcription. Numerous conducted studies have disclosed that TNF-α promotes HPV for immortalized proliferation in cervical cancer [19]. The TNF-α gene is placed within the MHC gene region on chromosome 6p21.3. This zone is a highly polymorphic region and TNF-α includes a wide range of polymorphisms [20]. Due to the great role of TNF polymorphisms on immune response, infection progress and susceptibility of cancers, many polymorphisms of TNF gene such as TNF-α -308G/A (rs1800629), -238G/A (rs361525), -863C/A (rs1800630), T-1031C and T-857C, in TNF-α and +252A>G in TNF- β has been studied [21,22,23,24].

In -238 and -308 SNPs the presence of guanine (G) instead of adenine (A) is the main cause of a noticeable increase in the expression level of TNF-α. The great effect of polymorphisms in the promotor region of TNF-α gene on the transcriptional level in various tumors, has drowned the scientist's attention. The most common of which are -308 and -238 in polymorphic loci.

TNF-α-308 is involved three genotypes of GG, GA, and AA and is highly attributed to the incidence, development, and prognosis of various malignant tumors.

According to the results of conducted studies and the critical effects of TNF-α on HPV infection and cancer susceptibility, it is crucial to detect the genetic agents of host role in the progress of diseases [15]. The current study aims to evaluate the relation of TNF-α -238 and -308 gene polymorphisms with susceptibility of cervical lesions affected by HPV. There are limited studies in this field being performed in Karaj City. Iran has a population of 33.5 million women ages 15 years and older who are at risk of developing cervical cancer. This is the first time that the cervical lesions of Karaj's women are evaluated from two aspects of TNF-α polymorphisms and HPV genotypes.

Materials and methods:

Study subjects

In this study, patients suspected of cervical cancer symptoms who were referred to Dr. Mohtasebi's obstetrics and gynecology clinic in Karaj from June 2015 to September 2015, were screened for histopathology tests. The population of this study included samples with metaplasia, pre-cancerous and cancerous lesions. For HPV test, the samples were prepared with simple random method using swap from endocervix and exocervix mucus in 10 ml Thinprep and they were transferred to the molecular laboratory. These Fifty-eight samples were selected among all specimens with the range of 17 to 52 years of age. In the pathology department, the sections of the specimens were preserved with the Formalin-Fixed Paraffin-Embedded technique (FFPE) and the sections were stained with Hematoxylin and Eosin for histopathology detections.

DNA Extraction

DNA Extraction from fresh lesions was carried out using the high-yield DNA purification kit, DNTPTM KIT (DN 8115, Cinnagen Inc., Iran), based on protocol. The quality of extraction was evaluated using NanoDrop.

Primer

Detection of HPV types was accomplished using Polymerase Chain Reaction (PCR) with Cinnagen (Cat. No. PR8252C, Sinaclone, Tehran, Iran) using specific primers. For Internal control, Beta-actin primers were used (Table 1a). To recognition of TNF-α-238 and TNF-α-308 polymorphism genotypes, in Multiplex-ARMS-PCR following primers (Table 1b).

The size of products for the G and A alleles in -238 polymorphism was 210 bp and for -308 polymorphism was 140 bp.

Genotyping TNF-α-238 and TNF-α-308 by Multiplex-ARMS PCR

TNF-α-238 and TNF-α-308 polymorphisms were determined using Multiplex-ARMS-PCR. Two reactions were designed in a final volume of 25 µl which contained

Table 1a. HPV and Beta-actin primers

	Forward primer	Reverse primer
HPV-11	5'CGC AGA GAT ATA TGC ATA TGC 3'	5' AGT TCT AAG CAA CAG GCA CAC 3'
HPV-16	5'ATATATGTTAGATTTGCAACCAGAGACAAC 3'	5'GTCTACGTGTGTGCTTTGTACGCAC 3′
HPV-18	5'CCGAGCACGACAGGAGAGGCT 3′	5'TCGTTTTCTTCCTCTGAGTCGCTT 3′
HPV-31	5'AACGCCATGAGAGGACACAAG 3′	5'ACACATAAACGAACTGTGGTG 3'
HPV-33	5'AAC GCC ATG AGA GGA CAC AAG 3'	5'ACA CAT AAA CGA ACT GTG GTG 3'
HPV-35	5' CCCGAGGCAACTGACCTATA 3'	5' GGGGCACACTATTCCAAATG 3'
Beta-actin	5'CCACACTGTTCCCATCTACG3'	5' AGGATCTTCATGAGGTAGTCAGTCAC 3'

HPV: Human papillomavirus

Table 1b. TNF-α-308 and TNF-α-238 primers for multiplex ARMS-PCR

	Forward primer	Common Primer
TNF-α-308G	5'ACCCTGGAGGCTGAACCCCGTCTC 3'	5' GCCCCTCCCAGTTCTAGTTCTATC 3'
TNF-α-308A	5'ACCCTGGAGGCTGAACCCCGTCTT3'	5' GCCCCTCCCAGTTCTAGTTCTATC 3'
TNF-α-238G	5' CACACTCCCCATCCTCCCTGCTTC 3'	5' GCCCCTCCCAGTTCTAGTTCTATC 3'
TNF-α-238A	5'CACACTCCCCATCCTCCCTGCTTT 3'	5' GCCCCTCCCAGTTCTAGTTCTATC 3'

ARMS-PCR: Amplification Refractory Mutation System
TNF: Tumor Necrosis Factor

13µl PCR Master Mix, 6 µl PCR grade water, 1µl of each designed primers (10pmol) including (common primer, TNF-α-238-A primer and TNF-α-308-A primer for reaction number one) and (common primer, TNF-α-238-G primer and TNF-α-308-G primer for reaction number two), 3µl genomic extracted DNA (100-200ng DNA).To optimize of Multiplex-ARMS-PCR circumstance, multiple annealing temperatures was used with gradient (from 55 to 62). The amplification has been done by Gene Touch thermo-cycler (Eppendrof). The best PCR program (the cycling condition) for amplification was used as bellow: Initial denaturation at 95C for 4 minutes, Denaturation at 95C for 30sec, repeated 35 cycles and followed by annealing temperature at 58 C for 30sec and extension at 72 C for 30 sec and the elongation step was set up for 4 min in 72 C The PCR product was separated by 2% agarose gel electrophoresis and observed by staining with ethidium bromide. Distilled water was loaded as negative control and two blinded duplicates in the Multiplex-ARMS PCR assay for quality control of our genotyping.

Statistical analysis

In the current study, the analysis of data was performed by the Chi-Square (x2) test using SPSS ver.7.3.1.10 and EPI info ver. 7. 2 to evaluate differences in the distributions of selected demographic variables between precancerous cervical intraepithelial neoplasia I, II, and II (CIN I, II and III) and Squamous cell carcinoma (SCC) cases with metaplasia group which were considered as normal cases. Besides, genotype and allele frequencies polymorphisms in TNF-α-238 and TNF-α-308 in the three mentioned groups were evaluated using Chi-squared test. A probability level of P-value less than 0.05 was considered to be statistically significant.

Results:

In current study, the number of 58 sample with the simple random sampling method were selected among of which 22 cases were categorized in metaplasia, 21 cases were in precancerous lesions group and 15 cases were affected by cervical cancer. From 22 cases of metaplasia, 21 cases of precancerous and 15 cases of cancerous lesions, 1, 13 and 12 cases were infected by HPV respectively.

The histopathology results of 58 samples included 37.93% metaplasia, 20.68% CIN I and II, 15.51% CIN III and 25.86% SCC class. The PCR evaluation of 58 samples for the presence of HPV show that 44.82% were infected by HPV in a way that, 26.92% of which were infected by HPV-16, 30.76% with HPV-18, 7.7% with HPV-11, 15.4% with HPV-31, 11.53% with HPV-33 and 7.7% with HPV-35 were infected (Figure 1a). Besides, 4.53% of HPV infected cases were classified in metaplasia, 58.33% were in CIN I and II, 66.66% in CIN III and 80% were in SCC category (Table 2).

In current study the level of GG genotype in the TNF-α -308 polymorphism in SCC group had significant difference compared with metaplasia group (P: 0.0031). Also, amount of (AG+GG) in recessive model (P:0.003) and (G) in allelic model (P< 0.001) showed a significant difference in SCC compared with metaplasia group (Table 3). The results of ARMS-PCR for the detection of -308 polymorphism in the TNF-α gene in the metaplasia group demonstrated that 14 cases had AA, 5 cases had AG and 3 cases had GG genotypes whereas, in the SCC group just 2 cases had AA genotype. 5 cases had AG and 8 cases had GG genotype. This relation in CIN1+ was 6 cases with AA genotype, 10 cases with AG genotypes and 5 cases with GG genotype. The comparison between two groups of CIN1+ and metaplasia revealed that there is a significant difference (X2 =16. 6, P<0. 0001) among genotypes of polymorphism in TNF-α-308 gene, and GG genotype frequency in the CIN group is more than metaplasia group and patients are 3 times more prone to be infected (Table 4a). Furthermore, the comparison between SCC and metaplasia group showed a significant difference (X2 = 56. 53, P<0. 000001) among genotypes of polymorphism in TNF-α-308 gene. GG genotype frequency in SCC group is more than metaplasia group and patients are 20 times more prone to be affected (Table 4b). However, there was not any significant difference in comparison of CIN1+ and SCC with metaplasia group for TNF-α-238 polymorphism.

Discussion:

Cervical cancer starts with a microinvasive phase that cannot be seen without specialized tools. Bigger lesions become noticeable and may spread into the vagina, bladder, rectum, pelvic walls, and more distant organs. This may be a multifocal condition, and additional locations might need therapy. Magnetic resonance imaging (MRI) is more frequently utilized to evaluate the severity of pelvic disease, while a chest X-ray can detect any lung metastasis. Options for treating cervical cancer depend on the disease stage, primarily including surgery, radiotherapy, and chemotherapy.

Cytology screening proved advantageous in a cohort study that indicated testing notably decreased the

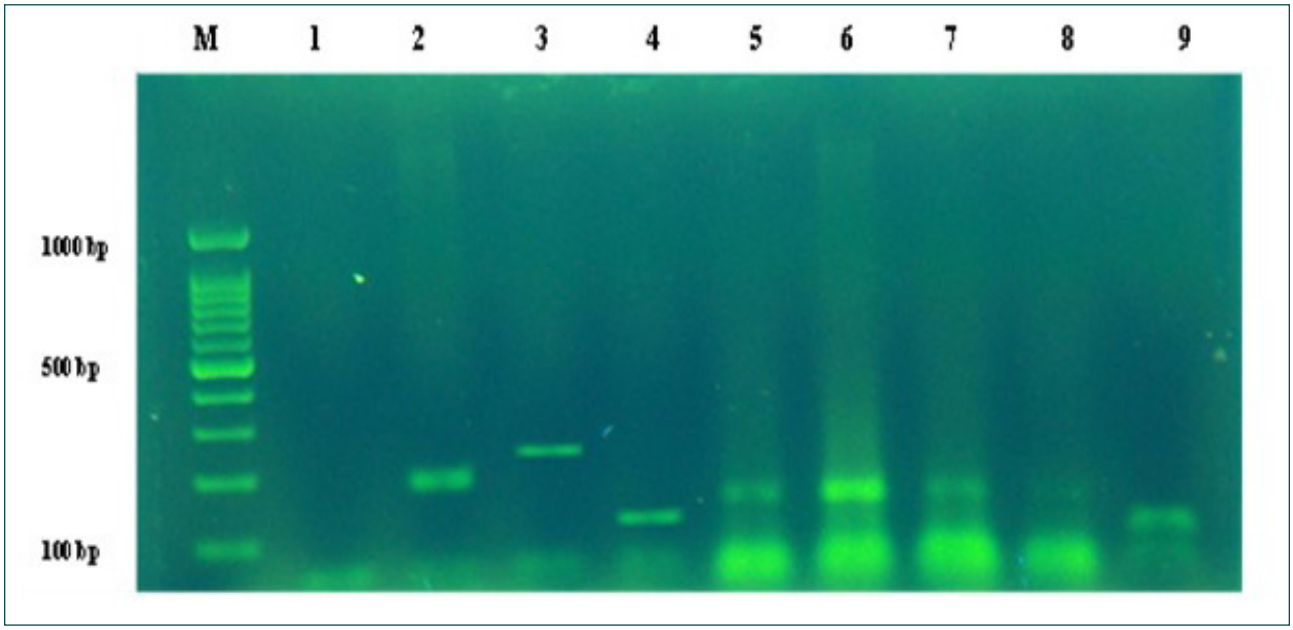

Figure 1a: Agarose gel electrophoresis result for HPV types. Beta actin is positive in all 9 wells. Well, number1: NTC. Well 2: HPV-16 positive (196bp). Well 3: HPV-35 positive (230bp). The wells 4 and 9: HPV-31 positive (153bp). The wells 5, 6, 7and8: HPV-18 (172bp).

Table 2. HPV types and lesion stages in specimens

	Metaplasia	CIN I and II	CIN III	SCC		
All (58) specimens	37.93%	20.68%	15.51%	25.86%		
HPV positive specimens	4.53%	58.33%	66.66%	80%		
HPV types in HPV positive specimens	HPV-16	HPV-18	HPV-11	HPV-31	HPV-33	HPV-35
	26.92%	30.76%	7.7%	15.4%	11.53%	7.7%

CIN: Cervical Intraepithelial Neoplasia
SCC: Squamous-cell carcinoma
HPV: Human papillomavirus

Table 3. Comparison of HPV genotypes between (SCC and CIN groups) and metaplasia group.

	Metaplasia	CIN1+	SCC	P1	OR1 (95%CI)	P2	OR2 (95%CI)	P3	OR3 (95%CI)
TNF -308	**Genotypic Model**								
AA	14	6	2	_	Ref.	_	Ref.	_	Ref.
AG	5	10	5	0. 04	4. 44 (1. 7-20. 65)	0. 056	6. 41(0. 095-61. 03)	0. 722	1. 47(0. 21-13. 98)
GG	3	5	8	0. 145	3. 67(0. 65-24. 43)	0. 0031	16. 02(2. 43-160)	1. 136	4. 43(0. 65-42. 99)
	Additive (Recessive) Model								
AA	14	6	2	_	Ref.	_	Ref.	_	Ref.
AG+GG	8	15	13	0. 026	4. 21(1. 18-16. 43)	0. 003	10. 57(2. 06-84. 41)	0. 317	2. 54(0. 45-20. 98)
	Allelic Model								
A	33	22	9	_	Ref.	_	Ref.	_	Ref.
G	11	20	21	0. 032	2. 69 (1. 09-6. 92)	P<0. 001	6. 79(2. 44-20. 11)	0. 064	2. 53 (0. 05-7. 08)

*P1= CIN 1+ vs. Metaplasia
*P2= SCC vs. Metaplasia
*P3= SCC vs. CIN1+
SCC: Squamous Cell Carcinoma
CIN: Cervical Intraepithelial Neoplasia
TNF: Tumor Necrosis Factor
HPV: Human papillomavirus

Table 4a. Comparison of CIN groups and metaplasia in TNF-α -308 polymorphism

TNFα -308	Metaplasia	CIN1+
AA	14(63. 63%)	6 (28. 57%)
AG	5 (22. 72%)	10 (47. 61%)
GG	3(13. 63%)	5 (23. 80%)

CIN: Cervical Intraepithelial Neoplasia
TNF: Tumor Necrosis Factor

Table 4b. Comparison of SCC group and metaplasia in TNF-α -308 polymorphism.

TNF -308	Metaplasia	SCC
AA	14 (63. 63%)	2 (13. 33%)
AG	5 (22. 72%)	5 (33. 33%)
GG	3 (13. 63%)	8 (53. 33%)

SCC: Squamous Cell Carcinoma
TNF: Tumor Necrosis Factor

likelihood of receiving a diagnosis of invasive cervical cancer compared to no screening (risk ratio 0.38; 95% confidence interval 0.23, 0.63). Combined data from twelve case-control studies also showed a notable protective effect of cytology screening (odds ratio 0.35; 95% confidence interval 0.30, 0.41). [25]

Features of the 29 Case-Control Studies in the Meta-Analyses involving 8850 cases and 9286 controls, revealed that the TNF-α rs1800629 (-308G>A) polymorphism increased the risk of cervical cancer under the allele genetic model (A vs. G: OR = 1.277, 95% CI = 1.104-1.477, P = 0.001) in the general population. This study which was conducted on the basis of ethnicity, also showed that a significant increase in the risk of cervical cancer was observed in the Caucasian and African female populations. This increase was observed under four different models, namely allele (A vs. G), homozygote (AA vs. GG), dominant (AA+AG vs. GG), and recessive (AA vs. AG+GG). African women exhibited a heightened risk of cervical cancer across three models: allele, homozygote, and dominant. Nonetheless, no major link was identified between the risk of cervical cancer and ethnicity among Asian women. However, no significant association was found between cervical cancer risk and ethnicity in Asian women. Moreover, following the classification of participants by their country of origin, a significant link between the TNF-α rs1800629 polymorphism and the likelihood of cervical cancer was identified in women from China and the USA. On the other hand, no similar association was observed among Iranian women. It is important to highlight that the previously mentioned link between the TNFα rs1800629 polymorphism and cervical cancer risk has been confirmed through thorough analysis of numerous studies, guaranteeing the findings' reliability and validity. [26]

In this study, it is demonstrated that about 80% of cervical cancer lesions (CC lesions) hold the most presence of HPV. This result is compatible with the study of Niakan et al. which was conducted in 1379 in the oncology section of Tehran Imam Khomeini Hospital. In this study, 60 samples of cervical cancer, Neoplastic, and carcinoma biopsies were evaluated by In_situ hybridization (ISH) and the results demonstrated the presence of the HPV genome in SCC biopsies which was about (70%) [27].

In other investigations carried out among women societies in various provinces of Iran, diverse results were detected. In the study of Allameh et al. which was carried out in the oncology clinic of Shahid Beheshti Hospital in 2011, prevalence of HPV in patients with abnormal cervical cytology was 90.8% from which 83.1% were positive for multiple HPV types 16, 18, and 11 or 6. The HPV subtype distribution among HPV-positive cases was 49.1% for HPV-16 and HPV-18, 10.1% for type 16, 1.69% for type 18, and 22% for type 11 or 6. [28]. In this study, the prevalence of HPV in precancerous groups such as CIN III was 66.6%, in CIN I and II was 58.3% and SCC was 80%. Subtypes of 18 and 31 of HPV accounted for more frequency along with malignancy progress while, HPV-33 and HPV-35 subtypes didn't have significant association with malignancy progress although they are categorized as high-risk subtypes. Thraore et al. during evaluation of association between TNF-α-308 G/A polymorphisms and high-risk subtypes of HPV, screened 91 HPV infected cases and 209 controls among sexually active women from Burkina Faso using TaqMan allelic discrimination. The results included: HPV 52 (21.19%), HPV 39 (11.86%) and HPV 33 (11.02%) as the most common HPV genotypes. Also, the results suggested no relationship between TNF-α-308 G/A polymorphisms and HPV infection [29].

In a survey carried out with Eslami et al. the number of 70 biopsy samples of cervical cancer were collected from Taleghani hospital between 2005 and 2007 and the presence and the subtypes of HPV was evaluated by Polymerase Chain Reaction. 49% of samples were found positive and HPV-16 subtype was the most prevalent type [10]. In this study however, HPV-18 was the dominant type of HPV in groups of CINI, II, III, and SCC expressing a compatibility with malignancy progress. In Munoz et al. study conducted in 1992, 436 histological cases with invasive cervical cancer and 387 controls were tested by HPV PCR. HPVs-16-18-31 and 35 subtypes showed a significant association with cervical cancer lesions [30]. As in our survey, the SCC group included HPVs-16, 18, 31, 33, 35 respectively, this study's results were compatible with Munoz's findings (Figure 1b).

In the current study, it was proven that in TNF-α -308 polymorphism, the A allele accounted for the most frequency (63.63%) in the metaplasia (control) group and the least frequency of this allele was in the CC group (13.33%). Notably, GG allele holds its high presence in the CC group (53.33%) and its tiniest presence in the metaplasia group (13.63%) (Figure 1c). In Kohaar et al. investigation, 165 histologically confirmed cases including 45 precancerous and 120 cancer patients against equal number (165) of healthy controls were assessed to detection of TNF-α promoter polymorphisms

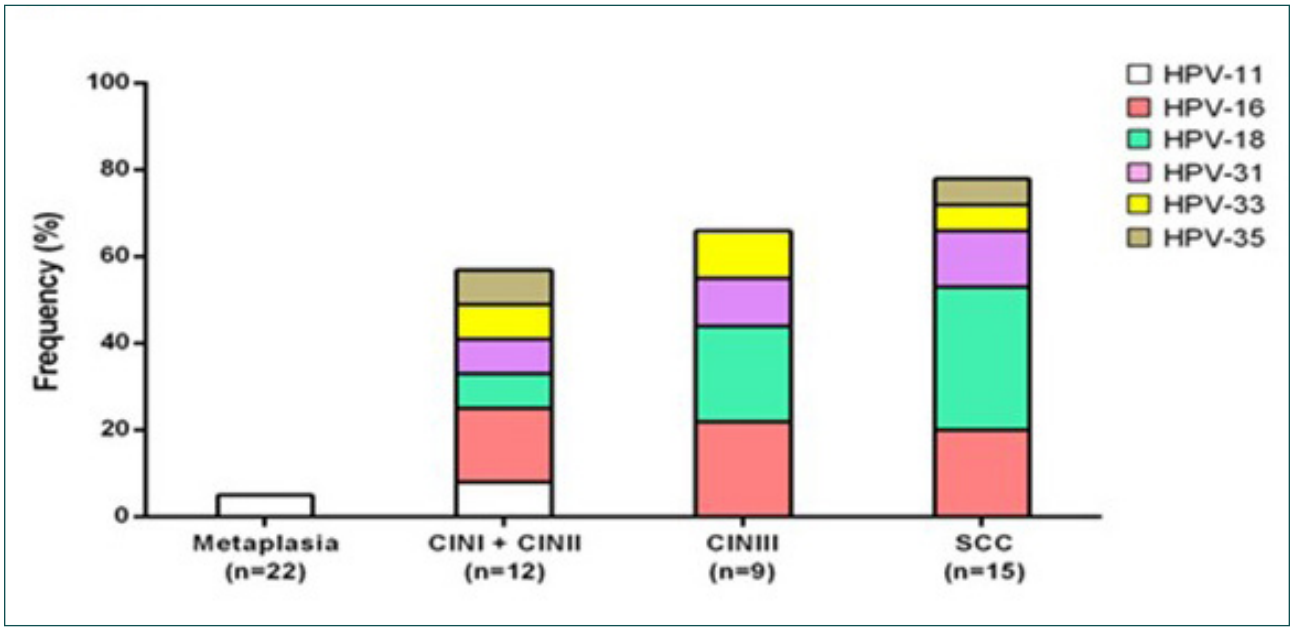

Figure 1b: The comparison of HPV types frequencies in four groups of Metaplasia, CIN I+ II, CIN III and SCC demonstrating the increase of high-risk genotypes of HPV infection such as HPV-18 and HPV-31 with the process of malignancy.

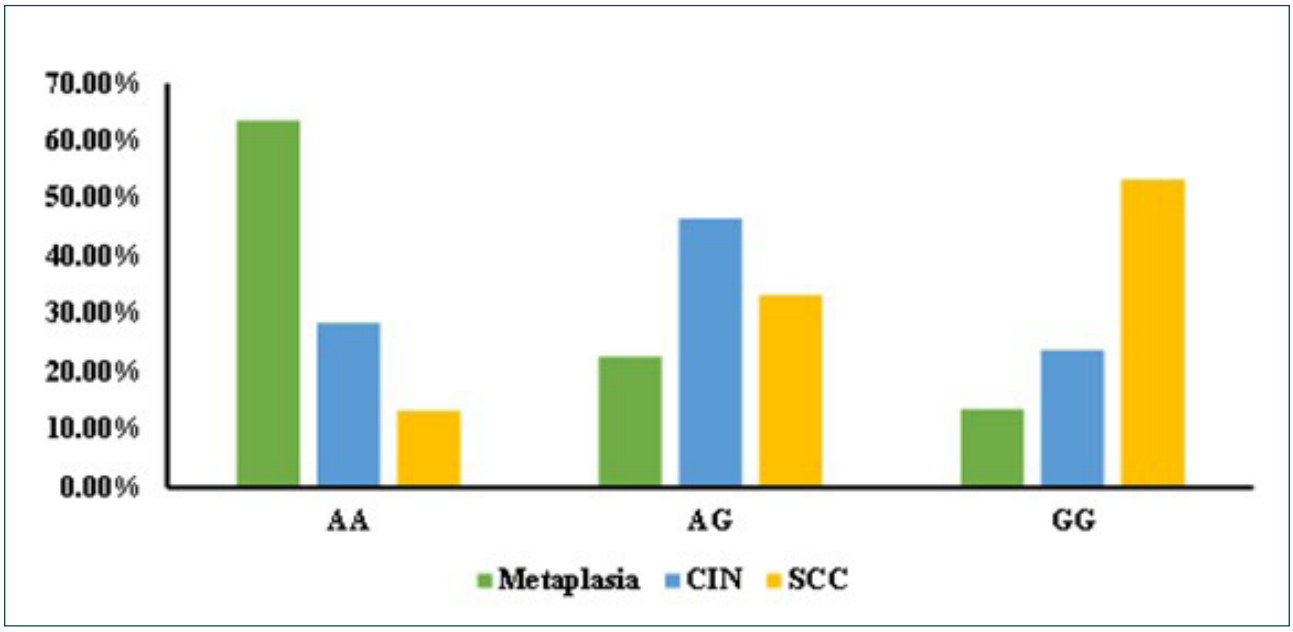

Figure 1c: The comparison of HPV genotypes in -308 polymorphism of TNF-α in three groups of Metaplasia, CIN and SCC. (P= 0.008).

using PCR-RFLP. The results revealed that the frequency of A allele in TNF-α–308 was remarkably higher in cases rather than control subjects (21% in cases vs. 9% in controls; $p < 0.01$) [31].

In the current study, the frequency of the A allele in TNF-α -308 promoter polymorphisms in metaplasia samples was 28.44%, in the CIN group was 18.96%and in the SCC group was 75.7%. Furthermore, the frequency of G allele in metaplasia group was 9.48%, in CIN was 17.24% and in SCC was 18.10% [OR=2. 69(1. 09-6. 92)

and P=0. 032]. Due to the higher frequency of the GG genotype in precancerous cases than metaplasia (control) group, G allele is considered as dangerous allele and it has made women 3 folds more prone to be infected by HPV. Furthermore, the increased frequency of GG genotype in SCC group compared with metaplasia has increased the chance of being infected by HPV about 20 times (X2 = 56. 53, P<0. 000001). In current study there was not significant relation in TNF-α-238 promoter polymorphisms among G/G, A/A and A/G genotypes with the illness progress in three groups.

In Kroeger et al. study, the presence of A allele in TNF-α-308 polymorphism of was related to increased level of cytokine production and disease severity while, in other surveys the results were highly different [32]. In general, frequency of A allele is various in different countries. In current study, the higher level of A allele in TNF-α-308 polymorphism of in metaplasia group may refer to the protective effect of this allele on the illness. In another study conducted by Liping Li et al. the correlation between the polymorphism of the TNF-α-308 gene and susceptibility to cervical cancer was evaluated using PCR-RFLP on 142 whole blood samples of patients affected by cervical cancer and 150 healthy controls. In this study, no significant differences were found in allele frequency and genotype of cervical cancer group and healthy group (P>0.05). A/A genotype elevated the risk of cervical cancer by about 1.46 folds with a 95% confidence interval of (0.32-6.67). However, different genotypes didn't show association with tumor types (P>0.05) [15].

In another survey, Sotirija Duvlis et al. assessed the association between HPV-positive CIN and CC and TNF-α-238 G/A and TNF-α-308 G/T polymorphisms Using multiplex SNaPshot analysis in 134 cases and 113 controls in women of Republic of North Macedonia. They demonstrated that the frequency of A alleles and AA genotypes in cases are not much higher than controls for both SNPs: AA of TNF-α-238 (0.7% versus 0%) and TNF-α-308 (1.5% versus 0.9%) and also for A allelic frequency (3.0% versus 1.7%) and (13.1% versus 10.6), respectively [33]. In the current study, statistical analysis in TNF-α-238 promoter polymorphisms showed that AG genotype was noticeable in metaplasia (45.45%), in CIN (47. 61%), and in the SCC group (46. 66%) (Figure 1d). Besides, women carrying A allele in -238 polymorphism were 3 times more prone to be infected by HPV-16 compared with normal cases (24% in patients with HPV infection and 9% in metaplasia group) (Table 4c and Table 4d). In a valuable study conducted by Guang Hui-Du and June Kang Wang in a large cohort of women of China, the association of TNF and IL10 genotypes with cervical cancer susceptibility was assessed. The polymorphisms in TNF (-238 G/A, -308 G/A) and IL10 (-592 C/A, -819 C/T, -1082 A/G) were genotyped. Results demonstrated that the frequency of TNF-α-238 A allele in patients was

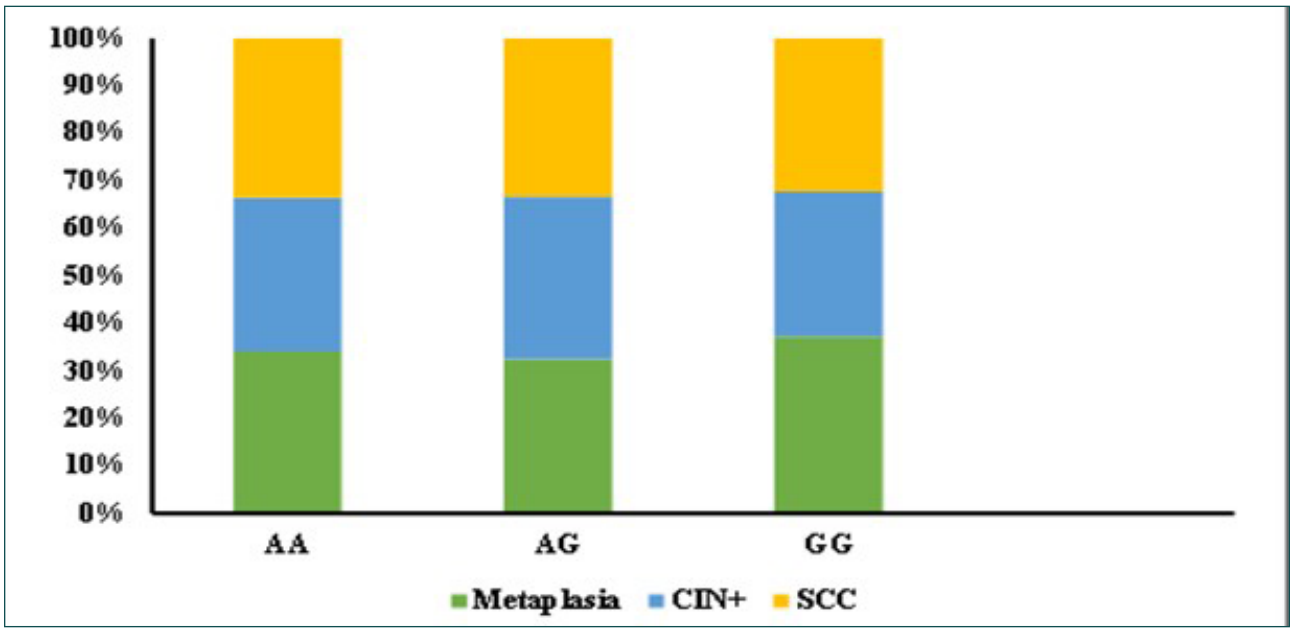

Figure 1d: The comparison of HPV genotypes in -238 polymorphism of TNF-α in three groups of Metaplasia, CIN and SCC.

Table 4c. Comparison of SCC groups and metaplasia in TNF-α -238 polymorphism

TNF -238	Metaplasia	SCC
AA	8(36. 36%)	5(33. 33%)
AG	10(45. 45%)	7(46. 66%)
GG	4(18. 18)	3(20%)

P=0. 86
OR=1.103 (0. 42-2. 85)
SCC: Squamous Cell Carcinoma
TNF: Tumor Necrosis Factor

Table 4d. Comparison of CIN groups and metaplasia in TNF-α -238 polymorphism

TNF -238	Metaplasia	CIN1+
AA	8(36. 36%)	7(33. 33%)
AG	10(45. 45%)	10(47. 61%)
GG	4(18. 18%)	5(23. 80%)

P=0. 762
OR=1.21(0. 33-4. 44)
CIN: Cervical Intraepithelial Neoplasia
TNF: Tumor Necrosis Factor

lower than controls however, the frequency of A allele in TNF-α-308 in patients was noticeably higher than controls ($p < 0.05$). They also figured out, TNF-α-308 AA and IL10-592 CA/AA genotypes may increase susceptibility to cervical cancer by altering the immune response of an individual [34].

Chagas BS et al. evaluated potential relationship between polymorphisms of IL10 and TNF-α promoter and HPV infection in the cervical carcinogenesis risk in 654 women from Brazil using PCR for HPV detection and MY11/09 PCR product for HPV genotyping. TNF-α SNP (rs1800629) genotyping was performed utilizing fluorogenic allele-specific probes. Results showed that there is a significant difference between HPV-58 infection and TNF-α (rs1800629) allelic ($p = 0.03$) and genotype ($p = 0.03$) frequencies between case and control group. In relation to TNF-α (rs1800629) allelic and genotypic and HPVs 18 and 31 infections no significant difference was found. In women affected by HPV-16 and HPV-18 with GTA and ATA haplotypes, there is a 2.32 and 3.67-fold, respectively, increased risk of susceptibility to cervical cancer. They indicated that inflammation-related genes polymorphisms display a risk to the susceptibility in cervical cancer progress in women infected by HPVs-16, 18 and 58 [35].

The privilege of this study is the simultaneous evaluation of two polymorphisms of -238 and -308 in TNF-α gene while, limited number of studies were included -238 polymorphism. And the weak point of this survey is the small society of study. Since in various races different polymorphisms in promotor region of TNF-α can be found, it is suggested to assess other polymorphisms of this region. Besides, given the association between TNF-α polymorphism and cervical cancer has remained contradictory in many results, it would be of the value if more examinations be conducted to elucidate this discrepancy.

Conclusion:

From this study it is deducted that, the G/G genotype in-308 polymorphism of TNF-α is predominant genotype in SCC group. The G allele causes the less expression of TNF-α and consequently G/G genotype can be considered as predisposing factor of acquiring infection. In general, it is concluded that the molecular test to detection of GG genotype in -308 polymorphism of TNF-α gene and HPV genotyping beside other Para-clinical tests could be considered as a prognostic feature for cervical cancer.

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Conflicts of Interest:
The authors declared no conflict of interest.

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