

# Expression of Toll-like Receptor 9 in Cervical Intraepithelial Neoplasia from Mexican Women

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## Abstract

**Introduction:** Cervical cancer is a major health problem, is the second leading cause of cancer death in women worldwide and the first in developing countries. Toll-like receptors play an important role in the immune system through the recognition of pathogen-associated molecular patterns and damage associated with endogenous molecules, so that the role of Toll-like receptors is being explored in cancer.

**Objective:** To evaluate the expression of Toll-like receptor 9 (TLR9) in cervical intraepithelial neoplasia grade I, II and III tissues using immunohistochemical staining.

**Material and Methods:** Retrospective, observational and descriptive study, the expression of TLR9 in cervical intraepithelial neoplasia grade I (n=124), II (n=68) and III (n=29) tissues were evaluated using immunohistochemical staining. All samples used in this study were from Mexican women.

**Results:** We found the expression levels of TLR9 was higher in CIN I ( $p < 0.001$ ) and CIN-II ( $p < 0.001$ ) than in CIN-III.

**Conclusion:** The low expression of TLR9 may play important roles in the development and progression of cervical intraepithelial neoplasia in Mexican women.

**Keywords:** TLR9; Cervical cancer; HPV and CIN

## Introduction

Worldwide, cervical cancer is the second most common cancer in women, according to the World Health Organization (WHO), every year 5,000 women worldwide develop cervical cancer and 270,000 die, 80% of these occur in developing countries [1]. High-risk Human Papilloma Virus (HPV) is the primary factor in cervical tumor genesis. Studies have shown that approximately 40% of sexual active women have high-risk HPV infection, but in 1% of patients, the HPV infection cannot be cured and ultimately leads to cervical cancer [2]. These findings suggest that the immune system play a critical role in the incidence of cervical cancer.

Innate immune system is the first line of defense against infection, playing a role in infections resistance before the acquired immune response is activated. One of the receptors on innate and acquired immune system cells and other tissues are Toll-like receptors (TLRs) [3]. The fundamental role of these receptors in host defense is the regulation of immune response, because the TLRs are responsible for the highly conserved pattern recognition in families of microorganisms known as Pathogen-Associated Molecular Patterns (PAMPs) or Microorganisms Associated Molecular Patterns (MAMPs) [4].

Furthermore, the TLRs are able to recognize endogenous molecules associated with cell damage known as Damage-Associated Molecular Patterns (DAMPs), the recognition of these patterns through TLRs triggers a signaling cascade that results in the synthesis and release of inflammatory mediators as cytokines and chemokines and cell activation [3,4]. TLR9 play a critical role in antiviral immunity by activating the production of interferon (IFN) type I (IFN- $\alpha$ , IFN- $\beta$ ) [5]. The presences of IFN induce antiviral, antitumor and immune-regulatory activity that protects surrounding cells [5]. The role of TLRs in tumorigenesis and progression of cancer is controversial [6]. Some studies have found that abnormal expression and signaling in cancer cells promotes tumor progression [6-8]. In this study we measured the

expression levels of TLR9 in Cervical Intraepithelial Neoplasia (CIN) of Mexican women.

## Material and methods

### Case information and specimen source

A total of 221 paraffin-embedded tissues were collected from the Archives of Pathology of "Hospital Juárez de México" between November 2008 and June 2010.

### Immunohistochemistry

The streptavidin/peroxidase immunohistochemical assay was performed on formalin-fixed and paraffin-embedded samples. Slides were conventionally dewaxed by xylene and hydrated with gradient alcohol, and antigen was then retrieved using a microwave. Endogenous peroxidase was blocked with 3% H<sub>2</sub>O<sub>2</sub>. Slides were incubated with primary antibody (rabbit polyclonal anti-TLR9 antibodies, H-100, Santa Cruz Biotechnology, Danvers, MA) as a negative control. In the following day, secondary antibody was added, followed by DAB detection (DakoCytomation, Cambridgeshire, UK), hematoxylin staining and conventional dehydration. Finally, the slides were clearly

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mounted. Immunohistochemical results were observed independently by two pathologists.

### Statistical analysis

The Fisher's exact test was used for multiple comparisons between each two groups. All the values presented in this study were two sided and the significance level was set to less than 0.05. The statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA).

### Results

The review of 221 Cervical Intraepithelial Neoplasia (CIN) tissues from 2008 to 2010. The 51.2% (n=124) were CIN-I, the 28.1% (n=68) were CIN-II and 12% (n=29) were CIN III. The age of the patients was 38 to 74 years with mean age of 57 years, most between 50 and 59 years (44.8%), all the patients were from Mexico.

The expressions of TLR9 were defined as light or dark brown granules in cytoplasm. Immunohistochemical staining was scored according to the percentage of positive cells and staining degree: 0, ≤ 10 % positive; 1, 11% to 25% positive; 2, 26 % to50% positive; 3, 51% to 75% positive and 4, ≥ 76% positive; Light yellow for score 1, yellow for score 2 and brown for score 3. The two scores were multiplied and the final scores ranged from 0 to 12. Then, a medium score was calculated. The tissues with scores equal to or more than median were defined as the high expression group, and those with scores less than median were defined as the low expression group (Table 1).

Immunohistochemistry results showed that the expression levels of TLR9 were higher in CIN-I than in CIN-III (p<0.001) and the expression levels of TLR9 were higher in CIN-II than in CIN-III (p<0.001). No difference was found in the expression levels of TLR9 in CIN-I with CIN II (p=0.48) (Figure 1).

### Discussion

The majority of HPV infections do not lead to cytological anomalies or cancer because they are cleared by the immune system in a relatively short time (6-18 mo). Only a small percentage of infections promote the development of low- and/or high-grade CIN, which may

regress or progress to invasive carcinoma [9]. Thus, as the failure of the immune response is a key component for the developments of these cancer enhancing immunity is imperative to prevent viral cellular transformations. Recent studies have demonstrated that recognition of PAMPs by TLR9 can promote immune response against viruses and deteriorate virus induced diseases [10]. After the TLR mediated signaling pathway is activated, proinflammatory cytokines are produced, antigen presenting cells are activated and innate immunity and acquired immune response is initiated. However, other studies have demonstrated that TLR4, TLR7, and TLR9 are highly expressed in a variety of solid tumors, such as ovarian cancer [11], gastric cancer [12], colon cancer [13] and prostate cancer [14], and can promote tumor development. Thus, in addition to their function of activating immune response, TLRs may establish a suitable microenvironment for tumor cell growth, which allows tumor cells to evade immune cells, infiltrate and metastasize, and undergo malignant progression [15].

In the preset study, we found that the expression levels of TLR9 were significantly higher in CIN-I and CIN-II tissues than in CIN -III in Mexican women. The expression levels gradually decreased along with the grade of CIN. The expression levels were mainly very low in CIN-III but high in CIN-II, suggesting that expression of TLR9 are early events in the development of cervical cancer in Mexican women.

Other studies that support our observations are HPV165 E6 and E7 directly inhibit TLR9- mediated pathways by down-regulating the transcriptions of the TLR9 gene, also the TLR9 level were reduced in HPV16 positive cervical cancer-derived cell lines [16].

### Conclusions

Our results suggest that low expression of TLR9 may play important roles in the development and progression of the Cervical Intraepithelial neoplasia grade. Therefore, further studying the differences between distinct populations and looking for the function of TLR9 in cervical carcinoma will provide a basis for cancer-specific gene and immune therapy.

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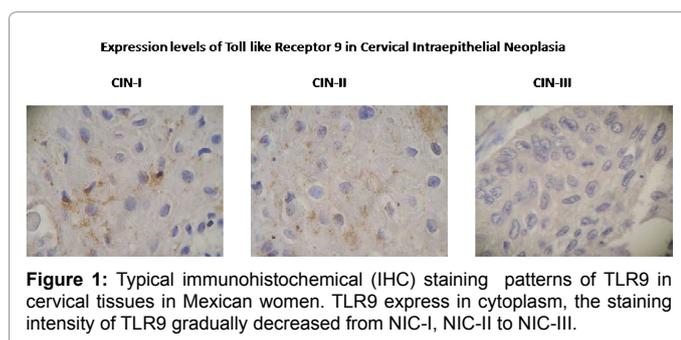
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Clinicopathologic feature	No. of Case	TLR9 expression	
		High	Low
CIN grade I	124	84 (67.7)	40 (32.3)
CIN grade II	68	48 (70.6)	20 (29.4)
CIN grade III	29	2 (6.9)	27 (93.1)

All values are presented as numbers of cases with percentage in parentheses. CIN: Cervical Intraepithelial neoplasia

**Table 1:** TLR9 Expression and the clinicopathological features of cervical tissues.



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