

## RESEARCH ARTICLE

# Evaluation of the Relationship between Cervical Intraepithelial Neoplasia Grades and Connexin 43

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## Abstract

**Objective:** Cervical intraepithelial neoplasia (CIN) is a premalign cervical disease. The microscopic features of CIN indicates an alteration leading to dedifferentiation and loss of maturation in the squamous epithelium. Disruption of connexins are frequently reported in malignant cell lines. Here, it is aimed to show the relationship between the dysplasia grades and connexin.

**Methods:** 79 cases were included in the study who were referred to the pathology department between 2014 and 2015 and who had CIN (grade 1, 2, 3). Sections of 3 micrometer thickness were taken from the paraffin blocks of the uterus on the polylysine slide. Cx43 antibody with ABC technique were performed to these sections. Staining cells were defined as positive. The cases were graded according to the intensity of the staining.

**Results:** The distribution of 79 dysplastic cases was as follows. 41 of these cases had CIN 1. The average age of these women was 44.93. 16 women were diagnosed with CIN2. The average age of these women was 42.06. 22 women were diagnosed with CIN 3. The average age of the women was 48.87.

**Conclusion:** In this study, complete loss of Cx43 expression was observed in all dysplastic cervical cases.

**Key words:** Cervical intraepithelial neoplasia, degree of dysplasia, Cx43

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## Introduction

Cervical cancer is seen all over the world, after breast and colorectal cancers in women. It is still the second most frequent cancer in developing countries, in the developed countries frequency ranks has decreased to the 10th from sixth with help of the main screening programs (Parkin et al., 2005; Ferlay et al., 2010).

Cervical intraepithelial neoplasia (CIN) is a premalign cervical disease, also called cervical squamous intraepithelial lesion (LSIL). The microscopic features of CIN indicate an alteration leading to dedifferentiation and loss of maturation in the squamous epithelium; which is characterized by proliferation abnormalities in the basal and parabasal layers (Atasü and Aydın, 1996).

Gap junctions (GJ) play an important role in the proliferation, differentiation, migration and other cellular functions of cells, and homeostasis and tumor suppression. GJs play a role in intercellular communication in many tissues and organs with

epithelium, muscle and nerve tissues (Hong and Lim, 2008).

The connexins (Cx's) are structural precursors of GJs. Cx's are widely distributed in all mammalian cells. Recent studies have shown that Cx's can affect cellular homeostatic balance independently of intercellular communication (Ferlay et al., 2010).

There are alternative mechanisms that can sustain tissue function if one of the molecules of the Cx family in many cell types is mutated or not synthesized. However, in numerous studies conducted in recent years, mutations in genes encoding Cx proteins have been found to result in severe and chronic illnesses. In many cases, single-point mutations cause dramatic consequences due to the insufficient amount of Cx and the lack of internalization (Atasü and Aydınli, 1996). The Cx classification is based on two systems. The first refers to the molecular weight predicted from the cDNA sequence. For example, Cx 26, Cx 32, Cx 43 refer to molecular weights of 32 kDa and 43 kDa, respectively (Beyer et al., 1987).

Although the presence of Cx is unknown, it has recently been shown that these proteins are superimposed on cell and tissue proliferation. Together with these studies, the values of Cx in cell and tissue types were tried to be revealed (Atasü and Aydınli, 1996).

There are different results in the literature showing the relation of cx 43 with the dysplasia grades. We aimed to investigate this relationship in this study.

### Methods

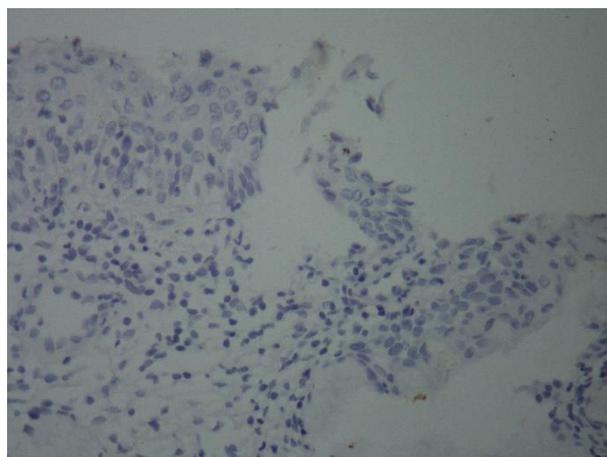
79 cases were included in the study who were referred to the pathology department between 2014 and 2015. In this cases had CIN grade 1, 2 and 3.

Sections of 3 micrometer thickness were taken from the paraffin blocks of the uterus on the polylysine slide. Cx 43 antibody with ABC technique were performed to these sections.

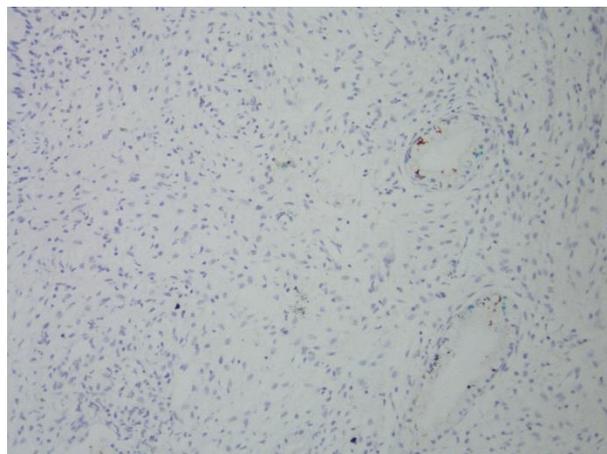
### Immunohistochemistry

The sections were held in the 60 ° C for 1 hour then were passed through xylol and alcohol steps. Tissue sections were incubated in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 10 minutes to remove the endogenous peroxidase and then were washed in distilled water for 5 minutes. Antigen was retrieved through retrieval step. Immunohistochemical staining was performed with Avidin-Biotin Peroxidase Complex (ABC) technique. The antigen was washed in PBS after the retrieval protocol.

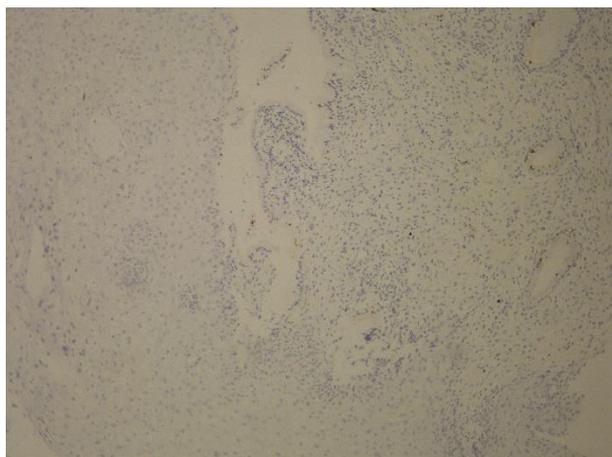
Primer antibody Cx43 (dilution ratio 1: 200) was applied. The sections were then plunged into AEC (3-amino-9-ethylcarbazole) chromogen substrate (10 minutes), washed with water, stained with hematoxylin (3 minutes) and covered with mounting medium. The stained sections were examined with a Nikon eclipse Niu microscope and photos were taken. Immunohistochemically stained preparations were examined. Cells showing staining were evaluated as positive. It was noticed that it was stained in endocervical glands and that there was no staining in the squamous epithelium (Fig. 1-3).



**Figure 1.** No staining in the presence of CIN-3 (Cx43x400)



**Figure 2.** Staining was observed in endocervical glands (Cx43x200)



**Figure 3.** No staining in CIN-1 epithelium, there is a weak staining in the endocervical epithelium (Cx43x100).

**Statistical Analysis**

Descriptive values that measured in the study are shown as mean, minimum, and maximum.

**Results**

41 of these cases were diagnosed with CIN 1. The average age of these women was 44.93. The ages were ranged between 31 to 66 years. 16 women were diagnosed with CIN 2. The average age of these women was 42.06 and the ages were ranged between 28 to 65. 22 women were diagnosed with CIN 3. The average age of the women was 48.87 and the ages were ranged between 32 to 63 years.

CIN1, CIN2, and CIN3 were found to have grade 0 (negative) in staining with Cx43. Positive staining was observed in endocervical glands in all cases. There was no meaningful relationship between dysplasia levels and staining. Since dysplastic areas were stained negative with Cx 43, they could not be evaluated statistically according to grades (table-1).

**Table-1.** Cx 43 staining results in dysplastic epithelium according to grades

CIN grades	Number	Cx 43 stain		Total
		Negative	Positive	
I	41	41	0	41
II	16	16	0	16
III	22	22	0	22
Total				79

**Discussion**

Some Cx's are very specific and are expressed in many tissues. One of them, Cx 43 has been reported to be expressed in 35% of the excess tissue (Beyer et al., 1995; el Aoumari et al., 1990).

The loss of cell junctional communication (GJIC) is due to abnormal proliferation and increased neoplastic phenotype. Several human tumors, including HeLa and cervical carcinoma line, have been reported to be inadequate for expression of the gap junction protein Cx43 and GJIC. To determine whether this is an early event in the carcinogenesis, King et al. screen a series of cervical biopsies using immunohistochemical techniques. They showed that there was a large reduction in Cx43 expression in the dysplasia regions as a result of the study (King et al., 2000).

There is extensive literature knowledge that suggests that these junctions are associated with cellular growth control and tissue differentiation, and tumor suppressor. It has been suggested that impairment of intracellular communication of GJ protein expression, abnormal cytoplasmic localization. Gap junctions are important events in carcinogenesis, invasion and metastasis (Nicolson et al.,1988; Carystinos et al., 2001). However, the role of Cx's in carcinogenesis and metastasis is controversial, since it is still unclear whether Cx expression is required for invasion and metastasis (Carystinos et al., 2001).

In a study by Bišanin et al., Cx43 expression was reported to be high in high grade dysplastic adenomas (p = 0.047), large adenomas (p = 0.015) and villous adenomas (p = 0.02) (Bišanin et al., 2016). In adenomas, Cx 43 expression was reported to be no differences between the degree of dysplasia (p = 0.87) (Bišanin et al., 2016).

In this study, Cx 43 expression was not observed in the dysplastic epithelium so there was no change in expression according to the degree of dysplasia.

In a study carried out by Hieber et al., carotenoids have been shown to increase Cx 43 expression in message and protein levels in suprabasal layers of human keratinocytes in human and mouse fibroblasts and in organotypic cultures (Hieber et al., 2000).

In a study has been reported to be a significant observation in terms of apparent suspension formation in the growth of human tumor cells (Sutherland and Bennett, 1984). Thus, Cx43 expression strongly inhibits the in vitro marker of malignancy (Sutherland and Bennett, 1984).

Expression of Cx43 in human carcinoma cells has been shown to decrease both in vivo and in vitro (Sutherland and Bennett, 1984; Nicolson et al., 1988; King et al., 2000; Hieber et al., 2000; Carystinos et al., 2001; King et al., 2002; Bišćanin et al., 2016).

Again, in a study on connexins and cancer, dysplastic epithelium staining was observed, as well as dysplasia-free glandular epithelium staining. There was no difference between staining grade and dysplasia grade (Bertram, 2004).

There have been recent publications showing that Cx 43 was negative or weakly positive in poorly differentiated carcinoma (Puzzo et al., 2016).

In the study performed by Wilgenbus et al., benign tumors and some malign tumors were studied. They reported that renal and breast cancer and sarcomas showed a significant decrease in gap-junction proteins as opposed to normal tissue (Wilgenbus et al., 1992).

Tada et al. observed that Cx expression was weak in BCC and SCC, and expression was absent in eccrine and apocrine glands (Tada and Hashimoto, 1997).

In a study by Schneider et al., Cx 43 reported cases of basal, parabasal, and middle-layer connexin (Schneider et al., 2002).

In a study on cervical dysplasia was reported that Cx 43 expression is very low in normal cervix (100%) but, Cx 43 expression in low-grade squamous intraepithelial lesions (64%) increased in the parabasal cells. As well as loss in staining (47%), weak-full-thickness Cx 43 staining (53%) were observed in high-grade squamous intraepithelial lesions. In the same study, it was noted that Cx expression disappeared as dysplasia increased (Hagemann et al., 2012).

In this study, it was seen that there was no staining in all the layers of the dysplastic epithelium. When evaluated according to dysplasia grades, it was also seen that there was a loss of full staining. Weak staining of endocervical glands was evaluated as an internal control. Normal cervix epithelium could not be evaluated because it was not included in this study. therefore, normal epithelium and dysplasia epithelium could not be compared.

### Conclusion

In this study, no staining with Cx43 was observed in dysplastic surface epithelium, compared with normal endocervical gland epithelium. This condition is thought to be related to the reduce of gap junction protein in dysplastic

epithelium. However, no difference was found between dysplasia grade and loss of expression

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**Ethics Committee Approval:** Ethics committee approval was received for this study from in Clinical Research Ethics Committee of ORDU University.

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