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Adenomatöz ve Hiperplastik Poliplerde Konneksin 32 ve Konneksin 43 Ekspresyonu

Expression of Connexin 32 and Connexin 43 in Adenomatous Polyps and Hyperplastic Polyps

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ÖZ

Amaç: Konneksinlerin tümör baskılayıcı bir gen yoluyla normal işleyen geçit bağlantıları sağladığı düşünülse de yeni çalışmalar çeşitli karsinom ve sarkomlarda konneksinlerin anormal artışına işaret etmektedir. Bu nedenle adenomatöz ve hiperplastik poliplerde connexin 43 (Cx43) ve connexin 32 (Cx32) ekspresyonunun araştırılması amaçlandı.

Materyal ve Metot: Bu retrospektif çalışmaya 2014-2015 yılları arasında adenomatöz polipli 32 olgu ve hiperplastik polipli 42 olgu dahil edildi. Örnekler Cx43 ve Cx32 ile immünohistokimyasal boyama yöntemi ile boyandı ve değerlendirildi.

Bulgular: Çalışmamızda, hiperplastik poliplerde adenomatöz poliplere göre Cx43 ve Cx32 boyanmalarının arttığını gözlemledik (sırasıyla p=0,039, p=0,048). İstatistiksel olarak yaş, cinsiyet, yerleşim yeri ve çap ile boyama paterni arasında anlamlı bir ilişki yoktu.

Sonuç: Adenomatöz poliplerde Cx43 ve Cx32 boyanmasının azaldığı ve bu durumun displazi ile ilişkili olduğu düşünüldü.

Anahtar Kelimeler: Adenomatöz polipler, hiperplastik polipler, konneksin 32, konneksin 43

ABSTRACT

Objective: Although connexins are thought to provide normally functioning gap junctions via a tumor suppressor gene, new studies point to an abnormal increase of connexins in various carcinomas and sarcomas. Therefore it was aimed to investigate the expression of connexin 43 (Cx43) and connexin 32 (Cx32) in adenomatous and hyperplastic polyps.

Materials and Methods: This retrospective study included 32 cases with adenomatous polyps and 42 cases with hyperplastic polyps, between 2014 and 2015. The samples were stained with immunohistochemical staining method with Cx43 and Cx32 and evaluated.

Results: In our study, we observed that Cx43 and Cx32 staining increased in hyperplastic polyps compared to adenomatous polyps (p=0.039, p=0.048 respectively). Statistically, there were no significant correlations between age, sex, location, and diameter with staining pattern.

Conclusion: Loss of Cx43 and Cx32 staining in adenomatous polyps was noted and thought to be related to dysplasia.

Keywords: Adenomatous polyps, connexin 32, connexin 43, hyperplastic polyps

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INTRODUCTION

Gap junctions (GJs) found in all cell types except red blood cells, platelets, some neurons, skeletal muscle fibers, and spermatozoids are essential for cell differentiation, tissue physiology and normal functions of body structures.[1] One of the most important functions of the cell is to provide homeostasis. If the homeostatic condition deteriorates, a variety of diseases can develop, including malignant tumors. Cell-to-cell communication is achieved via GJs. Gap junction is partly responsible for the proliferation of cell-to-cell (GJIC) controls, differentiation, migration, homeostasis, and tumor suppression.[2]

GJs allow a direct exchange of small molecules that plays a part in cellular differentiation, apoptosis, cell proliferation, and biological signal transduction.[3,4] GJ channel proteins are connexins (Cx), innexins, and pannexins.[5] Cx found in the normal and tumoral human cells. Depending on the biological system, Cx can both stimulate and inhibit tumor-initiating cells. However, connexin 43 (Cx43) and connexin 32 (Cx32), are important members of the Cx family.[6] Recently, many studies have been conducted to investigate the relationship between Cx32 and Cx43 with cancer. These studies reported that Cx32 and Cx43 showed different levels of expression in different cancer types.[7]

Colorectal cancers are an important public health problem. According to GLOBOCAN 2020 data, colorectal cancer is the second most deadly and third most commonly diagnosed cancer in the World.[8] Besides, few studies have been conducted investigating the relationship between colorectal cancers and Cx. The adenomas-carcinoma line has screening and surveillance potential. Therefore, the relationship between the change in Cx expression and the degree of tumorigenicity should be investigated. However, it is also important to understand which Cx expression and function affect which cancer phenotypes. Therefore, in this study, the importance of Cx43 and Cx32 expression and expression differences in hyperplastic polyps as non-precursors of colon cancer and adenomatous polyps as precursors of colon cancer were examined.

MATERIALS AND METHODS

Our study was approved by the Ordu Ethics Committee (Date: 29/04/2016, decision no: 2016/41).

This retrospective study included 32 cases with adenomatous polyps and 42 cases with hyperplastic polyps, between 2014 and 2015. 30 of the cases were female and 44 of the cases were male. The lesions were 5 mm or smaller in diameter in 51 cases and 6 mm and larger in 23 cases. This study is a retrospective cross-sectional study. In this study, biopsy materials sent to the pathology laboratory were used for analysis. The sections were kept at 60 ° C for 1 hour, followed by xylol and alcohol steps. The sections were incubated in 3% hydrogen peroxide solution for 10 minutes, then washed in distilled water for 5 minutes. The antigen retrieval was applied. Immunohistochemical staining was performed using the avidin-biotin complex technique. Then the sections were washed three times for 2 minutes with PBS (phosphate buffer solution). The sections stained with Cx32 (recombinant anti-connexin 32/GJB1 antibody [EPR8036(2)/ab181374]; dilution ratio 1:200) and Cx43 (recombinant anti-connexin 43/ GJA1 antibody [EPR21153/ab217676]; dilution ratio 1:200) by immunohistochemical staining method. The preparations were evaluated under a light microscope and graded as none staining, light, medium, severe as modified from Poyet et al.[9]

Statistical Analysis of Data: Chi-square analysis and Fisher's exact test were used to investigate the relationship between Cx43 and Cx32 staining results and the sex, age, type, subtype, and location of the patients after evaluation in the study. To ensure the continuity hypothesis, the number of expected values for each cell must be at least 5. For this reason, rows or columns with an expected value of <5 are combined with other rows and columns (values for children under 50 years, between 51-70 years, moderate staining with mild staining appearance, diameter less than 5 mm with findings of 6-10 mm).

Besides, the effect of the risk of staining for significant results was determined by bivariate logistic regression analysis. Multivariate logistic regression analysis was not performed for the study ($p<0.5$) only because the effect of the type was significant. All statistical calculations were made in the SPSS 19.0 V statistical package program and are expressed in terms of findings and percentages.

RESULTS

This study included 74 cases. 11 of the cases were over 50 years old and 63 of them were under 51 years old. According to the location, there were 50 cases with sigmoid and rectum involvement, while 24 cases had involvement in other parts of the colon.

The results of the connexin Cx43 and Cx32 staining after pathological examination on the samples taken from the patients, depending on the gender of the patients (Figure 1-4). It was determined that the results of Cx43 and Cx32 staining did not change according to the gender of the patients ($P=0.215$ and ($p=0.719$), respectively). It was determined that Cx43 and Cx32 staining results weren't significantly different in terms of ages of the patients ($p=0.758$, $p=0.437$, respectively).

Table 1 shows whether the results of Cx43 and Cx32 staining changed according to tumor type. The results of Cx43 staining were found to be altered according to tumor type ($p=0.039$). As a result of bivariate logistic regression analysis, the risk of hyperplastic polyp type is 7.593 times more than adenomatous polyp type. Cx32 staining also changed accor-

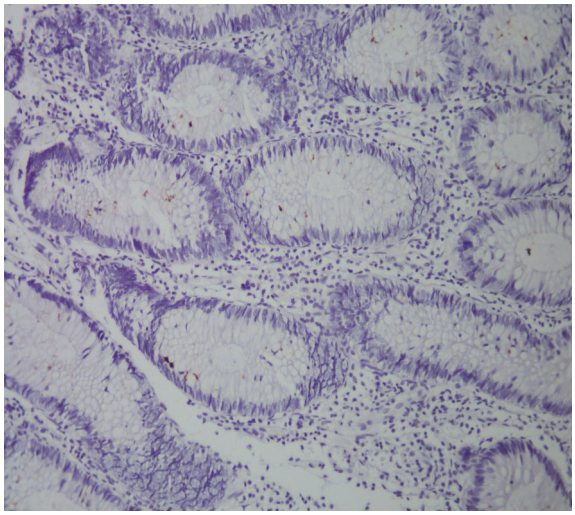

Figure 1. Cx32 positivity in adenomatous polyps (x200).

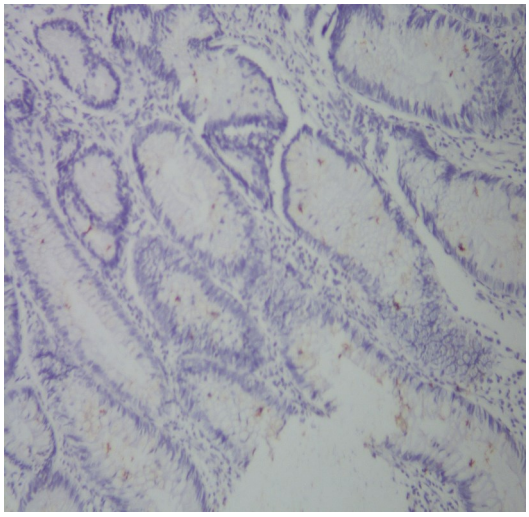

Figure 2. Cx43 positivity (mild) adenomatous polyps(x200).

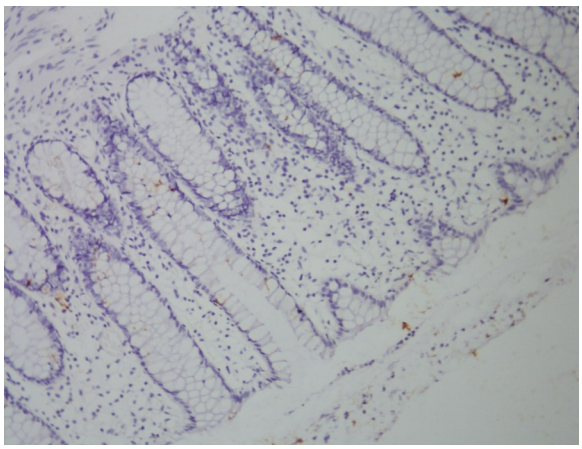

Figure 3. Cx32 positivity (mild) in hyperplastic polyps (x200).

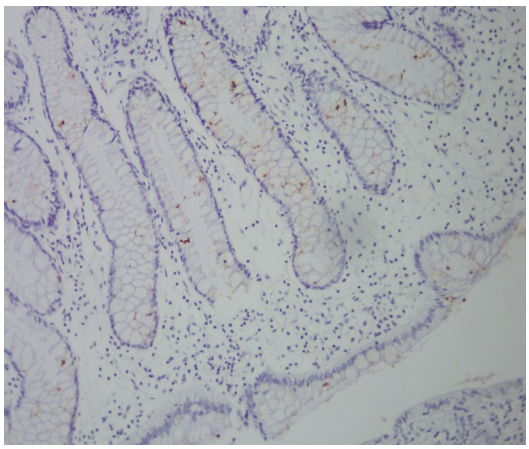

Figure 4. Cx43 positivity (moderate) hyperplastic polyps (x200).

ding to the tumor type of the patients (p=0.048). As a result of the bivariate logistic regression analysis, the risk of hyperplastic polyp type was 4.333 times higher than the adenomatous polyp type. When staining was evaluated according to the location of the tumor, it was found that there was no staining difference depended on location in both Cx43 and Cx32 groups (p=1.000, p=0.740, respectively). The size of

Table 1. Evaluation of Cx43 and Cx32 staining results according to tumor type.

Type	Staining Cx43		Total
	Negative n (%)	Positive n (%)	
Hyperplastic polyp	1 (2.4)	41 (97.6)	42 (100.0)
Adenomatous polyp	5 (15.6)	27(84.4)	32 (100.0)
Total	6 (8.1)	13 (91.9)	74 (100.0)
χ^2: 4.276 P= 0.039			
Staining Cx 32			
Type	**Negativen (%)**	**Positiven (%)**	**Total**
Hyperplastic polyp	3 (7.1)	39 (92.9)	42 (100.0)
Adenomatous polyp	8 (25.0)	24 (75.0)	32 (100.0)
Total	11 (14.9)	63 (85.1)	74 (100.0)
χ^2: 4.576 P= 0.048			

Cx43: Connexin 43; Cx32: Connexin 32.

the tumor didn't have any significant effect on staining density in Cx43 and Cx 32groups (p=0.659, p=0.485 respectively).

DISCUSSION AND CONCLUSION

In this study, we determined that Cx43 and Cx32 staining did not change according to the location, size, gender, or age of the patients but despite that changed according to tumor type. Cx43 and Cx32 staining increased in hyperplastic polyps compared to adenomatous polyps.

There is extensive literature knowledge related to GJ and cellular growth control and, tissue differentiation. It has been reported that conditions such as loss of GJ protein expression, abnormal cytoplasmic localization, and impairment of GJ intracellular communication may play an important role in carcinogenesis, invasion, and metastasis.[10] Normal membranous expression of Cx has tumor suppressor activity that regulates tumor progression by regulating cell proliferation and differentiation. However, the role of Cx in carcinogenesis and metastasis is controversial. Because it is still unclear whether Cx expression is necessary for invasion and metastasis.[11]

The role of Cx43 and Cx32 in colon physiology is not fully understood at this time. In the literature, studies on malignant tumors have shown that some have decreased, while others have increased in expression.[7,12] Studies related to dysplasia have been reported to be unclear or decrease in expression. Abnormal Cx43 and Cx32 expression have been found in variable tumor types and it is related to tumor vasculature and metastases.[12-14] It has been reported that the inactivation of Cx 43 by frequently mutating in colon tumors may play a role in the pathophysiology of colorectal carcinogenesis.[15] Mutations observed in polyps in adenomatous polyposis coli (APC) and familial adenomatous coli syndrome are among the main causes of colon cancers (40-80% and 100% respectively).[16-18] Van der Heyden et al.[19] observed that Wnt1 overexpression in PC12 cells mediated increased GJIC-mediated electrical and chemical connections. These results show that increased expression of Cx43 mRNA is associated. It has also been reported that the induction of Wnt1 expression in a mammary epithelial cell line results in an increase in the expression of the GJIC and Cx43 protein. In the absence of functional APC, β-catenin accumulates in the nucleus and is expressed here as a gap junction protein capable of transcription of many genes including Cx43, COX-2, cyclin-D1, and PPARδ.[20-22] For this reason, Cx 43 is thought to have a potential role in colorectal carcinogenesis.

Yusheng et al.[11] have demonstrated that colorectal adenocarcinomas frequently express both beta-catenin and Cx43 in the same or different cells with Cx43 and beta-catenin. Besides, deregulation of beta -catenin signaling has been reported to play an important role in the development of colon cancer.[23] However, these two factors have failed to correlate with in situ expression.

Hieber et al.[24] have shown that carotenoids increase Cx43 expression in the message and protein levels in suprabasal layers of human keratinocytes in human and mouse fibroblasts and organotypic cultures. This observation has been reported to be a significant observation in terms of apparent suspension formation in the growth of human tumor cells. Thus, it has been suggested that Cx43 expression strongly inhibits the in vitro marker of malignancy.[25] In another study in the same direction, Bertram[26] reported that increasing Cx43 expression reduced normal and neoplastic tissue proliferation, decreased dysplastic tissue, and reduced tumor progression. In a study by Puzzo et al.,[27] they reported that the expression of connexin Cx43 in poorly differentiated carcinoma was negative or poorly stained.

In the study performed by Wilgenbus et al.,[28] benign tumors, and some malign tumors were studied. Breast cancer, renal-cell cancer and sarcomas showed a significant decrease in gap-junction proteins CX 26, Cx32, and Cx43 as opposed to normal tissue. Danos et al.[29] reported a positive correlation between expression of connexin 43 and patient surveys and head and neck carcinomas.

In this study, hyperplastic polyps and adenomatous polyps were evaluated and it was noted that less expression was observed in adenomatous polyps than hyperplastic polyps. Therefore, we suggest that Cx43 and Cx32 expressions can be used as a potential therapeutic and prognostic markers.

Ethics Committee Approval: Our study was approved by the Ordu Ethics Committee (Date: 29/04/2016, decision no: 2016/41).

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Ruch RJ, Porter S, Koffler LD, Dwyer-Nield LD, Malkinson AM. Defective gap junctional intercellular communication in lung cancer: loss of an important mediator of tissue homeostasis and phenotypic regulation. Exp Lung Res. 2001;27(3):231-243. doi:10.1080/019021401300053984
2. Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annu Rev Biochem. 1996;65:475-502. doi:10.1146/annurev.bi.65.070196.002355
3. Kanczuga-Koda L, Sulkowski S, Lenczewski A, et al. Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer. J Clin Pathol. 2006;59(4):429-433. doi:10.1136/jcp.2005.029272
4. Krutovskikh VA, Piccoli C, Yamasaki H. Gap junction intercellular communication propagates cell death in cancerous cells. Oncogene. 2002;21 (13):1989-1999. doi:10.1038/sj.onc.1205187
5. Scemes E, Suadicani SO, Dahl G, Spray DC. Connexin and pannexin mediated cell-cell communication. Neuron Glia Biol. 2007;3(3):199-208. doi:10.1017/S1740925X08000069
6. Beyer EC, Davis LM, Saffitz JE, Veenstra RD. Cardiac intercellular communication: consequences of connexin distribution and diversity. Braz J Med Biol Res. 1995;28(4):415-425.
7. Mulkearns-Hubert EE, Reizes O, Lathia JD. Connexins in Cancer: Jekyll or Hyde? Biomolecules. 2020;10;10(12):1654. doi:10.3390/biom10121654
8. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021. doi:10.3322/caac.21660
9. Poyet C, Buser L, Roudnicky F, et al. Connexin 43 expression predicts poor progression-free survival in patients with non-muscle invasive urothelial bladder cancer. J Clin Pathol. 2015;68 (10):819-824. doi:10.1136/jclinpath-2015-202898
10. el-Sabban ME, Pauli BU. Adhesion-mediated gap junctional communication between lung-metastatatic cancer cells and endothelium. Invasion Metastasis. 1994-1995;14(1-6):164-176.
11. Han Y, Zhang PJ, Chen T, Yum SW, Pasha T, Furth EE. Connexin43 Expression increases in the epithelium and stroma along the colonic neoplastic progression pathway: Implications for its oncogenic role. Gastroenterol Res Pract. 2011;2011:561719. doi:10.1155/2011/561719
12. Aasen T, Leithe E, Graham SV, Kameritsch P, Mayán MD, Mesnil M, Pogoda K, Tabernero A. Connexins in cancer: bridging the gap to the clinic. Oncogene. 2019 Jun;38(23):4429-4451. doi:10.1038/s41388-019-0741-6
13. Sheen IS, Jeng KS, Wang PC, et al. Are gap junction gene connexins 26, 32 and 43 of prognostic values in hepatocellular carcinoma? A prospective study. World J Gastroenterol. 2004;10(19):2785-2790. doi:10.3748/wjg.v10.i19.2785
14. Elzarrad MK, Haroon A, Willecke K, Dobrowolski R, Gillespie MN, Al-Mehdi AB. Connexin-43 upregulation in micrometastases and tumor vasculature and its role in tumor cell attachment to pulmonary endothelium. BMC Med. 2008;6:20. doi:10.1186/1741-7015-6-20
15. Dubina MV, Iatckii NA, Popov DE, Vasil'ev SV, Krutovskikh VA. Connexin 43, but not connexin 32, is mutated at advanced stages of human sporadic colon cancer. Oncogene. 2002;21 (32):4992-4996. doi:10.1038/sj.onc.1205630
16. Cottrell S, Bicknell D, Kaklamanis L, Bodmer WF. Molecular analysis of APC mutations in familial adenomatous polyposis and sporadic colon carcinomas. Lancet. 1992;340(8820):626-630. doi:10.1016/0140-6736(92)92169-g
17. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res. 1998;58(6):1130-1134.
18. Otori K, Konishi M, Sugiyama K, et al. Infrequent somatic mutation of the adenomatous polyposis coli gene in aberrant crypt foci of human colon tissue. Cancer. 1998;83(5):896-900. doi:10.1002/(sici)1097-0142(19980901)83
19. van der Heyden MA, Rook MB, Hermans MM, et al. Identification of connexin43 as a functional target for Wnt signalling. J Cell Sci. 1998;111(12):1741-1749.
20. Husøy T, Knutsen HK, Cruciani V, et al. Connexin43 is overexpressed in Apc (Min/+)-mice adenomas and colocalises with COX-2 in myofibroblasts. Int J Cancer. 2005;116(3):351-358. doi:10.1002/ijc.21025
21. Araki Y, Okamura S, Hussain SP, et al. Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res. 2003;63(3):728-734.

22. Shtutman M, Zhurinsky J, Simcha I, et al. The cyclin D1 gene is a target of the beta-catenin/ LEF-1 pathway. Proc Natl Acad Sci USA. 1999;96(10):5522-5527. doi:10.1073/pnas. 96. 10.5522
23. Morin PJ. Beta-catenin signaling and cancer. Bioessays. 1999;21(12):1021-30. doi:10.1002/ (SICI)1521-1878(199912)22:1<1021::AID-BIES6>3.0.CO;2-P
24. Hieber AD, King TJ, Morioka S, Fukushima LH, Franke AA, Bertram JS. Comparative effects of all-trans beta-carotene vs. 9-cis beta-carotene on carcinogen-induced neoplastic transformation and connexin 43 expression in murine 10T1/2 cells and on the differentiation of human keratinocytes. Nutr. Cancer. 2000;37:234 -244.
25. King TJ, Fukushima LH, Hieber AD, Shimabukuro KA, Sakr WA, Bertram JS. Reduced levels of connexin 43 in cervical dysplasia: inducible expression in a cervical carcinoma cell line decreases neoplastic potential with implications for tumor progression. Carcinogenesis. 2000;(21) 6:1097-1109.
26. Bertram JS. Dietary carotenoids, connexins and cancer: what is the connection? Biochem Soc Trans. 2004;32(Pt-6):985-989. doi:10.1042/ BST0320985
27. Puzzo L, Caltabiano R, Parenti R, Trapasso S, Allegra E. Connexin 43 (Cx43) expression in laryngeal squamous cell carcinomas: Preliminary data on its possible prognostic role. Head Neck Pathol. 2016;10(3):292-297. doi:10.1007/ s12105-016-0685-x
28. Wilgenbus KK, Kirkpatrick CJ, Knuechel R, Willecke K, Traub O. Expression of Cx26, Cx32 and Cx43 gap junction proteins in normal and neoplastic human tissues. Int J Cancer. 1992;51 (4):522-529. doi:10.1002/ijc.291051040
29. Dános K, Brauswetter D, Birtalan E, et al. The Potential prognostic value of connexin 43 expression in head and neck squamous cell carcinomas. Appl Immunohistochem Mol Morphol. 2016;24(7):476-481. doi:10.1097/ PAI.0000000000000212