Original Article

Down Regulation Role of DKK3 Protein in Clinical Diagnosis and Differentiation between Colonic Adenomatous Polyps and Colorectal Carcinoma: Immunohistochemical Procedure

Ammar Eesa Mahdi [1,*], Ali Zaki NAJI [1], Hadi M. Al-Mosawi [2]

[1] Basic science Department, College of Dentistry, University of Babylon, 51002 Hilla, Babylon, Iraq.
[2] Department of Pathology, Hammurabi College of Medicine, University of Babylon, 51002 Hilla, Babylon, Iraq.
[*] Corresponding author: ammarn729@gmail.com

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Abstract

Background: Colorectal cancer (CRC) is a substantial international health alarm, distributed as major 3rd effect of death. CRC is a distinctive carcinoma which can be controlled and decreased by earlier management of more risky colonic adenomas. **Objectives:** Study goals are recognized the potential role of Dkk3 in clinical diagnosis and differentiation between colorectal carcinoma and colonic adenomatous polyps by immunohistochemistry procedure. **Materials and Methods:** this research comprised from 44 colonic adenomatous polyps and 38 colorectal carcinoma which subdivided into well differentiated 11 (28.9%) cases, moderate differentiated 19 (50%) cases, 8 (21.1%) cases poorly differentiated. Paraffin specimen blocks were sending for DKK3 protein IHC. Intensity Expression of DKK3 were divided into negative intensity (0), weak intensity (1+), moderate intensity (2+), and strong intensity (3+). **Results:** 38 and 44 patients for CRC and colonic adenomatous polyps , and age factor were highly significant (p value = < 0.001) older age group (62.42±13.88) in CRC than colonic adenomatous polyps . Male sex group shows insignificant association and more distribution in both CRC and colonic adenomatous polyps. DKK3 IHC were a significant association between CRC and colonic adenomatous polyps , all colonic adenomatous cases demonstrate strong intensity of DKK3, while CRC reveal negative intensity 20 (52.6%) and weak intensity 18 (47.4%). **Conclusion:** this existing research showed principal male sex and older age in CRC, DKK3 protein biomarker had important functions as a down regulation whose is associated with cancer transformation in CRC. However, more progressive future studies are required to found specific diagnostic and therapeutic role of DKK3 in different human cancer particularly for risk cancer as CRC.

Keywords: Colorectal malignancy, Colonic adenomatous polyps, DKK3 biomarker.

Introduction

Colorectal cancer (CRC) is a substantial international highly risk medical disease [1], distributed as major 3rd effect of death that associate with this cancer , and same time CRC is tend to be rising by 2040 year [2,3]. CRC is a distinctive carcinoma which can be controlled and decreased by earlier management of more risky colonic adenomas [4]. 5-survival rate of CRC is same with other tumors that are related to degree of spread inside human and shows about 65% [5]. Diagnostic tool of CRC are colonoscopy which is a extensively recognized diagnostic tool and give good results at initial stages of CRC, however colonoscopy have some complications such as hemorrhage throughout cutting specimens or polypectomy [6]. Update, innovation molecular procedure have most important diagnostic tool in detection a many cancers types including CRC , in addition to its role in diagnosis of genetic basis of pathophysiology of CRC [7]. Main problem in

Main problem in diagnosis of CRC had no symptoms or clinical feature otherwise CRC is getting a wide cancer spreading inside patients and reaching advanced progressive stage, so CRC surveillance can be decreased the death rate and is giving excellent response to cancer treatment in earlier stage [8]. Family of Dickkopf (DKK) are extracellular set which has an Wnt antagonists [9] and divides into Dkk-1,Dkk-2,Dkk-3,Dkk-4, and Soggy (Sgy) protein which has a distinctive protein linked to Dkk-3 and related to class of Tumor endothelial markers (TEMs) [10]. Hyper-methylation of the DKK3 gene promoter was a leading causing of down-regulation of DKK3 protein [11] which can expressed various carcinoma as in digestive tract [12], and this Hyper-methylation of the DKK3 were regard as bad prognostic factor in acute lymphoblastic leukemia [13], hepatic cancer [12], stomach cancer and more specifically in CRC [14]. Proangiogenic factor of DKK3 showed by St Croix et al. [10] in which DKK3 increases their expression in CRC endothelium in comparison to endothelium that found in normal tissue of colon. Update, DKK3 have 2 role in Wnt antagonist in CRC, one is tumour suppressor and other is proangiogenic factor in vascularization, tumor suppressor role of DKK3 showed decreased colonic tissue proliferation and promote apoptosis [15]. This tumor suppressor role of DKK3 occur due methylation of DKK3 promoter gene and subsequently, DKK3 showed low expression in cancer [16, 17]. Carcinogenic mechanism of Dickkopf homolog 3 protein (DKK3) is not interpreted until now, in which DKK3 act as tumor suppressor protein by decreases capacity of colonic tissue to proliferate, and this can be used as good indicator for therapeutic aims for CRC [18]. The aim of study is exploring potential role of Dkk3 as tumor suppressor proteins in differentiate between colorectal carcinoma and colonic adenomatous polyps by applying immunohistochemistry technique, Because of few researches involved studies of structural and functional role of DKK3 in development of colorectal carcinoma and colonic adenomatous polyps.

Materials and Methods

This prospective project research was a finished through 2 years' time, gleaned 44 colonic adenomatous polyps and 38 colorectal carcinoma which subdivided into well differentiated 11 (28.9%) cases, moderate differentiated 19 (50%) cases, 8 (21.1%) cases poorly differentiated. Sex distribution are 29 female and 53 male, with ranging age between 57-82 years old, all cases are collected from Al-Imam Al-Sadiq General Hospital, Marjan Medical City Hospital and private clinics.

Paraffin piece blocks for all cases was cutting for 2 purposes , the first is for hematoxylin and eosin (H&E) slides for approved diagnosis by 2 experienced histopathologists and other purpose is for positively charged glass slides for DKK3 protein immunohistochemistry . Staging and grading of CRC and were relying on TNM staging cancer system (8th edition, 2017) [19] and WHO grading cancer system (2019) [20]. The kit of anti-DKK3 antibodies were for vitro studies only, AA 1-350 antibodies, ABIN7103807 LOT (HD12JA2040), Schloss-Rahe-Str., Germany. Slides were evaluated independently by two or more blinded pathologists and how inter-observer discre-pancies were resolved (not applying H-score or Immunoreactive Score [IRS] system), but only intensity alone was used. Intensity Expression of DKK3 staining in immunohistochemistry were divided into negative intensity (0), weak intensity (1+), moderate intensity (2+), and strong intensity (3+) [21].

Statistical analysis

Statistical interpretation was achieved by SPSS (version number 26) , Pearson's chi-squared test was analysis association of categories. Mann whitney test was analysis non-symmetrical continuous data. Significant P value is < 0.05.

Ethical approval

Ethical research was involved all patients who diagnosed colorectal carcinoma and colonic adenomatous polyps., permission and agreement were done in college of dentistry/ university of Babylon according to documented paper (NO. 3 at 4 February, 2024)

Results

Total cases of this study were 82 cases which were divided into 38 and 44 patients for CRC and colonic adenomatous polyps. Characteristics of colorectal carcinoma (38 cases) study was brief described in table 1, staging system of CRC was more presentation for T3 stage 20 (52.6%), N0 stage 21 (55.3%), and M0 stage 31 (81.6%). Grade of CRC was more presenting grade 2 19 (50%) colonic adenomatous polyps

Table 1: Clinicopathological Characteristics of Colorectal Carcinoma Patients

Parameters		CRC No. (%)
T stage	T1	6 (15.8 %)
	T2	12 (31.6%)
	T3	20 (52.6%)
N stage	N0	21 (55.3%)
	N1	12 (31.6%)
	N2	5 (13.1%)
M stage	M0	31 (81.6%)
	M1	7 (18.4%)
Grade	G1	11 (28.9%)
	G2	19 (50%)
	G3	8 (21.1%)

Demographic age data were resulting significant difference (p value = < 0.001) older age group (62.42±13.88) in CRC in compare to middle age group (47.56±8.78) in colonic adenomatous polyps. 56-75 age group were more presenting 26 (68.4%) in CRC while 35-55 age group was high percentage 27(61.4%) in colonic adenomatous polyps with highly significant association (p value = < 0.001). Male sex group shows insignificant association (p value = 0.111) and more distribution in both colorectal carcinoma and colonic adenomatous polyps (28 (73.7%) and 25 (56.8%) respectively) (table 2)

Table 2: Comparison of Demographic Parameters between Colorectal Carcinoma and Colonic Adenomatous Polyps

Parameters		CRC No. (%)	colonic adenomatous polyps No. (%)	*P* value
Age/years	Mean ± SD	62.42±13.88	47.56±8.78	**< 0.001
	Median	64	45	
Age groups/ years	35-55	8(21.1%)	27(61.4%)	*< 0.001
	56-75	26 (68.4%)	17(38.6%)	
	>76	4 (10.5%)	0	
Sex	Male	28 (73.7%)	25 (56.8%)	*0.111
	Female	10 (26.3%)	19 (43.2%)	

* Chi-Square Test

** mann-whitney u test

By means of using DKK3 protein immunohistochemistry (table 3), there were a significant association between CRC and colonic adenomatous polyps (p value = <0.001), all colonic adenomatous cases 44 (100%) demonstrate strong intensity of DKK3, while CRC reveal negative intensity 20 (52.6%) and weak intensity 18 (47.4%).

Table 3: Association of DKK3 IHC between colorectal carcinoma and colonic adenomatous polyps

Parameters		CRC No. (%)	Colonic adenomatous polyps No. (%)	*P value
Dkk3 IHC staining	Negative	20 (52.6%)	0	< 0.001
	Weak positive	18 (47.4%)	0	
	strong positive	0	44 (100%)	

*Chi-SquareTest

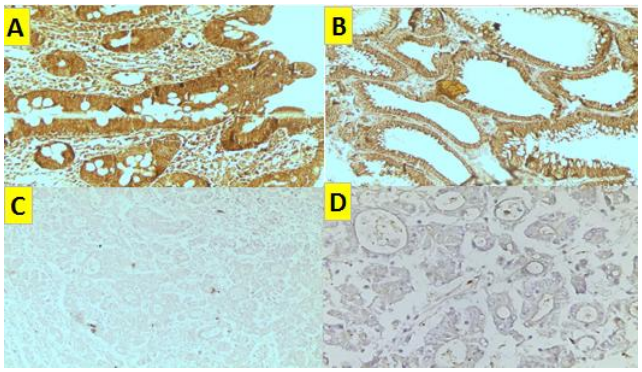

Figure 1: DKK3 intensity IHC in the colonic neoplasms; (A) and (B) colonic adenomatous polyps demonstrates strong intensity of DKK3; (C) colorectal carcinoma demonstrates negative intensity of DKK3; (D) colorectal carcinoma demonstrates weak intensity of DKK3.

Discussion

CRC is a leading cause of increasing death in worldwide [22,23], while colorectal adenoma is regarding as precancerous source of development of adenocarcinoma through DNA and pathological abnormities but its pathogenesis of molecular changes was still misguiding [24]. DKK3 protein had structural amino acid sequences differ in comparison to Dkk-1, Dkk-2, and Dkk-4 [9]. In our study, Demographic data were showing significant in older age in CRC in compare to middle age in colonic adenomatous polyps. 56-75 age group and 35-55 age group were more presenting in CRC and colonic adenomatous polyps respectively with highly significant association .Male sex group shows insignificant association and more distribution in both colorectal carcinoma and colonic adenomatous polyps. Also, expression of DKK3 in GIT tissue (stomach and colorectal tissue) was negative correlation with more advanced grade and advanced metastatic stages [25, 26] while sex and age showed no correlation with DKK3 immunohistochemical staining protein [24]. In this study, DKK3 protein biomarker that were uregulated in colonic adenoma in compared to downregulated in CRC caused a highly significant association with that illustrated by immunohistochemical analysis. So by this analysis, DKK3 had good physiological and molecular structure in colonic adenoma. These results were revealed decreased intensity of DKK3 IHC as tumor become progress from benign colonic tumor to malignant colonic cancer, and consequently low level of DKK3 enhanced development of CRC. In Yang et al. study , Dkk3 biomarker was expressed in low level that malignant colorectal tumors in experimental adenocarcinoma cell lines [15], and the expression of DKK3 was decreased in colorectal neoplasm in contrast normal adjacent tissue with association more advanced cancer grade and stages [27,26]. Pathophysiological role of DKK3 in colorectal carcinoma was illustrated as suppressing growth by arrest tissue proliferation and G2 growth phase , in addition to their apoptosis effect of DKK3 (cytosolic and secretory Dkk3) in colorectal carcinoma [24]. Also the role of DKK3 in colonic carcinoma was illustrated arrest tissue proliferation and cancer invasion, enhanced apoptosis [15]. A RT-PCR technique was applied in Shuang Zhao et al study [24] in which, DKK3 messengerRNA showed low level colorectal carcinoma in comparison to neighboring normal colonic tissue. Earlier noninvasive diagnosis of CRC can made by DDK3 protein, PKM2 enzyme and Insulin like growth factor binding protein 2, giving up 95% specificity and ranging sensitivity 57%-

76% for 1st and 2nd CRC stage metastasis, and this were regarding equal result diagnostic value for CRC in compare to fecal immunochemical test (iFOBT or FIT) [28]. Treatment of a highly progression types of CRC were noted that role of recombinant DKK3 protein when it was overexpressed prevent immigration and invasion role of CRC [24]. Moreover, recombinant and ectopic types of DKK3 had weak ability to immigration and invasion role of CRC and consequently prevent more progression of advanced CRC [24].

Conclusion

Current research showed predominant male sex and significant older age group in CRC, DKK3 protein staining had significant downreguted in CRC in comparison to upregulated in colonic adenomatous and consequently so carcinogenic role of DKK3 were tumor suppressor protein in CRC. However, advanced future researches are necessary to establish the role diagnostic and therapeutic role of DKK3 in various human carcinoma especially more risk cancer as CRC.

Data Availability

All data and materials requested to help in writing the structure of this research was involved

Conflicts of Interest

Researchers illustrate that aren't any conflict of interest.

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