IMMUNOHISTOCHEMICAL EXPRESSION OF CDX2AMONG SUDANESE PATIENTS WITH CLCORECTAL ADENOCARCINOMA

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ABSTRACT

Colorectal cancer is the fourth most common cancer in the world with approximately 875.000 new cases per year in the worldwide accounting for 8.5% of all new cancer. This study was aimed to detect CDX2 expression among Sudanese patients with colorectal cancer. This study was conducted at Soba University Hospital during the period (June 2018 to November 2019). Forty samples of paraffin wax blocks from patient diagnosed as colorectal carcinoma collected in tissue micro-array slide. The study was used a standard avidin- biotin- peroxidase complex method with monoclonal antibodies against CDX2 for its detection. SPSS version 21 was used to analyze the data. The age range was from 31-80 years old with mean of 55 years old and was devided into two groups less than 40 years old and more than 40 years old. Association between age group and CDX2 expression, showed that (33.3%) had presence of CDX2 and 4(14.3%) had absent of CDX2 among less 40 years old while (66.7%) had present of CDX2 and (85.7%) had absent of CDX2 among more than 40 years old. There was no significant difference in proportion between the two groups. Association between gender of patient and CDX2expression, the showed that male (12.5%) had present of CDX2 and (45.5%) had absent of CDX2, and show female (17.5%) had present of CDX2 and (25.0%) absent of CDX2.Association between stage of the disease and CDX2 expression, (30.8%) of C colorectal cancer patients in stage C had present of CDX2 and (69.2%) of them had absent of CDX2, while (28.6%) of colorectal cancer patients in stage D had present of CDX2 and (71.4%) of them had absent of CDX2. **Conclusion:** in this study showed CDX2 expresses in patients less than 40years with colorectal adenocarcinoma less than patients above 40years, in female more than male and expresses in stage C more than stage D.

KEYWORDS: Colorectal cancer, CDX2.

INTRODUCTION

Colorectal cancer is the fourth most common cancer in the world with approximately 875.000new cases per year in the worldwide (WHO 1996), accounting for 8.5% of all new cancer. Colorectal cancer is second most common cause of cancer related death. High risk areas are considered North America, Europe and Australia. Colorectal cancer, also known as bowel cancer, colon cancer, or rectal cancer, is any cancer that affects the colon and the rectum. these cancer starts in the colon or the rectum. Most colorectal cancer begin as growth called polyp on the inner lining of the colon or rectum some types of polyp can change into cancer over the course of several years, but not all polyps become cancer. The chance of changing into cancer depends on kind of polyps the 2 main types of polyps are (Abhishek Bhandari, 2017).

The CDX2 are home box genes are expression in the colorectal epithelium and encoded nuclear transcription factors. Over expression of Cdx genes have been shown previously to result in cellular differentiation (Abhishek Bhandari, 2017).

Tow gen has been identified in humans (CDX-1 and CDX-2) and a third gen (CDX4) in mice(Ferlay J, Bray F, 2004).

CDX1 and CDX2 are both broadly expressed in fetus but by the early neonate period become restricted to the small and large bowel and pancreas(Bosman FT, 2010).

Both genes have been reported to exhibit a gradient of expression with cdx-1 being present at the highest level caudally whereas cdx2 expression peaks in the proximal colon and decreases caudall(WHO, 2012).

CDX2 expression in colorectal cancer tissue and its possible implication in cancer development, proliferation, and migration. Only studies where the authors performed or verified their result in human

colorectal cancer tissue were included (Johnson JJ, 2007).

MATERIALS AND METHOD
This study has been conducted in Khartoum state from Soba hospital. (From June 2018 till to November 2019).

Samples were collected from Sudanese patients include both males and females.

Forty samples of paraffin wax block from patients, diagnosed ascolorectal adenocarcinoma, collected in tissue micro array slide.

Method
Section (3um) from formalin-fixed, paraffin-embedded tumors is cut and mounted onto salinized slides(thermo). Following deparaffinization in xylene, slide was rehydrated through graded series of alcohol and was placed in running water. Sample was steamed for antigen retrieval using PT-Link. Briefly, slides was placed in tank containing enough sodium citrate buffer(PH9.0) to cover the sections, then was boiled at high Temp(95) for 20 minutes then allowed sections to cool at RT. Endogenous peroxidase activity was blocked with 3% hydrogenperoxidase and methanol for 20min, then Slides was incubation with 100-200ul of primary anti body (CDX-2monoclonal mouse anti human, ready to use) for 30min at room temperature in a moisture chamber, and then was wash with PBS for 3min, binding of antibodies will be detected by incubation for 20min with dextrin polymer(thermo)

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- Finally, the section was washed in three changes of PBS, followed by adding 3,3diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce for up to 5 min. slide was counterstained with haematoxlin.

Data Analysis
- Data was processed using Statistical Packages for Social Sciences (SPSS) version 21.

RESULTS
The study include 40 patients with colorectal cancer, the age range was from 31-80 years old. Table (3.1) shows distribution of colorectal cancer patients according to age group. 40 colorectal cancer patients 8 (20%) were less than 40 years old and 32 (80%) were more than 40 years old with P-value 0.170.

Table (3.2) shows distribution of colorectal cancer patients according to sex group. 23(57.5%)male and 17(42.5%) ware female with P- value 0.185.

Table (3.3) shows distribution of colorectal cancer patients according to stage of the disease. 26 (65%) of colorectal cancer patients under study were in stage C while 14 (35%) of them were in stage D with P-value 0.591.

Table (3.4) association between age group and CDX2 expression, the analysis showed 4 (33.3%) had present of CDX2 and 4 (14.3%) had absent of CDX2 among less 40 years old while 8 (66.7%) had present of CDX2 and 24 (85.7%) had absent of CDX2 among more than 40 years old. There was no significant difference in proportion between the two groups with P-value 0.170.

Table (3.5) association between gender of pation and CDX2expression, the analysis show male 5(12.5%) had present of CDX2 and 18(45.5%) had absent of CDX2, and show female 7(17.5%) had present of CDX2 and 10(25.0%) absent of CDX2 with P- value 0.185.

Table (3.6) association between stage of the disease and CDX2 expression, 8 (30.8%) of C colorectal cancer patients in stage C had present of CDX2 and 18 (69.2%) of them had absent of CDX2, while 4 (28.6%) of colorectal cancer patients in stage D had present of CDX2 and 10 (71.4%) of them had absent of CDX2 with P-value 0.591.

Table (3.1) distribution of colorectaladenocarcinoma` patients according to age group.

Variable	Frequency	Percentage (%)
<40 Years	8	20.0%
>40 Years	32	80.0%
Total	**40**	**100.0%**
P-value	*0.170*	

Table (3.2) distribution of colorectaladenocarcinoma patients according to sex group.

Variable	Frequency	Percentage (%)
Male	23	57.5%
Female	17	42.5%
Total	**40**	**100%**
P-value	*0.185*	

Table (3.3) distribution of colorectaladenocarcinomapatients according to Dukes stageof the disease.

Variables	Frequency	Percentage (%)
Dukes' C	26	65.0%
Dukes' D	14	35.0%
Total	**40**	**100.0**%
P-value	*0.591*	

Table (3.4) association between age group and CDX2 expression.

Age	CDX2		Total
	Present	**Absent**	
<40 Years	4 (33.3%)	4 (14.3%)	8 (20.0%)
>40 Years	8 (66.7%)	24 (85.7%)	32 (80.0%)
Total	*12 (100.0%)*	*28 (100.0%)*	*40 (100.0%)*
P-value	**0.170**		

Table (3.5) association between gender and CDX2expression.

Gender	*CDX2*		*Total*
	Present	*Absent*	
Male	5 (12.5%)	18 (45.0%)	23 (57.5%)
Female	7 (17.5%)	10 (25.0%)	17 (42.5%)
P-value	**0.185**		

Table (3.6) association between duke's stage of the disease and CDX2 expression.

Stage	CDX2		Total
	Present	*Absent*	
Dukes' C	8 (30.8%)	18 (69.2%)	26 (100.0%)
Dukes' D	4 (28.6%)	10 (71.4%)	14 (100.0%)
Total	**12 (30.0%)**	**28 (70.0%)**	**40 (100.0%)**
P-value	**0.591**		

DISCUSSION
-About age, the result of the study show that the incidence of colorectal adenocarcinoma increase more in patients aged more than 40 years old with percentage 80%, while previous studies reported that the incidence rates of colorectal adenocarcinoma increase among people 50 years old and above which agrees with my result. (AL rayan and Ofer Purim, Noa Gordon, and Baruch Brenner), and more than 65 years.

-The result of the study show that the most common gender among the patients were males with percentage of 57.5%, and the female of 42.5%, and the previous studies also showed that the majority of patients were male which agrees with my results.(AL rayan and ofer Purim).

-The present in this study show that most of the colorectal adenocarcinoma patients were in Dukes'C with percentage of 65% followed by Dukes D with percentage 35%. my result agree by (Weimin Xu, 56(40.6%) had stage II, 59(42.8%) had stage III, and 13(9.4%) had stage IV).

-the result show that the expression of CDX2 in colorectal adenocarcinoma with pation age, CDX2 had present in 12(100.0%) samples and absent 28(100.0) samples with P-value (0.170%) the result agree by (ALrayan ALtahir), (negative expression 20(50%) and positive expression 5(12.5%).

- The result show that the females had a higher percentage 7(17.5%) than males 5(12.5%) in CDX2 expression, while other previous study showed that

CDX2 loss in colorectal cancer is independently association with females, which disagree by (Yoshifumi Baba, Katsuhiko Nosho, and Kaori Shima).

-The result of the study show that the expression of CDX2 in colorectal adenocarcinoma Dukes' C 8(30.8%) more than Dukes' D 4(28.6%). As it appear in previous studies that admitted by (Werimin Xu, Yilian Zhu, and Peng Du-jounral). (59(42.8%) had stage III and 13(9.4%) had stage IV).

CONCLUSION
- Colorectal adenocarcinoma incidence more in males than females.

-It is more in patients less than 40 years old.

-The most common grade in this study C and D.

-Stage C is higher than stage D in colorectal adenocarcinoma.

- CDX2expresses in patients less than 40 years old with colorectal adenocarcinoma more than patientsabove than 40 years old.

- CDX2 expresses in females more than males.

-CDX2 expresses in Duckes 'C more than Duckes' D

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