The Effect of Biomarker (IL-8) on the Tissue of Colorectal Cancer Patients by using IHC Technique

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Abstract

Aim: The search to evidence a link between the molecular markers (IL-8) with colorectal cancer. The aim of the presented work is examining the relation regarding the biomarker (IL-8) in colorectal cancer (CRC) tissues as well as its relation with the other clinic pathological variables.

Methods: The detection of IL-8was confirmed using histopathological examination of colonic biopsy of tissue section fixed on slides previously coated and analyzed by immunohistochemistry method (IHC), hence to detect the variable regarding IL-8 expression in normal as well as CRC tissues including (adjacent normal tissue and healthy controls tissues); in addition to its association with other clinic-pathological variables (intensity, gender and age).

The results of the study specified the enrichment regarding IL-8 expression in the samples of CRC with the use of IHC approach. No considerable relation has been indicated between the expression of IL-8 with the other clinical-pathological variables (gender and age), yet there has been considerable relation between the expression of IL-8 and the stain's intensity (grade), while it has been increased in tissues of CRC; this might be specified as one of the risk factors with regard to the CRC's development and the metastasis in the samples of CRC, also it might be significant marker to predict insignificant prognosis in patients experiencing CRC, also it can be utilized as possible therapeutic target in the CRC.

Keywords: *Immunohistochemistry technique, Biomarker, Colorectal cancer, Metastasis.*

Introduction

The cancer's incidence is increased yearly. CRC can be considered as the second most general cause regarding mortality from cancer in Western world [1]. A lot of factors (genetic and environmental) have been involved in mortality as well as propagation resulted from CRC. From the many trophic factors, chemokines were of major role.

IL-8 can be defined as chemokine that is created through a lot of tumor in addition to normal cells, also its major role is in the amplification and initiation regarding acute inflammatory reactions. Furthermore, IL-8 was involved in the chronic inflammatory process in addition to the diseases with the chronic inflammatory component including cancer (colorectal), the level has been increased with the progression and metastasis of disease [2,3].

Interleukin-8 (IL8), belong to neutrophil-specific CXC sub-family of chemokine (ELR) with defining CXC amino acid motif including (Leu, Arg, and Glu), which might not only be acting on Leukocyte chemotaxis, infectious diseases and inflammatory responses [4]. IL8 is generated by many different normal cells and tumor cells and its main goal is initiating and amplifying acute inflammatory reactions. IL8 was implicated as well in the chronic inflammatory diseases and procedures with chronic inflammatory components like the cancer [3]. It has been known to be possessing pro-angiogenic and tumorigenic characteristics. IL8 over-expression was discovered in numerous human tumours, inclusive the CRC, and is related to insufficient prognoses [5]. The cells of the tumor generate IL8 as an autocrine factor

of growth that promotes the growing of the tumor, metastatic spread, and invasion of tissues [3].Ning and coworkers [5] reveal that the use of a xenograft model of the tumor, cells that express IL8 produced considerably larger tumors compared to control cells with enhanced density of the micro-vessels.

Whereas the applications related to the immunological approaches to the histopathology caused marked improvement in microscopic diagnosis that is related to the neoplasm. Even through that the histological analysis regarding Haematoxylin &Eosin (H&E) stained tissue section remain the core related to practice of neck and head surgical pathology [6], due to the fact that immunohistochemistry (IHC) remains the major applied and provided approach in pathology for determining the expression status regarding tumor-associated proteins, also for studying the clinical prognostic relevance related to the biomarkers [7].

This work has been developed for examining the differences in the expression of IL-8 between the tissues of CRC in addition to the matched adjacent normal mucosa tissues, as well as the relation between expressions of IL-8 as well as the clinic pathological features in the CRC.

Materials and Methods

Surgical specimens

Fresh specimens (80) of colorectal tissues were collected from patients with CRC (n=42) beside healthy control individuals (n=10). Likewise; (n=28) CRC samples from (n=42) were matched with adjacent normal tissue (n=28). All these specimens were obtained from patients underwent colonoscopy and segmental colonic resections, those that were diagnosed as having colorectal cancer with different grades or healthy tissues were considered as a control group.

All these tissue samples were collected from patients; admitted maintained in different hospitals in Baghdad city, during the period of 2018 -2019.

All sought after datum on demographic and clinical histopathological parameters which was gained from the patient's medical records and designed information sheet. The exclusion criteria were on patients for CRC patients including chemotherapy or radiation treatments prior surgery, also excluded the patients who had another malignancies or polyps in another organs [8]. All participants received conventional bowel preparation without preoperative antibiotic administration.

Histopathological Examination

It was performed according to the method of [9].The histopathological examination of the colon tissues which were selected from the patients of CRC were determined after fixing, and sectioning the organs, then staining them as well as Immunohistostaining .All these were performed in the Educational Laboratories in the City of Medicine as follows:

1-Preparation of Histological Sections:

At the moment of eradication the part of Colon tissue or Rectum was removed and then washed with PBS, preserved and fixed in 10% formalin for (24- 48) hr. Then the section was washed up with tap water and processed with a set of increasing ethanol concentrations for 2hr at each concentration. After that, the tissue was clarified in xylene for 2hr,then it was impregnated in melting paraffin at 60-70ºC for 1hr.The tissue was embedded in paraffin blocks and waited to be solidified, finally it was sectioned using a microtome at slices of (5-6μm) and they were mounted on the slides. The sections of tissue have been stained with the eosin stains and hematoxylin, after that the cell morphology has been identified within light microscopy.

2- Immunohistochemical examination:

Specimen preparation for IHC staining:

The tissue sections of human cancer subjected to immunohistochemistry analysis with the use of GenTex System (Gen Tex / USA) as well as PolyExcel HRP/ DAB Detection System (Pathinsitu / USA) on the basis of the instructions of manufacturer. Put briefly, the tumor blocks have been formalin-fixed, paraffin-embedded and cut into 4-μm-thick sections.

Antibody dilutions:

IL-8 is concentrated antibodies, hence diluted them with antibodies diluent according to company (Pathinsitu and Gen Tex) sheet enclosed with them:

IL-8 was diluted by ratio of 1:100-1:1000 (according

to the sheet between 100-1000), by taking 1xPBS, 20%Glycerol(pH7), 0.025% ProClin 300 which was added as a preservative.

Controls of marker:

Tissues of Gastric Ca known to express the antigens of interest have been utilized as positive controls. Whereas for negative control, omitting primary antibody and after that has been replaced with PBS.

Immunohistostaining steps:

This was accomplished according to [10].

The slide's evaluation has been made through the pathologists which is blinded for the characteristics of patients. Slides have been scored in the following way on the basis of an approach suggested via [11]: Intensity related to staining has been classified from 0 to 3, as 0(-) negative,1(+)low or weak, 2(++)moderate, and 3(+++) high or strong. The staining's extent has been indicated as the percentage regarding positive cells with regard to the whole tumor area, has been classified from 0 to 3, as 0(0%), 1(10-20%), 2(20-50%), 3(>50%). The final staining score has been evaluated through adding staining intensity as well as the staining's extent.

Statistical Analysis

Data analysis has been conducted with the use of available statistical package of SPSS-25. The data have been provided in simple frequency measure, percentage, standard deviation, mean, in addition to range (minimum-maximum values).

The significance of difference regarding various percentages (qualitative data) has been tested with the use of Pearson Chi-square test (c^2-test) with application regarding Yate's correction or Fisher Exact test when appropriate. Statistical significance has been specified when the P value has been equal or not more than 0.05.

Results

H&E stained

The results of intensity of the stains (grade of differentiation) was classified from 0 to 3, as 0(-) negative, 1(+) poorly, 2(++) moderate, and 3(+++) well differentiated.

The expression of IL-8 in CRC

The results regarding cytokine IL-8 illustrated an increased in IL-8 expression [from score (+1, +2, +3)] as seen in (Figures 1A,1B,and1C) respectively ,as well as positive stained cells in cancer cells of patient compared with healthy control, and adjacent normal tissue. The results of statistical analysis indicated a significantly increased in IL-8 expression in CRC tissue in comparison to adjacent controls as well as healthy control individuals as showed in (Tables 1, and 2); while no significant association appeared between adjacent normal tissue compared with healthy control tissue (Table 3).As well as no considerable relation has been indicated between IL-8 expression and other clinic pathological variables (age, gender) (Table 4).

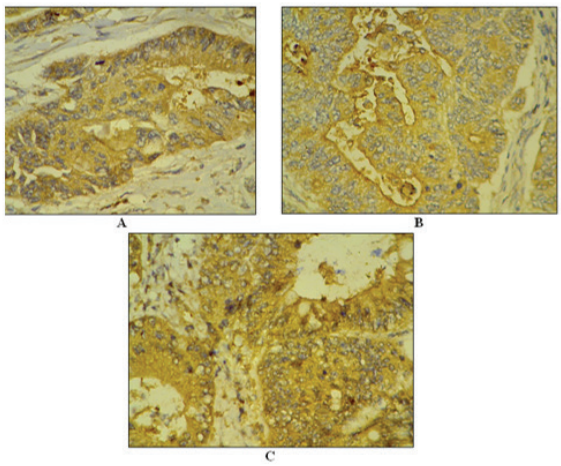

Figure 1: IL-8 IHC Scores expression (400x): A- Score +1 expression; B- Score +2 expressions; and C- Score +3 expressions.

Table 1: The data regarding the significant expression of IL-8 scoring and intensity of stain in patient's cancer tissue compared to healthy control individual's tissue.

		Patient "Cancer tissue" (n=42)		Healthy control (n=10)	
		No	%	No	%
IL-8 Score	[0]	-	-	8	80.0
	[+1]	2	4.8	2	20.0
	[+2]	13	31.0	-	-
	[+3]	27	64.3	-	-
P value compared to healthy control tissue		0.0001*		-	
IL-8 Intensity	[0]	-	-	8	80.0
	[+]	9	21.4	2	20.0
	[++]	17	40.5	-	-
	[+++]	16	38.1	-	-
P value compared to patient normal tissue		0.0001*		-	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.					

Table 2: The data regarding the significant expression of IL-8 scoring and intensity in patient's cancer tissue compare to patient's adjacent tissue

		Patient "Cancer tissue" (n=28)		Patient "Adjacent normal tissue" (n=28)	
		No	%	No	%
IL-8 Score	[0]	-	-	14	50.0
	[+1]	-	-	9	32.1
	[+2]	11	39.3	5	17.9
	[+3]	17	60.7	-	-
P value compared to patient adjacent tissue		0.0001*			
IL-8 Intensity	[0]	-	-	14	50.0
	[+]	6	21.4	12	42.9
	[++]	13	46.4	2	7.1
	[+++]	9	32.1	-	-
P value compared to patient adjacent tissue		0.0001*			
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.					

Table 3: The data regarding of no significant association of expression IL-8 scoring and intensity between the adjacent normal tissue of the patients and healthy control individuals

		Patient "Adjacent normal tissue" (n=28)		Healthy control (n=10)	
		No	%	No	%
IL-8 Score	[0]	14	50.0	8	80.0
	[+1]	9	32.1	2	20.0
	[+2]	5	17.9	-	-
	[+3]	-	-	-	-
P value compared to healthy control tissue		0.191		-	
IL-8 Intensity	[0]	14	50.0	8	80.0
	[+]	12	42.9	2	20.0
	[++]	2	7.1	-	-
	[+++]	-	-	-	-
P value compared to healthy control tissue		0.234		-	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.					

Table 4: The data regarding no significant association between the expressions of IL-8 scoring compared with age and gender

		IL-8 for Patient "Cancer tissue" (n=42)						IL-8 for Healthy controls (n=10)					
		[0&+1]		[+2]		[+3]		[0&+1]		[+2]		[+3]	
		No	%	No	%	No	%	No	%	No	%	No	%
Age (years)	<50y	1	50.0	5	38.5	6	22.2	5	50.0	-	-	-	-
	50---59	1	50.0	5	38.5	14	51.9	2	20.0	-	-	-	-
	60---69	-	-	3	23.1	4	14.8	2	20.0	-	-	-	-
	=>70y	-	-	-	-	3	11.1	1	10.0	-	-	-	-
	P value	0.696											
Gender	Male	1	50.0	8	61.5	18	66.7	4	40.0	-	-	-	-
	Female	1	50.0	5	38.5	9	33.3	6	60.0	-	-	-	-
	P value	0.866											
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.													

Discussion

The over-expression regarding IL-8 was recognized in various human tumors, such as CRC, also it is related with the poor prognosis[5].

With regard to colonic mucosa, the upregulation that is related to IL-8 happen in relation to the inflammation's degree in ulcerative colitis and the Crohn's disease [12, 13]. With regard to the human colon cancer cell lines, constitutive expression related to IL-8 was associated to the metastatic potential [14], also was indicated to be of high importance in the progression of distant metastases. Some researches indicated that the induction related to the IL-8 signaling elevated the NF-κB transcriptional activity [15, 16]. A study conducted via [17] examined the patterns related to the gene's expression in colon cancer cells in addition to the corresponding normal mucous cells through DNA microarray analysis, indicating an elevation in the IL-8 expression in cancer cells in comparison to adjacent normal mucosa. Furthermore, a study by [18] indicated that increased levels of *IL-8* mRNA in tumor tissue in comparison to corresponding normal mucous tissue, more often positive staining with regard to IL-8 protein specified in poorly and moderately differentiated tumors in comparison to excellently differentiated tumors (P=0.024). A research carried out via [14] assumed that IL-8 expression in colon carcinoma cells is of high importance in metastasis and growth, also such data in accordance with the results of [19]. Results specified no considerable relation between IL-8 expression scoring a gender or age related to the patients.

While another supported that CXCL8 expression has been considerably upregulated in the tumoral samples in comparison with the normal tissue, and such upregulation elevated with the age of patients[2]. The results suggested that IL-8 staining has been more common in well-differential and moderate tumors in comparison to adjacent and healthy controls which were stained poorly or mildly and negative differentiated. While Čačev *et al*.[18] showed that the positive immunohistochemically IL-8 staining has been more common in poorly and moderately differentiated tumors in comparison to excellently differentiated ones.

Conculsion

The highly elevated expression of IL-8 in CRC tissues; may be specified as risk factor with regard to the CRC's development and metastasis in CRC samples and may be of high importance marker to predict poor prognosis in the patients experiencing CRC that might be applied as possible therapeutic target in CRC.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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