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Histopathological Study on Importance of Mortalin as a Tumor Marker in Patients with Colorectal Adenocarcinoma

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Abstract

The majority of incidences of colorectal cancer (CRC) are found in Western nations, and it is the third most prevalent cancer and the fourth most common cause of cancer-related death . The molecular chaperones of the (HSPs) family are important effectors to protect intracellular proteins from misfolding or aggregation, inhibit cell death signaling cascades, and preserve the intracellular signaling pathways that are critical for cell survival. Mortalin is a member of the Hsp70 family ,which is encoded by a nuclear gene and it plays a crucial role in the proliferation and carcinogenesis of cancer cells, and overexpression of mortalin in CRC is associated with a poor prognosis .

Key words:

mortalin, colorectal cancer, heat shock protein, prognosis of colorectal cancer

The expression of tumor markers (mortalin) was examined by immunohistochemical analysis of sections generated from biopsies taken from colorectal lesions of patients with various stages of CRC.

Abbreviations:

CRC: colorectal cancer

Hsp70: heat shock protein 70

Conflict of interest: all authors declare that there is no conflict of interest Contributor ship: All authors contribute for collection of dat, analysis and writing the manuscript

Introduction

The fourth most common cancer in the world is colon cancer, whereas the eighth most common disease is rectum cancer. As the third most common type of cancer in the world, CRC are more common in men than in women and three to four times more prevalent in males (Ferlay et al ., 2015). Iraq having a low incidence rate of CRC but with a steady increase over time. A total of 7,246 cases of CRC were registered in the cancer registry for the period 2002-2011 and 706 cases between 2012 and 2014, and the ratio between male to female varied from 1.17:1 to 1.28:1. CRC is significant disease in Iraq, where the middle age patients presented were the highest percentage and the trend of CRC showed an accelerated increase after 2007. (Taha et al., 2019; Ashraf and Riyadh , 2021; Hasan et al., 2022). Mortalin protects cells from glucose deprivation, prevents apoptosis, and promotes carcinogenesis. It also plays a significant role in the

refolding of mitochondrial proteins (Ming Xu et al .,2020). Recent studies have also shown that upregulating mortalin contributes to human carcinogenesis, while downregulating mortalin results in growth arrest in immortalized human cells. As a result, mortalin plays a crucial role in the proliferation and carcinogenesis of cancer cells, and overexpression of mortalin in CRC is associated with a poor prognosis ((Rajani et al.,2021).

Material and methods

Patients and controls

The study involved (146) men and women, who were clinically diagnosed with CRC by the general surgery doctor .The patient age range between 36 and 80 years .This study designs as prospective study , all samples were taken from Al-Sadder teaching Hospital and Basrah teaching hospital and colonoscopy unit in this hospital during the period from December 2021 to December 2022. For histopathological and immunohistochemical analysis, the tissue samples are collected from patients with CRC who undergoing surgical resection (colectomy) and colonoscopy. All data were collected throw direct interview with the patients as a questioner. The collected samples were kept in (10%) buffered neutral formalin for histopathological analyses, .The biopsy samples were collected after r surgical operations were kept in (10 %) buffered neutral formalin for (24) hours and transferred to (70 %) ethanol then processed to embedded in paraffin blocks until the decided examination in this study , so formalin-fixed, paraffin-embedded tissue samples of (52) patients with colon or rectal cancer of TNM system were chosen for histopathological analysis and staining. The tissue specimens of CRC were blocked into a thickness of 3 or 4 mm and fixed in 10% neutral buffered formalin for 24 hours , then the tissues were dehydrated by series of alcohols and cleared in xylene, after that infiltration by molten paraffin at 60°C was done , meanwhile the tissue paraffin blocks were cut into sections of 4 µm, and placed on Fisher-brand positively charged slides and then placed on the racks, and then dried at room temperature overnight ,then The slides were stained with Mayer's hematoxyline and eosin for examination(Launa,1968).

Immunohistochmical (IHC) staining analysis

The protocol of IHC will be according to (Ramos-Vara and Miller,2014).

Deparaffining and rehydrating the tissue sections, that incubated with 3% H2O2. Antigen retrieval was done in sodium citrate buffer (pH 6.0) for 20 min at 97°C. Then, the slides were covered with mortalin kit, (rabbit polyclonal, dilution 1:100 at room temperature for 15 minutes. Then the slides were covered with secondary antibody, after that the slides were immunostained with 3,3'- diaminoben zidine chromogens and then counterstained with Mayer's hematoxylin. In addition, positive tissue sections were covered with Phosphate-buffered saline solution (PBS) instead of the primary antibody for the negative controls.

The intensity of mortalin immunostaining, therefore the intensity was scored as (negative immunostaining), weak, moderate and strong immuostaining (Karina et al., 2012) .

Results

Demographic features

Age Distribution

The (Table 1) showed the distribution of (146) total patients in our study according to their age groups, the highest percentage recognized in age group (55-65) years old was (27.4%), while the lowest percentage was (4.7%) for the age group (35-44).

Table (1): Illustrated age group

Age Groups in years	No of patient	percentage
35-44	7	4.7%
54 -45	40	27.3%
55- 64	45	27.4%
65-74	40	27.3%
75-80	14	8.5%
Total	146	100%

Family history

The study found that (19) cases (13.01%) had positive family history of (CRC), while (127) cases (86.98 %) had negative family history of (CRC), there is a significant difference between two groups ($P < 0.05$, (Table 2).

Table (2): Distribution of patients according to family history.

Family history	no. of cases	percentage
Positive	19	13.02%
Negative	127	86.98 %
Total	146	100%

Gender Distribution

The study found that incidence of CRC was more in men than in women for all stages and for all age groups, (Table 3).

Table (3): Distribution of patients according to sex distribution

Sex distribution	no. of cases	percentage
Female	55	37.67%
Males	91	62.33%
Total	146	100%

Histopathological study

Histologic staging

Dependent on Tumor-Node -Metastasis (TNM) system was used to classify the stage of CRC, in our patients out of (146) cases , , staging system can be applied to (52) cases only, so this study had reported only 5 case (9.62 %) diagnosed as stage I, (15) cases with (28.85%) are clarified as stage II and (23) cases with (44.23 %) are stage III and(9) cases with (17.3 %) are stage IV, there were a significant differences among different stages at (P⩽0.05), (Table 4).

Table (4): Types of tumor histologic staging in (CRC) patients.

Stage	no. of patient	percentage
Stage I	5	9.62%
Stage II	15	28.85%
Stage III	23	44.23%
Stage IV	9	17.3%
Total	52	100%

Pathological Grading

In this study grading system can be applied only to 52 cases, and we find that 5 case (9.62%) were diagnosed as grade I (well differentiated), 34 cases with (65.38%) are grade II (moderately differentiated), and 13 cases with (25 %) recorded as grade III (poorly differentiated), there were a significant difference among different stages at (P⩽0.05), (Table 5).

Table (5): distribution of patients according to grades .

Grades	no. of patient	percentage
Well differentiated (gradeI)	5	9.62%
Moderately differentiated (gradeII)	34	65.38%
Poorly differentiated (gradeIII)	13	25%
Total	52	100%

Histopathological observation

The tumor glands presented either an empty lumen or a lumen that was occupied by necrotic cells, cytoplasmic fragments, nucleus residue and fibrous material, making the so called “dirty necrosis”, while the tumor stroma is varied in size and most often, had a desmoplastic aspect, with the development of a dense connective tissue, rich in fibroblasts and collagen fibers (figure 1). At the same time, within the tumor stroma, there was a chronic inflammatory infiltrate formed of lymphocytes. The malignant cells looked pleomorphic ,larger in size , arranched in clusters of neoplastic cells that lack glandular structure in advanced stages with abundant cytoplasm , hyper chromatic nuclei and evidence of abnormal mitotic figure which appeared in most nuclei with evidence of venous invasion(figure 2)

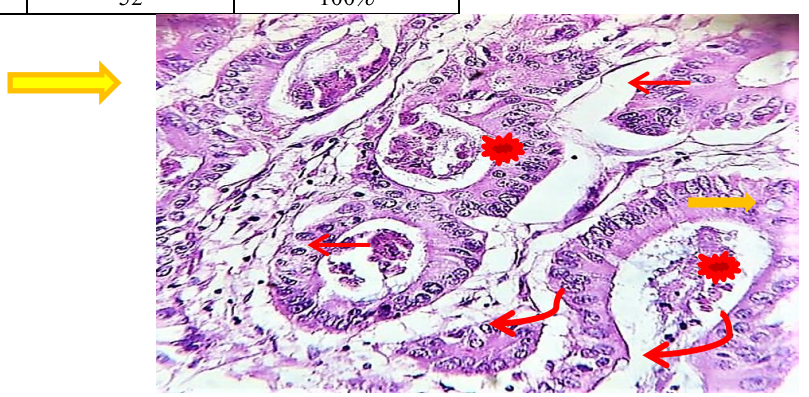

Figure (1): Section of colonic adenocarcinoma moderately differentiated , showing pyknotic nuclei ( ) and abnormal mitotic figures( )with necrotic debris (dirty necrosis) within the lumen of adenocarcinomatous glands ( ) in desmoplastic stroma () (H&E)stain (40X).

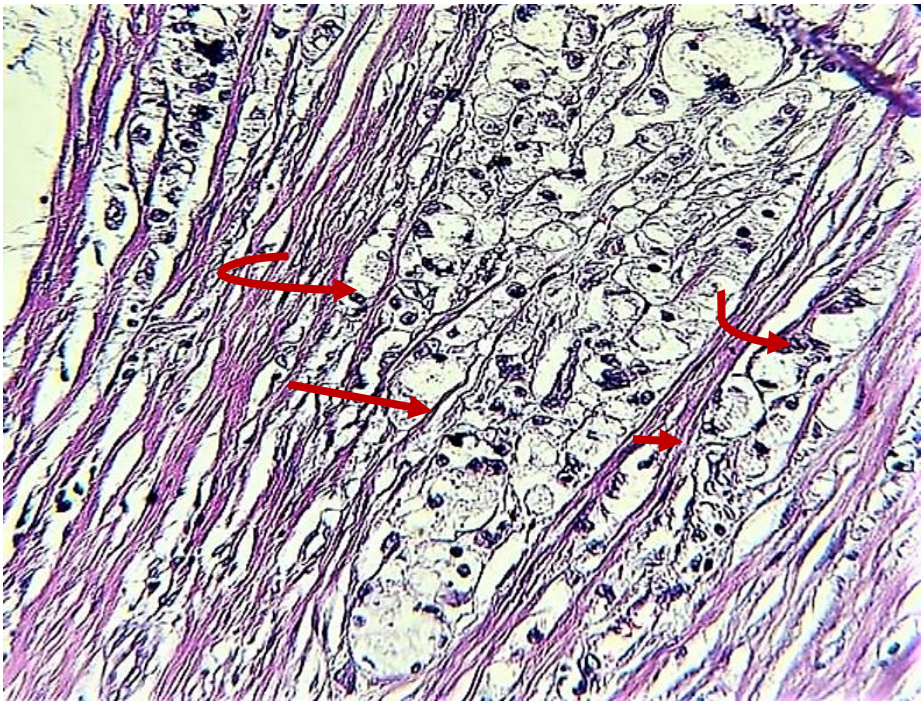

Figure (2): Section of colonic adenocarcinoma poorly differentiated showing cords of polygonal to rounded neoplastic cells with abundant cytoplasm ( ) , eccentrically located nuclei , associated with hyperchromatosis ,and abnormal mitotic figures ( ) .(H&E)stain (40X)..

Immunohistochemical expression of mortalin in CRC patients

The results showed that mortalin expression with CRC (grade II) tumors was 65.4% in total staining intensity reaction, which is the moderate reaction was seen in (26.9%),while strong reaction was seen in (38.5%), followed by CRC (grade III) ,which was 25% in total staining tumors showed a strong reaction in (19.23%) and moderate reaction in (5.8%), and finally CRC(grade I) which was (9.6%) in total, the weak reaction was seen in (1.92%), the moderate reaction was seen in (1.92%) and strong expression was seen in (5.8%) (Table, 6).

According to the stages of CRC, the study included (5 patients) with (stage I), (15 patients) with stage II,(23 patients) with stage III and 9 patients with stage IV.

The staining reaction for mortalin expression in(stage III) was the highest percentage as (44.2%) in total , the moderate staining reaction was (15.4?%),while the strong reaction was(28.8%), followed by stage II the total rate of staining reaction was (28.8%) in total , that subdivided into, the weak reaction was (1.92%),the moderate was (11.5%) while strong staining reaction was (15.4%), then the stage IV was reported as (17.3%) in total , the moderate expression was (5.8%) and the strong was(11.5%) then stage I was reported the lowers percentage (9.6%) in total ,the weak staining reaction was (1.92%) ,while the moderate was (1.92%), and strong reaction was (5.8% (Table 7). The statistical analysis showed a high significance level with Probability value < 0.05 for both mortalin expression according to different stages and grades.

Table (6): Reaction intensity for mortalin expression according to the grades of CRC adenocarcinoma in IHC assay.

Histopathology	Mortalin expression negative	% Negative	Mortalin Expression Positive						% positive
			Weak No. & %		Moderate No. & %		Strong No. & %		
CRC adenocarcinoma grade I	0	0	1	1.92	1	1.92	3	5.8	9.6
CRC adenocarcinoma grade II	0	0	0	0	14	26.9	20	38.5	65.4
CRC Adenocarcinoma grade III	0	0	0	0	3	5.8	10	19.2	25
Subtotal	0%	0	1	1.92	18	34.6	33	63.5	100
Total	52								

$P < 0.05$ (High significant level).

Table (7): Reaction intensity for mortalin expression according to the stages of CRC adenocarcinoma in IHC assay

Histopathology	Mortalin expression negative	% Negative	Mortalin Expression Positive						% positive
			Weak No. & %		Moderate No. &%		Strong No. &%		
CRC adenocarcinoma stage I	0	0	1	1.92	1	1.92	3	5.8	9.6
CRC adenocarcinoma stage II	0	0	1	1.9	6	11.5	8	15.4	28.8
CRC adenocarcinoma stage III	0	0	0	0	8	15.4	15	28.8	44.2
CRC adenocarcinoma stage IV	0	0	0	0	3	5.8	6	11.5	17.3
subtotal			2	9.62	18	34.62	32	61.5	100
Total	52								

$P < 0.05$ (High significant level).

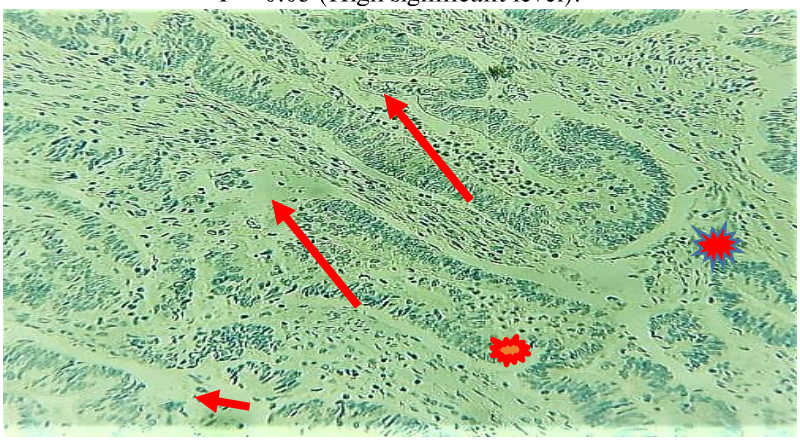

Figure (3): Immunohistochemical staining of (mortalin) expression in colonic adenocarcinoma showed negative staining in tumor cells ( )and stroma ( ) . (IHC) staining. (X10).

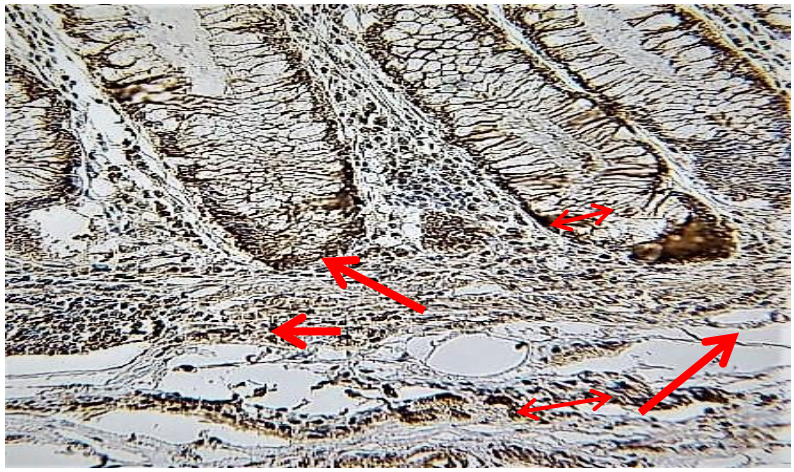

Figure (4): Section of CRC diagnosed as grade 2, showed by IHC staining the expression of mortalin as moderate positive staining of tumor cells ( ) and in stroma ( ) . (IHC) staining. (X20).

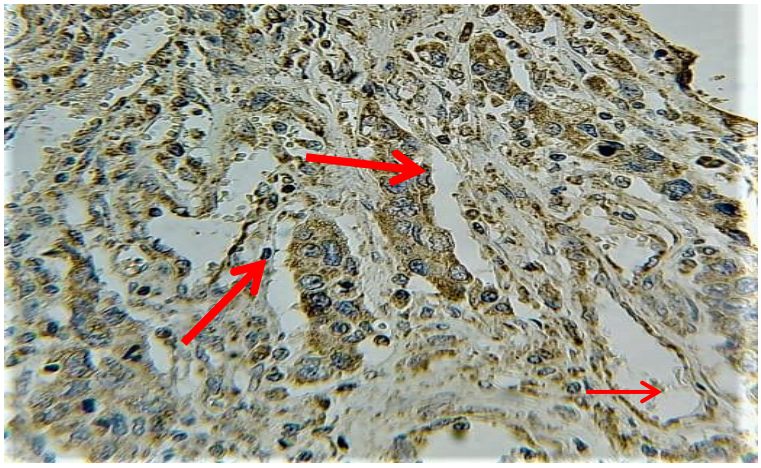

Figure (5): Section of colonic adenocarcinoma poorly differentiated showed by IHC staining , the mortalin expression as positive moderate staining in cytoplasm of malignant cells ( ). (IHC) staining. (X40).

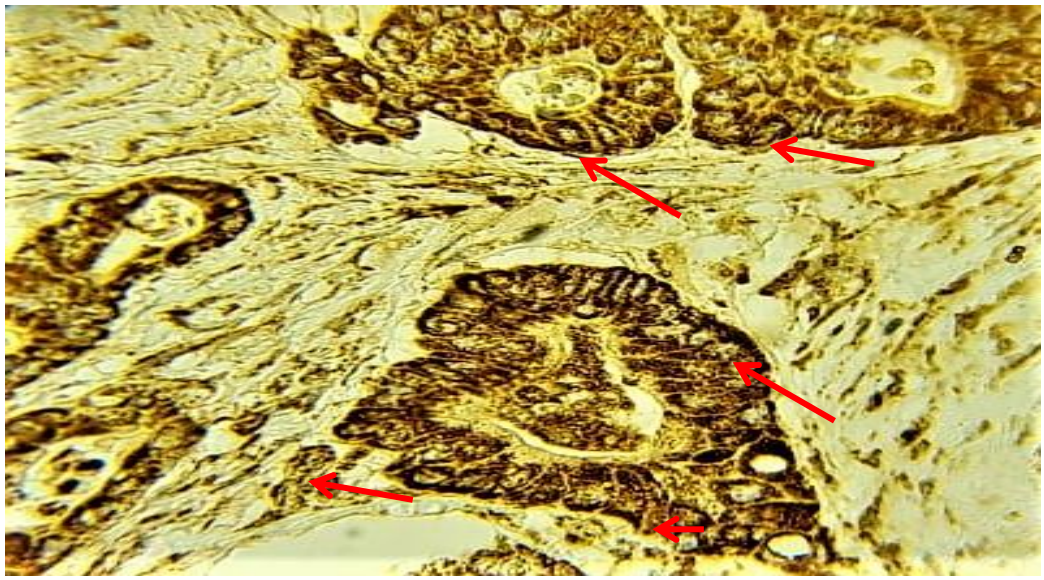

Figure (6): Section in colonic adenocarcinoma moderately differentiated , showed by IHC staining , the mortalin expression as strong positive staining in tumor cells ( ), (IHC) staining. (X20).

Discussion

Mortalin was observed to promote CRC cell proliferation, and was reported to be significantly overexpressed in CRC patients, and that overexpression was associated with mortality (Batoul Abi Zamer et al.,2021). The significance of mortalin as a prognostic tumor marker for CRC was demonstrated in this study, and it was discovered that the level of mortalin in colon and rectum tissue is significantly correlated with the tumor stage in those patients. This is in line with a study by Ming Xu et al. (2019), which found a correlation between the severity of CRC and high mortalin expression, which was positively correlated with a low survival rate and and this may be due to the role of mortalin with (CRC) and the cancerous cells that produce it in addition. Additionally, the study demonstrated that mortalin's reaction intensity was useful in comparison to other parameters and is used as a tumor marker for the expression of CRC and this result is in agreement with study by Yoon et al., in 2022 which confirm that the colorectal adenocarcinoma showed an overexpression of mortalin. According to our data, which accord with Ryu et al. (2014), the expression of mortalin turned positive in all adenocarcinoma cases, making it possible to distinguish between benign tumors and malignant tumors. Cancer cells that express a mortalin mutant have been found by Ryu et al. to have increased malignant characteristics.

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