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BIOCHEMICAL MARKERS IN PATIENTS OF COLORECTAL CANCER IN IRAQ

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***Abstract*:**

The word cancer is a very broad term that covers more than 200 types of malignant tumors. Each of them has particular characteristics that in some cases are completely different from the rest of the other cancers, and can be considered independent diseases. When the malignant tumor is located in the colon or rectum, we speak of colorectal cancer .From the transformation of a normal first cell to the appearance of a detectable cancer, it takes a long number of years, since it is a process that involves multiple alterations in the genes.Through this study, we want to know the biochemical markers of patients with colorectal cancer We have then proposed OLFM4 as a candidate biomarker potential of the detection early of cancer colorectal. For addition, we have noted that the level of expression was most strongly deregulated in the mutated tumors on the oncogene KRAS.

Key word: *cancer , colorectal, biomarker, biochemical markers.*

I. Introduction :

The word cancer is a very broad term that covers more than 200 types of malignant tumors. Each of them has particular characteristics that in some cases are completely different from the rest of the other cancers, and can be considered independent diseases, with their causes, their evolution and their specific treatment. Therefore, it is not appropriate to compare with each other the process that two people who have a cancer diagnosis may be going through. Despite suffering from a disease that has the same name, its symptoms, treatments and evolution can be totally different and what works for one may not have any value for another.Our organism is made up of a group of cells, only visible through a microscope, which divide periodically and regularly in order to replace those that are already aged or dead and thus maintain integrity and correct fun - operation of the different organs (Magri, 2019).

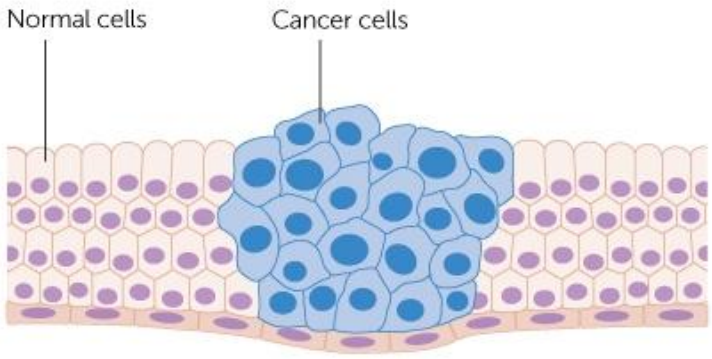

Figure 1.1 :Schematic representation of Cancer Cell

The division process is regulated by a series of control mechanisms that tell the cell when to start dividing and when to stop. When these control mechanisms are altered in a cell, it and its descendants initiate an uncontrolled division that will eventually lead to a tumor or nodule. When the cells that make up this tumor do not have the ability to invade and destroy other organs, we are talking about benign tumors. But when these cells, in addition to growing uncontrollably, undergo new alterations and acquire the ability to invade surrounding tissues and organs (infiltration), and to move and proliferate in other parts of the body (metastasis), it is called a malignant tumor, which is what we call cancer(Alhilfi et al , 2019).

The evolution of the disease is subject to multiple factors that will interact with each other. These factors vary depending on both the tumor and the patient. It is important to keep in mind that although it is the same tumor as yours, you should not compare yourself with another person or with the information you obtain, it does not have to be exactly the same as yours and the evolution can vary greatly.

Colorectal cancer

When the malignant tumor is located in the colon or rectum, we speak of colorectal cancer .

From the transformation of a normal first cell to the appearance of a detectable cancer, it takes a long number of years, since it is a process that involves multiple alterations in the genes.

Although people often talk about colorectal cancer, the term encompasses two tumors with very different behavior: colon cancer and rectal cancer.

Most colorectal cancers appear on a polyp that existed years before in the mucosa of the colon or rectum, which over time evolves into a malignant tumor due to the action of different agents on it.

Colorectal cancer Status in Iraq

According to the latest published WHO data from 2017, deaths from colorectal cancer in Iraq have reached 1,015 (0.58% of all deaths). The mortality rate by age is 5.79 per 100,000 population (Mohamed Ban Jasim, et al ,2015). Colorectal cancer ranks as the 7th most common cancer among Iraq people, affects male above 60 years and most are presented with left-sided tumours at an advanced stage and poor differentiation. Similar results were reported in the previous study from Ira(. In Iraq, several descriptive studies were conducted in colorectal cancer, the colorectal cancer was low but has increased in the last few years (Mohamed Ban Jasim, et al 2019). The first reported incidence of colorectal and esophageal cancer in Iraq was published in 1979 and

1980 by(Al-Bahrani et al.1980)who reported low frequency of colorectal cancer and a high frequency of esophageal and stomach in Iraq. Meanwhile, Al-Humid study in 2008 reported that there were 511 patients diagnosed with colorectal cancer from 1965 to 1990. The descriptive Iraqi studies of colorectal cancer included age, gender, signs, and symptoms. Colorectal cancer has increased in Iraq. Patients with middle ages presented the highest percentage. Education should be applied for colorectal patients about bowl habit and symptoms including constipation and bleeding per rectum. Colorectal carcinoma is most common Gastrointestinal Tract (GIT) cancer in Misan population. Middle aged groups are common age for colorectal cancer. Comparative studies in the Iraqi Cancer Registry during the 30 year period (65-94) showed an increased incidence of colorectal cancer from 25% to 50% and a decrease of gastric cancer from 78% to 50%. The incidence of colorectal cancer in Iraq is 2.6% compared to 6-13% in the developed countries and 17-51.1% in the industrialized nations.

Colorectal cancer Symptoms

Like all malignant tumors, colorectal cancer produces a series of symptoms, which can vary depending on its location within the large intestine:

- Blood in the stool: It is one of the most frequent symptoms of colorectal cancer. It can be red blood, more frequent in tumors of the rectum, sigma and descending colon, or black blood, which mixes with the faces giving rise to black stools also called melena. Manes appear more frequently when the tumor is located in the ascending colon.

After a certain time of bleeding and when the bleeding is not detected or the doctor is not consulted for diagnosis and treatment, anemia usually appears, which can produce, to a greater or lesser extent, a series of symptoms such as the feeling of shortness of breath, tiredness, palpitations, dizziness.

Please note that this symptom may be intermittent, it is

In other words, a polyp or cancer does not have to bleed every day. It is advisable to consult your doctor about any anal bleeding, even if it only lasted a few days.

-Change in the rhythm of bowel movements:diarrhea or constipation appears in people with a previous normal intestinal rhythm, although, ----- more often than not, periods of constipation are interspersed with periods of diarrhea.

- Narrower stool: Generally this occurs because the tumor is narrowing the intestine and does not allow the passage of stool normally.

- Tenesmus or feeling of incomplete evacuation: It usually appears in tumors located in the rectum or in the left colon.

- Abdominal pain: It is usually a frequent symptom, although generally, it is a non-specific pain. When the tumor partially closes the caliber of the intestinal tube, a colic-like abdominal pain is produced. When the closure is complete, it is accompanied by constipation and vomiting.

- Extreme tiredness or weight loss without apparent cause: These are general and nonspecific symptoms that frequently occur in certain diseases, among which are tumors.

Colorectal cancer Diagnosis

When there are symptoms that may lead to suspicion of possible injury to the colon or rectum, the doctor will order a series of tests to determine the diagnosis.

Firstly, it will prepare a clinical history in which data from the patient is collected, and later it will carry out a physical examination, in which it will assess the general condition of the person. This examination, which includes digital rectal examination, is usually performed when the patient reports any of the symptoms described above.(AL-Janabi NA ,et al,2019)

Digital rectal examination involves manual examination of the anus and part of the rectum. The doctor puts on a glove and uses lubricant to gently slide the finger through the anus and explore the area.

Although it is an uncomfortable test, it is important that you know that in general, it is not painful. Take a deep breath before you start and try to distract your attention from what is happening. The more relaxed you get to have your muscles faster and easier the test will be.

However, digital rectal examination is insufficient to reach a correct diagnosis, so it is necessary to perform a colonoscopy:

- Colonoscopy is a test that allows you to visualize the mucosa of the entire colon and rectum through a long, flexible tube (endoscope) that is inserted through the anus.
- For the performance of the same it is necessary that the person follows a special diet, at least for 2-3 days and the day before the test use laxatives, so that both the colon and rectum are free of feces and can observe the mucosa without hindrance.
- If during the colonoscopy a mass or an altered area is observed, a sample will be taken from that area (biopsy) and examined later under a microscope; in order to determine if the abnormality is due to cancer or any other benign process.
- Currently, the colonoscopy is performed in a hospital under sedation (the patient is not asleep, feels relaxed and does not perceive pain), in such a way that discomfort caused by the distention of the digestive tract is avoided. by inserting the endoscope inside.
- Although the use of sedation is becoming more frequent, you may not be sedated. In this case, you should start a series of relaxation exercises that reduce the discomfort caused by the test.(AL-Hilfi hider Qasim,et al ,2019)

Types of Colorectal cancer

The vast majority of malignant colorectal tumors develop over existing lesions in their mucosa, such as polyps or inflammatory diseases. Cancer that appears in healthy mucosa is exceptional.(Magri , 2019)

If we look at malignant cells under a microscope, colorectal cancers can be of various types. Among them, Adenocarcinoma that occurs in the glands that cover the interior of the colon and rectum stands out for its incidence. It is the most frequent type of cancer since it appears in 90-95% of cases.

Other tumors that may occur, although much less frequently, are the following:

- Sarcomas: tumors of the connective tissue of the digestive tract.
- Lymphomas: cancer of the defense cells of the stomach and intestine.
- Carcinoid tumors: of the hormone-producing cells of the digestive system.
- Melanomas .

II. Material and Methods :

Detection of CRC in Iraq patients

Colorectal cancer represents a real health problem in the Iraqi population, the frequency of which is gradually increasing. The risks of this pathology are linked to several factors including age, sex, heredity, diet, smoking and alcohol. Present work is based on a retrospective and prospective study with the aim of determining cases of colorectal cancers attended to the teaching hospital Baghdad, Iraq.

Methodology

Study material

It is a cross-sectional study carried out in two phases:

- The first consists of collecting data at the Radiotherapy department (laboratory of the AL-KINDY Hospital, Baghdad, Iraq) including 70 samples collected (samples of patients with rectal cancer which is an entity colorectal cancers whose treatment is based on surgery and radiotherapy unlike colon cancer where treatment is based on surgery and chemotherapy).

1-Work methodology

2-Collection of data

For the realization of this work; researcher have consulted:

- Hospital records.
- The medical records of the radiotherapy department
- The computer system.

2.1 Inclusion criteria

Any patient with rectal cancer regardless of sex, age, at the time of diagnosis.

2.2 Variables studied

The various data collected related to:

- Patient identification: Name and surname, sex, age, date and duration of hospitalization.
- Tobacco consumption (passive or active) over a lifetime
- Leisure or professional physical activities

- Eating habits
- Predisposing conditions, personal history and associated comorbidities, family history.
- Tumor markers found

Study on Colorectal Cancer Tumor Biochemical Markers

The first part of this work led us to study the global proteome of colorectal tumors according to their histological stage, in order to search for potential early biomarkers. This led us to identify several potential markers, one of which, OLFM4 biochemical marker. We have also recalculated our iTRAQTM experiences according to the KRAS status of the patients to find that our protein of interest was actually increased in tumors mutated on KRAS. We then wanted to know if this protein was detectable in the patient's serum before any further analysis. Another interest of this work led us to take a deeper interest in the proteins secreted in the context of biomarkers. For this, we analyzed the immediate cellular environment of the tumor, the tumorstroma, in which probably a large amount of the proteins secreted by the tumor is located. The aim was to detect secreted proteins that could be potential biomarkers and that would have escaped analysis of tumors and their environment. Another interest is in glycosylated proteins present in colorectal tumors. These proteins have significant advantages in terms of stability in biological media ensuring them a half-life sufficient to be accumulated in the blood of patients, and detected by routine methods such as ELISA. However, the continuation of proteomic research is essential because, certainly, there is a space in the CRC screening that needs to be filled by reliable biomarkers (College of medicine mausle,2019)

Biomarker/Regulation	Technique	Sample
- Actin beta-like 2 (ACTBL2)	Two-dimensional gel electrophoresis coupled to mass spectrometry (2DE-MS)	CRC tissue
- Dipeptidase 1 (DPEP1)	Fourier transform mass spectrometry (FTMS)	CRC tissue
- Olfamectomedin-4 (OLFM4) - Kininogen-1 (KNG1) - Transport protein Sec-24 (Sec-24)	Liquid chromatography–mass spectrometry (LC-MS). Further immunohistochemistry validation.	FFPE CRC tissue
- Cyclophilin A - Annexin A2 - Aldolase A	LC-MS	FFPE CRC tissue
- Leucine-rich alpha-2-glycoprotein 1Epidermal growth - factor receptor - Inter-alpha-trypsin inhibitor heavy-chain family member 4 - Hemopexin - Superoxide dismutase 3	Targeted liquid chromatography-tandem mass spectrometry	Serum
- Mannan binding lectin serine protease 1 - Osteopontin - Serum paraoxonase lactonase 3 - Transferrin receptor protein 1 - Amphiregulin	Liquid chromatography/multiple reaction monitoring-mass spectrometry and proximity extension assay	Plasma
- Serine/threonine kinase 4 (STK4 or MST1)	Mass spectrometry (MS/MS). Also verified with Western blotting and enzyme-linked immunosorbent assay (ELISA).	Serum
- Macrophage mannose receptor 1 (MRC1) - S100 calcium-binding protein A9 (S100A9)	High-performance liquid chromatography (HPLC) and Western blotting.	Serum
- Alpha-1-antitrypsin (SERPINA 1) - Alpha-1 antichymotrypsin (SERPINA 3) - Antithrombin-3 (SERPINC1)	Multiplexed quantification with isobaric tag for relative and absolute quantitation (iTRAQ)	Serum

Biomarker	Relevance	Technique	Sample
- Poly (C)-binding protein 1 (PCBP1)	Oxaliplatin resistance	2D gel electrophoresis followed by MALDI TOF/TOF mass spectrometry	Cell lines and tumoural tissue
- Apolipoprotein E 180 (APOE) - Angiotensinogen (AGT) - Vitamin D binding protein (DBP)	Survival outcomes in patients treated with bevacizumab	Gel electrophoresis (2D-DIGE), followed by LC-MS/MS	Serum
- Phosphorylated EGFR (pEGFR)	Response to Cetuximab	Quantitative proteomic analysis	Plasma
- Proteasome subunit alpha type 1 (PSA1 - Leucine aminopeptidase 3 (LAP3) - Annexin A3 (ANXA3) - Maspin (serpin B5)	Proteomic profiling of antibody-inducing cancer-associated immunogens	Mass spectrometry to evaluated antibody-reactive proteins. Western blotting and immunohistochemistry validation	Serum and CRC tissue
- Interferon induced protein with tetratricopeptide repeats 1 (IFIT1) - FAST Kinase Domains 2 (FASTKD2) - Phosphatidylinositol-5-phosphate 4-kinase type-2 beta (PIP4K2B) - AT-rich interactive domain-containing protein 1B (ARID1B) - Solute carrier family 25 member 33 (SLC25A33) - Caldesmon 1 (CALD1) - Carboxypeptidase A3 (CPA3) - Beta-1,3-galactosyltransferase 5 (B3GALT5) - CD177 - Receptor-interacting serine/threonine-protein kinase 1 (RIPK1)	Response to neoadjuvant chemoradiotherapy in rectal cancer	Mass spectrometry	FFPE CRC tissue

The current research interest is to set up a new analysis method allowing them to be studied more specifically in order to improve our chances of detection during CRC analysis experiments.

III. Discussion and Results :

Cancer and a multifactorial pathology responsible for 9 million deaths in recent years this pathology can affect any part of the body. Rectal cancer remains a very common cancer in Iraq, it represents 30 to 40% of colorectal cancers. Its diagnosis is often late, hence the high rate of advanced stages. The one aim of our study was to assess the quality of management of rectal cancer.

1. The average age of our patients was 66 years, with extremes ranging from 23 to 90 years, a male predominance was found in our series with a sex ratio of 1.1.
2. The clinical symptomatology had no particularities, it was dominated by rectal bleeding, transit disorders, palpation of a rectal mass and abdominal pain.
3. This work allowed us to lead an approach aimed at determining the risk factors and protective factors likely to be linked to the violation by the CRC. For that, researcher tried to cover the maximum of elements having a relation with colorectal cancer. From the results of our study which includes 70 cases of rectal cancer,

It was deduce that:

a. The various factors involved in the occurrence of colorectal cancer are: age, consumption of alcohol and that of smoking tobacco, overweight and obesity, diabetes hypertension, family history of a CRC thus a diet rich in red meats.

This modest study opens up several perspectives:

- Early detection based on an assay of specific markers for each type of cancer
- We must address this problem by recommending: Screening based on a prior epidemiological survey, to assess risk factors in a target population in particular.

- a good lifestyle,
- a healthy diet,
- avoid a sedentary lifestyle,
- exercise physical activity,
- Consult your doctor in case of abdominal discomfort or blood in the stool.

- All of these factors are preventive factors against colorectal cancer
- All of these data we have led in proposing several bases of data. Our analyses we have one highlight of candidate biomarkers already described in the literature indicating the relevance of our approach.
- We have then proposed OLFM4 as a candidate biomarker potential of the detection early of cancer colorectal. For addition, we have noted that the level of expression was most strongly deregulated in the mutated tumors on the oncogene KRAS.
- Our studies preliminary to the patients by ELISA appears to show a relevance of this biomarker in the detection early of cancer colorectal. Its plasma level is also higher in patients with tumors mutated on KRAS. Its expression seems to be regulated by the transcription factor NF- κB.
- It also seems to intervene in the regulation of cell contacts by decreasing them, thus probably promoting tumor spread. By also analysing its glycosylation sites led us to show that they were necessary for its secretion.
- We have also demonstrated fact that there could have glycosylation's on sites corresponding the known consensus sequence.
- Other proteins from our analyses could also constitute good biomarker candidates, and also deserve to be studied in this context. This is in particular the case of the protein TGFBi, the expression of which is very often modified in our analyses. The continuation of this work could consist in carrying out assays of these markers in the blood of sick and non-sick people, in order to try to determine their relevance. These markers could, if they prove to be relevant, be combined to provide a diagnostic aid, and prognostic for clinicians.

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