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Evaluation of Serum Cathepsin B as a Biomarker in Colorectal Cancer Patients

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ABSTRACT

The present study included (55) colorectal cancer (CRC) patients (men and women) and (35) voluntary healthy subjects. The serum cathepsin B was calculated by ELISA in order to investigate the relationship between this biomarker and gender, age, histopathological type, stages, grades, tumor location, as well as body mass index (BMI). The findings indicated significant increases ($p<0.05$) in the sera levels of cathepsin B of CRC patients in comparison with the control groups. Cathepsin B levels differed significantly ($p<0.05$) according to age, histopathological type, stages, and grades, while there were non-significant differences ($p<0.05$) for gender, tumor location, and BMI. These findings imply that cathepsin B may be implicated in colorectal tumor malignancy at multiple levels, and that the specific activity of this marker could be of relevance as an independent predictive factor for CRC disease progression. Furthermore, our findings support the clinical application of cathepsin B as a routine test in medical laboratories, as this biomarker has a significant prognostic impact in patients with CRC.

1. INTRODUCTION

Cancer is a condition characterized by unregulated cell growth and proliferation, in which cells have eluded the body's usual growth control mechanisms and developed the ability to divide continuously. It's a multistep process that needs the accumulation of numerous genetic alterations with time [1]. When this type of growth occurs in the colon or rectum, it is termed colorectal cancer [2]. Colorectal cancer (CRC) is one of the most common cancers in the world. CRC is the fourth most frequent cancer worldwide, accounting for 9.7% of all cancer cases [3]. Every year, almost 1.4 million new cases of CRC and 700,000 deaths due to CRC are recorded around the world [4]. The American Cancer Society estimated that 1.8 million cases of CRC occurred in 2018 [5]. Women have a 4.76% % lifetime risk of developing CRC, whereas men have a 5% risk. Overall, men have much greater rates of both incidence and mortality than women [6]. In Iraq in 2018, CRC accounted for 6.15% of cancers in both genders, 5.13% in women and 7.52% in men [7]. The advancement of molecular biology has improved our understanding of CRC pathogenesis in recent decades. CRC is a hereditary disease, and it is caused by changes in a number of oncogenes and tumor suppressor genes [8]. Colorectal carcinomas can be classed as sporadic, inherited, or familial, depending on the origin of the mutation [9].

Colorectal carcinoma can result from many causes, including both genetic and lifestyle-related factors. Consequently, CRC is on the rise in developed countries, due to an aging population, unfavorable urban eating choices, and an elevation in risk factors like smoking, lack of physical activity, and obesity [10]. The biomarkers play an important role in cancer early detection. Tumor markers are chemicals detected in tumor cells or bodily fluids and have improved the way oncologists practice. They can be applied for screening, diagnosis, prognosis, and therapeutic efficacy evaluation [11].

For CRC, a number of serum and cell/tissue-based biomarkers have been identified. Biochemical indicators may be useful in detecting early disease, assisting with diagnosis, evaluating prognosis, and indicating potential response to specific treatments of CRC [12]. Cathepsin B is a cysteine proteinase found in lysosomes and may have a role in cancer progression [13]. However, little is known about the relationship between serum cathepsin B and CRC. In the current study, we aimed to examine the levels of cathepsin B in sera with some clinical and pathological factors in CRC patients and also to explore the relationship between this biomarker and disease diagnosis and progression. In fact, there is a great need for sensitive and specific biomarkers of the disease.

2. Materials and Methods

2.1 Experimental design

The present study was conducted in the laboratory of advanced research of the Department of Laboratory Investigations/ Faculty of Science / University of Kufa. All the samples were obtained from patients at the oncology unit of AL-Furat Al-Awsat Center for Tumors in AL-Najaf province between 1/12/2020 and 1/3/2021. Only Iraqi patients diagnosed with colorectal cancer, verified by histopathology report, were included in the study. The present study included (55) patients (men and women) diagnosed with colorectal cancer and (35) healthy subjects. All the patients were examined and diagnosed by specialist physicians, and cases without histological report were all excluded from the study. Both the patients and the healthy groups were exposed to questioners about age and body weight. Also, the participants were informed about this study and consent was ensured. The scientific ethical committee approved the project.

2.2 Study groups

The patients with CRC in this study involved males and females and were divided into subgroups according to the gender, age, histopathological type, stages, grades, tumor location, and body mass index (BMI). The samples were collected from healthy volunteers only if they had no history of chronic diseases or acute infections and weren't smokers. The ages of the healthy subjects selected in this study were identical with the ages of the patients.

2.3 Estimation of serum cathepsin B level

Five milliliters of venous blood were taken from the cubital vein of resting patients and the healthy group using a disposable syringe. After clotting, the samples were isolated at room temperature by centrifuge at 3000 revolutions per minute (rpm) for 15 minutes and transported into Eppendorf tubes by micropipette and stored in freezing conditions at -20^{O}C until they were analyzed [14]. The enzyme- linked immunosorbent assay (ELISA) was used to determine the level of cathepsin B in the serum.

2.4 Statistical analysis

For statistical analyses, the Statistical Package for the Social Sciences (SPSS, ver. 23) was utilized. The t-test was used to compare two groups, while the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was employed to compare several groups. The mean±standard

deviation was used in the statistical analysis. A probability of <0.05 was considered significant for all tests.

3. Results and Discussion

The findings of this investigation reveal an elevation in cathepsin B levels that is statistically significant ($p<0.05$) in the sera of patients with CRC disease when compared with healthy subjects (control group), Figure (1).

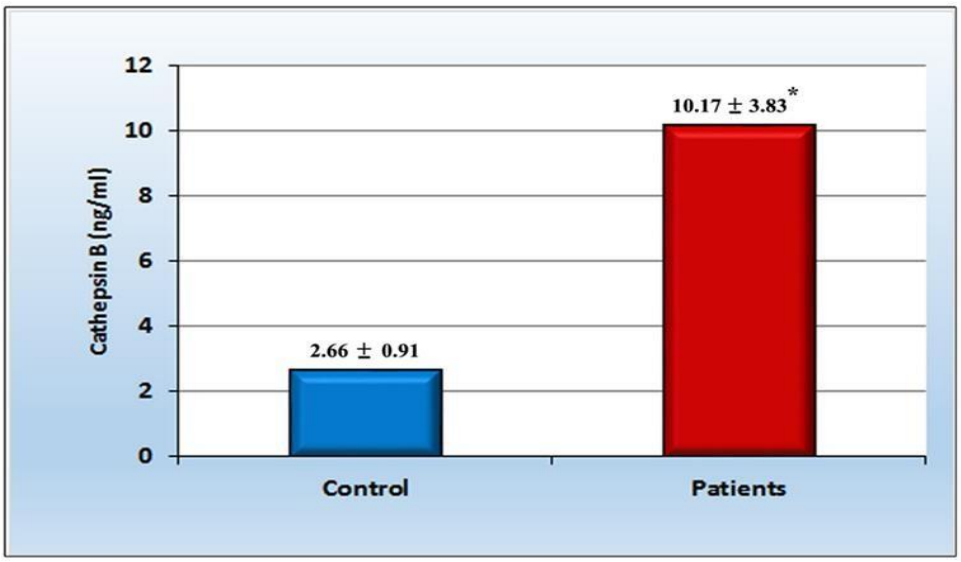

Figure (1): Cathepsin B levels in serum of with CRC patient group and control group.

- Values are means ± SD.
- (*)=Significant differences exist at the $p<0.05$.

The cathepsin B levels in serum were considerably higher in patients with CRC than in the comparable control group. The findings of our study are in agreement with those obtained by other investigations [15-17]. Contradictory results have been recorded by, who found a decrease in cathepsin B and an increase in cathepsin D levels in the sera of CRC patients [18]. Previously published research has indicated an increase in serum cathepsin B patients with many forms of malignant tumors, including melanoma [19], lung [20], urothelial bladder [21], and nasopharyngeal carcinoma [22]. The presence of cathepsin B in cancer patients' sera and its relationship to clinical features is less clear. Specifically, showed that higher cathepsin B expression is associated with tumor development and poor survival in CRC patients [23]. Suggested that cathepsin B levels are raised in colorectal neoplasms and contributed to the destruction of the extracellular matrix and the proliferation of tumoral cells. According to cathepsin B denaturates and destroys fibronectin, collagen, laminin, proteoglycans, and other extracellular matrix components, and aberrant cathepsin B activity may be associated with the development of human malignancies [24], [25].

In another study, showed that the formation of free radicals occurs concurrently with the development of cancer [18]. Reactive oxygen species (ROS) are thought to induce oxidative damage to cell membranes, resulting in an increase in membrane permeability. Consequently, regarding the elevated levels of cathepsin B in our study, the increased concentration may be due to the influx of cathepsin B into extracellular fluid and into the serum on account of impairment of the function of the barrier between the intracellular and extracellular environments. The available data indicate that cancer cells overexpress and produce a high fraction of cathepsins. However, complete excision of tumors may help to minimize ROS formation by inactivating cathepsin B. In another study, following radical tumor resection, the serum level of cathepsin B

was restored to the control level [26].

On the other hand, it has been reported by other researchers that immunohistochemical values of cathepsin B are significantly increased in patients with CRC. Observed that cathepsin B activity was significantly increased in adenocarcinomas compared to tumor-bearing tissue [27]. In addition, it was reported that cathepsins B and D activity was statistically considerably increased both in the cytosol of neoplastic tissue and in the complete cellular homogenate of CRC [28]. According to cathepsin B mRNA levels were considerably greater in tumor CRC samples than in surrounding normal colorectal tissue [17]. Besides, it was discovered that malignant tissues had an approximate 3.7-fold elevation in cathepsin B mRNA levels when compared to noncancerous tissues [13].

The results of the relationship between the levels of cathepsin B in the serum and some clinical features in CRC patients were presented in Table (1).

The statistical analysis of the current research revealed that there is no significant variance ($p<0.05$) in cathepsin B concentrations in serum between males and females. These study findings were in accordance with those of, who observed no significant association with respect to gender [15]. A previous study carried out by, also confirms the result of this study, which revealed that no association has been found between cathepsin B serum level and gender [29].

The current study found a significant increase ($p<0.05$) in serum cathepsin B levels in >50 year group when compared to ≤50 group. This result disagreed with studies by, who indicated that there was no significant association between cathepsin B and the age of CRC patients [29], [15].

The findings of this study indicated a significant ($p<0.05$) increase in serum cathepsin B level in mucinous adenocarcinoma in comparison with non-mucinous adenocarcinoma. In fact, there is limited information on the connection between cathepsin B concentrations and histopathological type. In any case, in an immunohistochemical study, showed that mucinous carcinomas express more cathepsin B than non-mucinous carcinomas [30]. It found that cathepsin B was intimately linked to mucinous carcinoma invasion and metastasis.

The results demonstrate there were significant changes ($p<0.05$) in cathepsin B levels in the stages and grades of patients with CRC disease. Numerous studies have been dedicated to finding the correlation between serum cathepsin B expression and these clinical indices of malignant progression of CRC disease. It is well known that the presence of cathepsin B in the serum of patients with CRC correlates with advanced tumor stage as assessed by the TNM scores or Duke's scale [29], [18].

Made a similar observation, reporting that increased serum cathepsin B levels are associated with late tumor stage and decreased survival in CRC. Recently, reported that end stage with lymph node metastases had considerably greater cathepsin B serum concentrations and cathepsin B gene expression than early-stage patients [17].

Table (1): Comparison between cathepsin B levels in sera of CRC patients and the healthy group according to clinical features.

Clinical Features	Cathepsin B level (ng/ml)	Number	Percentage %
CRC group	2.66 ± 0.91	55	61.2%

healthy group	10.17 ± 3.83*	35	38.8%
Gender			
Males	9.60 ± 2.95	25	45.45%
Females	9.67 ± 4.17	30	54.54%
Age (year)			
≤ 50	8.56 ± 4.48	19	34.54%
> 50	11.12 ± 3.70*	36	65.45%
Histopathological type			
Mucinous adenocarcinoma	12.94 ± 5.26	10	18.18%
Non-mucinous adenocarcinoma	9.49 ± 3.10*	45	81.8%
Stages			
I	5.90 ± 1.18[a]	9	16.36%
II	9.72 ± 1.22[b]	19	34.54%
III	14.43 ± 1.30[c]	13	23.63%
IV	17.65 ± 1.73[d]	14	25.45%
Grades			
Grade I	6.98±1.65[a]	29	52.72%
Grade II	11.39 ±1.98[b]	19	34.54%
Grade III	16.50 ±1.54[c]	7	12.72%
Tumor location			
Colon	10.36 ± 4.03	30	54.54%
Rectum	10.18 ± 3.03	25	45.5%
Body Mass Index (BMI)			
Normal weight	9.76 ± 3.72	19	34.54%
Overweight	10.61 ± 4.24	18	32.72%
Obese	9.96 ± 1.60	18	32.72%

- Values are means ±SD.
- (*)=Significant differences exist at the $p<0.05$.
- Different small letters indicate statistically significant differences at the $p<0.05$.

It is reported that not only the accumulation of cathepsin B and D in tumor cells correlates with the degree of neoplasm growth, but also that enhanced serum activity of the indicated proteases achieves the same thing [18]. In addition to the previously mentioned, another study discovered that increased cathepsin B synthesis and release in cancer cells promote tumor cell proliferation, invasion, and metastasis [31]. It is suggested that cathepsin B, cathepsin L, and plasminogen activator inhibitor type- 1 (PAI-1) likely play a role in the progression of premalignant colorectal adenoma to CRC [15].

When the role of lysosomal enzymes in tumor growth and invasion is considered, the rise in cathepsin B activity may be ascribed to the main tumor's ability to infiltrate normal tissue as well as the incidence of metastatic tumors. As well as cathepsin B secreted by tumor cells may lead to the cells' separation from the original tumor and therefore to invasion into normal tissue [26].

As it is known, cathepsin B is capable of degrading the extracellular matrix and basement membrane, hence facilitating cancer cell invasion and metastasis [30], [18]. According to, cysteine proteases (cathepsin B, cathepsin L) perform a critical role in this process by destroying numerous components of the extracellular matrix surrounding the cell [15]. On the other hand, cathepsin B activity was associated with tumor invasion and differentiation grade in adenomas to advanced malignancies [31]. Similarly, observed that moderately differentiated adenocarcinomas expressed more cathepsin B than well differentiated adenocarcinomas [32]. The patients with increased cathepsin B and carcinoembryonic antigen (CEA) levels were identified as having a poor prognosis [29]. It has been suggested that poorly differentiated clumps of cancer cells near the invasive front, referred to as "tumor budding", may be indicative of CRC malignancy [28]. Besides, one study found no significant association between tumor grade and survival [15]. The present study showed that there were no significant differences ($p<0.05$) in the sera of cathepsin B levels in patients with colon carcinoma compared with rectum carcinoma, which is consistent with the study of who stated that no significant variation in tumor site was noticed [15]. The relation between cathepsin B and body mass index (BMI) has not been recorded to our knowledge. On the basis of our findings, there were no significant differences ($p<0.05$) in cathepsin B levels in the sera of CRC patients.

The prevalence of gastrointestinal cancers is strongly related to body weight. Therefore, overweight or obesity can have a variety of severe health consequences, including an increased risk of acquiring certain types of tumors, such as CRC [33]. A recent study suggested that lysosomal dysfunction, reflected by decreased cathepsin B expression and increased cathepsin, is observed in the abdominal subcutaneous adipocytes of obese and overweight males and females, and may be associated with decreased overall glucose homoeostasis [34]. In our opinion, the relationship between cathepsin B levels and obesity in CRC patients is unclear and further study is needed in the future.

4. Conclusion

In conclusion, the results showed a significant increase in serum cathepsin B levels in patients. Therefore, cathepsin B may be involved in colorectal tumor progression. This biomarker may be of interest as an independent prognostic factor for malignant progression of this neoplastic disease.

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