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# Cathepsin D as a Biomarker in Colon Cancer Patients

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***Abstract***

*Colon cancer is a relatively common life-threatening malignancy for both sexes. Cancer cells are depicted by the upregulation of lysosomal proteases – cathepsin D. The study aimed to determine human cathepsin D activity in the control group as well as its role in metastasis and invasion of colon cancer. Enzymatic assay(manual) was used to measure the activity of cathepsin D and catalase, other parameters were measured using analytical kits provided by reputable companies. Carcinoembryonic antigen CEA has been determined as a biomarker for colon cancer, patients were subdivided into two classes based on carcinoembryonic antigen values: group 1, ≤5 ng/mL group 2 >5 ng/mL. The results explained that the normal value of Cathepsin D for the control group was (8.61±0.294 ng\ml) and the activity of the enzyme was not affected by gender, age, smoking and BMI. The results also proved a highly significant increase in cathepsin D activity in the patient's group (17.81±0. 652 ng/ml) compared to the control. A remarkable elevation in the action of cathepsin D in colon cancer patients was associated with a positive relationship with carcinoembryonic antigen levels. This supports the application of cathepsin D as a marker of tumor occurrence as well as evidence of tumor metastasis and invasion after treatment in colon cancer patients.*

***Keywords:*** *Colon Cancer, Cathepsin D, Carcinoembryonic Antigen, Catalase, Gamma-glutamyl Transferase.*

## Introduction

Cathepsins are a family of lysosomal proteases that are functional in a considerably acidic milieu [1]. More than twenty different cathepsin kinds have been identified in all living things since the first cathepsin was discovered in late 1920. Fifteen types of proteolytic enzymes known as cathepsins are found in humans and are structurally categorized according to whether they have, a serine cathepsin A and G, aspartate cathepsin D and E, or cysteine in catalytic active site residue cathepsin B, C, F, H, K, L, O, S, V, X, W, or Z [2]. Nonetheless, cathepsins A, B, and X may also function as carboxypeptidases and cathepsin H functions as an aminopeptidase, most cathepsins serve as endopeptidases [3].

All human tissues express cathepsin D (cath-D), a ubiquitous aspartic endoproteinase (E.C 3.4.23.5) [4]. It functions physiologically in lysosomes, where it breaks down unfolded or useless proteins through proteolysis. In crucial biological processes like expansion and tissue homeostasis, the enzyme is thought to function in protein hydrolysis outside of its acidic environment [5]. It's interesting to note that cathepsin D has drawn more attention due to its presence in the surrounding milieu of the tumour microenvironment, suggested parts in tumour formation and metastasis, and potential as a therapeutic target [6].

Today, it is understood that the enzyme is active at moderate pH in the cytoplasm of apoptotic cells in case of neurodegeneration, and during the evolution of cancer [7]. It is also active both proteolytically and non-

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proteolytically. An acidic environment is crucial for the stimulation of secreted inactive proteins to active forms [8]. At pH 3.5, Cath-D activity is strictly controlled [9]. Finally, Recent research has documented that improved synthesis and elaboration of this cathepsin from tumour cells resulted in tumour cell growth, invasion and metastasis [9, 10].

Colon cancer is the principal cause of cancer-related deaths in the Western world, with the highest rates in North America, Europe, and Australia [11, 12]. It is the fourth most common cancer in England and Wales and is the second life-threatening cancer [13]. Many different risk factors have been studied for colon cancer. There is a large variation in incidence rates between different populations in the world, which suggests influences of both genetic and environmental factors [14, 15]. In colon cancers males have a slightly higher risk of developing colon cancer than females [16]. Modifiable risk factors include factors related to lifestyle, such as obesity, sedentary lifestyle, lack of physical activity, nutrition, high consumption of red and/or processed meat, diet low in dietary fibre, excessive drinking of alcohol, and smoking [17, 18]

All stages of carcinogenesis can involve interactions between various environmental variables. Recently, it has been discovered that a person's vulnerability to developing colon cancer depends on how their genetic predisposition balances with these factors, including dietary components and lifestyle choices [19-23]. The current study aims to prove cathepsin D activity in colon cancer (Cca) patients and its relationship with the development and Metastases of tumours in addition to studying several biochemical factors and variables.

## Materials and Methods

### Study Groups

A total of 142 participants were selected for the study with an age range of 30-70 years. They were divided into two groups,

G1: The control group consisted of healthy individuals with a total of 52 members (n=52) (males 29 and females 23).

G2: patients group which were Colon cancer Patients with a total of 90 members (n=90)(males 54 and females 36).

The sample was selected from Oncology and Nuclear Medicine Specialized Hospital in Mosul city. Information for individuals was placed in a questionnaire form prepared for this purpose and included age, sex, body mass index, smoking, and fasting.

### Study Parameter

Multiple parameters that are linked with colon cancer were examined in this study (Table 1).

**Table 1**. Parameters and Methods Used

| **Parameters name** | **Methods Used** | **References** |
|---|---|---|
| Cathepsin D | Enzymatic assay (Manual) | [19] |
| Alkaline phosphatase | Kit from Guidonia Montecello /Italy | [20] |
| GGT | Kit from Roche/Germany | [21] |
| Catalase | Enzymatic assay (Manual) | [19, 22] |

| | | |
|---|---|---|
| CEA | Kit from Düsseldorf / Germany | [23] |

**Statistical Analysis**

Data expressed as mean ± standard error. The differences between groups were based on t-test values of less than 0,05 analyzed by SPSS (version 22, USA). To evaluate correlation, the correlation coefficient (r) was used.

**Ethical Considerations**

The approval of the health institution, as described in the attached file, was obtained to collect blood samples for colon cancer patients, in cooperation with the medical and nursing staff.

## Results

The results demonstrated that the normal value of Cathepsin D for the control group was (8.61±0.294ng\ml). The effect of numerous biomarkers on cathepsin D activity was evaluated in the control group, and the results demonstrated that the actions of the enzyme are not affected by gender or age. On the other hand, the results also showed that the activity of enzymes is not affected by smoking and BMI (Figure 1).

The results also proved a highly significant increase in cathepsin D activity in patient groups (17.81±0.652ng/ml) compared to the control group at P=0.001 (Figure 2). Results also explained a very high significant decrease in catalase activity of the patients group (32.03±1.984 KU/L) compared to the Control group (58.09±3.572 KU/L, P=0.001) (Figure 2).

Results showed a very high significant increase in activity of Gamma Glutamyl transferase in the patients group( 114.9±8.558 U/L) compared to the control group (18.21±1.18 U/L, P= 0.001) (Figure 2).

Results of Alkaline phosphatase demonstrated a significant increase in activity of the patients group (75.85±3.99 U/L) compared to the control group(62.11±1.954 U/L) at P=0.001 (Figure 2).

The results of carcinoembryonic antigen (CEA) showed a very high significant increase in the level of CEA in the patient group (17.62±8.28 ng/mL) compared to the control group (1.99±17.62 ng/mL) at P=0.001 (Figure 2).

The group of colon cancer patients was divided into two subgroups according to CEA values, the first being patients with CEA values >5 and the second with CEA values ≤5, The percentage of patients whose CEA values were less than 5 was 75.6% The percentage of patients whose CEA values were greater than 5 was 24.4%, and they are certainly at risk for tumor to grow again. The results also showed that activity values of cathepsin D in colon cancer patients are related to values of CEA (Table 2).

**Table 2.** CEA and Cathepsin D values

| CEA | | CEA | Cathpsine D |
|---|---|---|---|
| ≤5 | Mean | 2.7641 b | 14.8270 b |
| | N | 68 | 68 |
| more than 5 | Mean | 63.5391 a | 27.0182 a |
| | N | 22 | 22 |

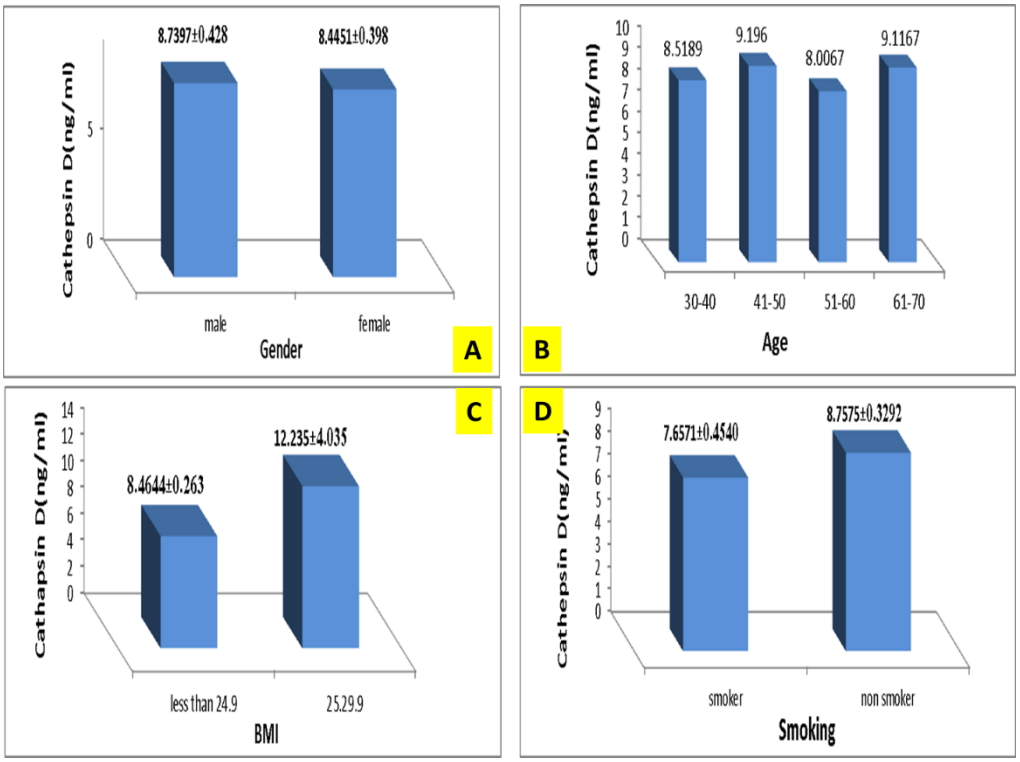


**Figure 1.** The Correlation of Cathepsin D And Demographic Parameters, (A) Gender, (B) Age, (C), Bmi, (D) Smoking

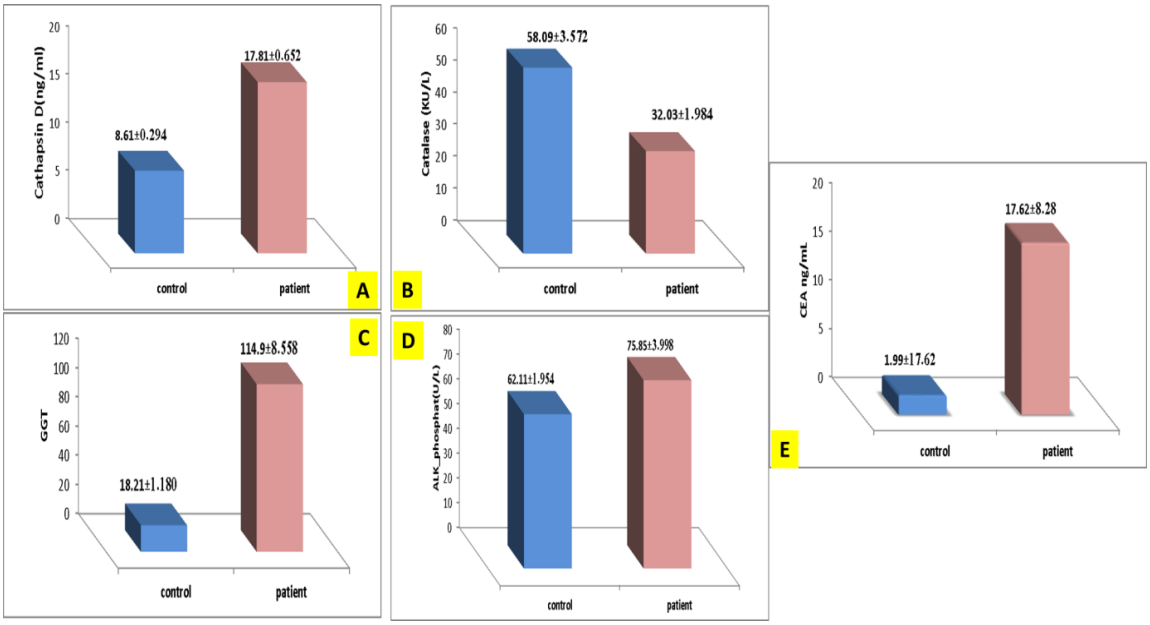


**Figure 2.** Measured Parameters in Patients Versus Control. (a) Cathepsin, (b) Catalase, (c) ggt, (d) alp (e) cea.

## Discussion

In the current study, we evaluated the serum level of cathepsin D in colon cancer patients in comparison to the control group [24]. The results of the present study highlighted that cathepsin D concentrations are significantly elevated in colon cancer patients, and the activity of enzymes in the control group is not affected by their age, gender, BMI and smoking. Furthermore, the elevated cathepsin D was reciprocally related to the progression of cancer. It is also proportionally balanced with carcinoembryonic antigen and colon cancer markers, these results are consistent

with [25]. Colon cancer is associated with elevated serum levels of cathepsin D which is potentially explained in the context of upregulation of cathepsin D in neoplasm, twined with loss of integrity of extracellular matrix (ECM) protein assembly. Cathepsin D as an endopeptidase disintegrate ECM, basal epithelium proteins as well as many intracellular proteins and endocytosed proteins. It is potentially enclosed in phagosome-like vesicles with acidic pH where ECM components are present, they are stuck. Hence, cathepsin D may indulge in proteolysis and cancer development [26].

The decreased activity of catalase in colon cancer patients is due to several reasons, catalase is the primary enzyme that controls $H_2O_2$ levels and may aid in the spread of cancer. Despite this, it has been demonstrated that the majority of cancers have low levels of catalase. This may be because malignant cells have impaired peroxisomal biogenesis. Given that peroxisome levels are typically lower in malignant tissues, catalase may also be expressed in other cell components, such as the cytoplasm and mitochondria [27].

Through the glutathione metabolism-related oxidative stress pathway, high blood GGT levels may raise the risk of cancer formation. Enhanced GGT activity may be a reaction to oxidative stress, according to several preclinical investigations; as a result, prolonged formation of reactive oxygen species by enhanced GGT activity may result in genetic instability and the development of tumours [28].

The current study's findings indicate that serum levels of ALP in colon cancer patients were significantly higher than in controls, which is consistent with findings made by earlier researchers who found that high activity levels of these enzymes were associated with a higher risk of developing colon cancer. These findings suggest that high activity levels of these enzymes may be used as tumour markers in the diagnosis, prognosis, and treatment of colorectal liver metastases that are incurable. Elevated ALP levels may suggest that cancer has progressed to bones or that liver damage is conceivable in people with colon cancer. This is likely caused by the breakdown of tissues, which allows enzymes to leak and appear in blood circulation [20].

A significant increase in CEA values appeared in the patient group compared to the control group, and a percentage (24.4%) of patients had high CEA values compared to a percentage (75.6%), whose values were normal. CEA levels that remain high or increase after treatment mean that treatment isn't working, or cancer is growing again, which is due to the role of CEA as a human fibroblast activation factor, in conditioning the target tissues for the engraftment of CEA-expressing cancer cells, through the differentiation of tissue-resident fibroblasts, leading to local change in the composition of the interstitial milieu [29, 30].

## Conclusion

Cathepsin D has a dual functionality, it increases in colon cancer patients compared with control and it has very high values in patients with metastasis and invasion of colon cancer.

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