# Determination of Meprin Alpha Enzyme and its Relationship with Some Antioxidants and Interleukin 12 in the Blood Serum of Patients with Colon Cancer

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## Abstract

**Background:** Colon cancer is an uncontrolled growth of cells that begins in a part of the large intestine called the colon. The colon is the first and longest section of the large intestine. The possible causes of colon cancer are genetic, environmental and nutritional factors. This study was prepared to help in identifying the causes and predicting the occurrence of colon cancer.

**Objective:** To identify the possible factors as causes of colon cancer in a group of patients in Diyala Governorate - Iraq.

**Patients and Methods:** In this study, blood serum samples were collected from 60 patients with colon cancer. 30 serum samples were collected from non-colon cancer patients who were considered a control group. The levels of alpha-meprin enzyme, catalase enzyme, nitric oxide and interleukin 12 were measured by immunoassay using the ELISA device.

**Results:** The results showed that the levels of Meprin alpha and interleukin 12 had statistically significant values, as their levels were higher than the control group, while the levels of catalase and nitric oxide were low when compared to the control group.

**Conclusion:** It was found that there is a significant positive correlation between meprin alpha and interleukin 12, and there was an inverse correlation between meprin alpha and catalase and nitric oxide. The results of our study indicated that the reason for the increase in meprin alpha and interleukin 12 is related to colon cancer.

**Keywords:** Antioxidants, Interleukin 12, Meprin alpha, Catalase, Nitric oxide.

## Introduction

Colon cancer is a type of cancer that affects the large intestine and usually arises from benign tumors that turn into cancerous cells over time (1). Colon cancer affects the colon and causes a health problem. It is a very common disease, with high rates of infection and death reaching almost half of those infected (2). Colon cancer was the cause of 10% of cancer deaths in 2010. Colon cancer is becoming more common among people aged 45 to 50, regardless of age (3) Colon Cancer is one of the most common types of cancer in the world, with more than a million new cases each year around the world (3). Malignant tumors of the lymphocytes are known as lymphomas. They begin in B or T lymphocytes. The gastrointestinal tract is the most common site for lymphomas outside the lymph nodes (4). Therefore, it is considered the third most common type of cancer and the fourth most

common type of cancer in terms of cancer-related deaths (5). Among the causes of infection are behavioral factors that affect the development of colon cancer, including age, lifestyle, environmental, and genetic factors (6). Meprin alpha is a metalloproteinase that contains zinc and is associated with the cell membrane, from which it is secreted. Many of these enzymes (metalloproteins) are secreted from cell membranes; some of them are associated with the cell membrane, and their work is both outside and inside the cell. These metalloproteins are crucial in various disorders and diseases, including inflammatory conditions and cancers (7). Interleukin 12 is a key member of the IL-12 cytokine family and a powerful stimulator of antitumor immunity. IL-12 was initially identified in the late 1980s as a factor that acts as a stimulator of natural killer cells with multiple biological effects on peripheral blood lymphocytes (8). It is mainly produced by antigen-presenting cells such as dendritic cells, monocytes, macrophages, and B cells. It consists of two covalently linked chains of 40 KDa and 35 KDa. IL-12 has been reported to have a potent antitumor effect (9, 10). Catalase plays an important role in decomposing hydrogen peroxide into oxygen and water. Catalase removes high concentrations of hydrogen peroxide due to its antioxidant action. Catalase is distinguished from other enzymes by its highest turnover rates, with a single molecule capable of converting 83,000 hydrogen peroxide molecules into water and oxygen per second. Catalase also resists the formation of Peroxynitrite by oxidizing nitric oxide in the presence of hydrogen peroxide. Overall, these catalytic properties enhance the antioxidant defense systems of cells mediated by catalase (11). Nitric oxide plays a significant role in the physiology of living organisms. It has a significant regulatory role at the physiological level in vital processes such as neurotransmission. It also has an essential role in

responding to inflammation and cardiovascular diseases and is involved in defense mechanisms and regulating inflammatory and immune mechanisms (12). Arginine acts as a building block for protein and a precursor for nitric oxide synthesis (13). Nitric oxide is a vasodilator, neurotransmitter, metabolic regulator, and bactericidal, fungicidal, and virucidal agent (14). This study investigates the meprin alpha enzyme's effect on cancer cells, the associated biochemical alterations in colon cancer patients, and its potential future implications.

## Patients and Methods

**Study design:** Blood samples were taken from 60 patients with colon cancer, and 30 samples were used as a control group for the period from 11/1/2023 to 3/1/2024. Blood samples (4-5 ml) were drawn from patients and healthy people and transferred to gel tubes. Serum was obtained by centrifugation at 3000 rpm for 15 minutes. The serum was stored at -20 °C until the tests were performed.

**Serological tests:** Serological tests were performed in Baghdad, Iraq's Scientific Center for Chemical Analysis laboratories. The effectiveness and levels of the study variables were determined using the sandwich method and the ELISA device. The concentration of the study variables in the blood serum was estimated using a ready-made test kit from the American company Clond-Clond Corp, which produced a yellow compound whose absorption intensity could be measured at a wavelength of 450 nanometers.

## Statistical analysis

The results were analyzed statistically, and the values were expressed as (mean ± standard deviation), and the level of statistical significance was determined using the SPSS program (statistical package for the social sciences), and the difference between the two groups were not considered statistically significant unless (P value) was less than

0.05, and Pearson's correlation coefficient (r) was used to test the relationship between the two coefficients.
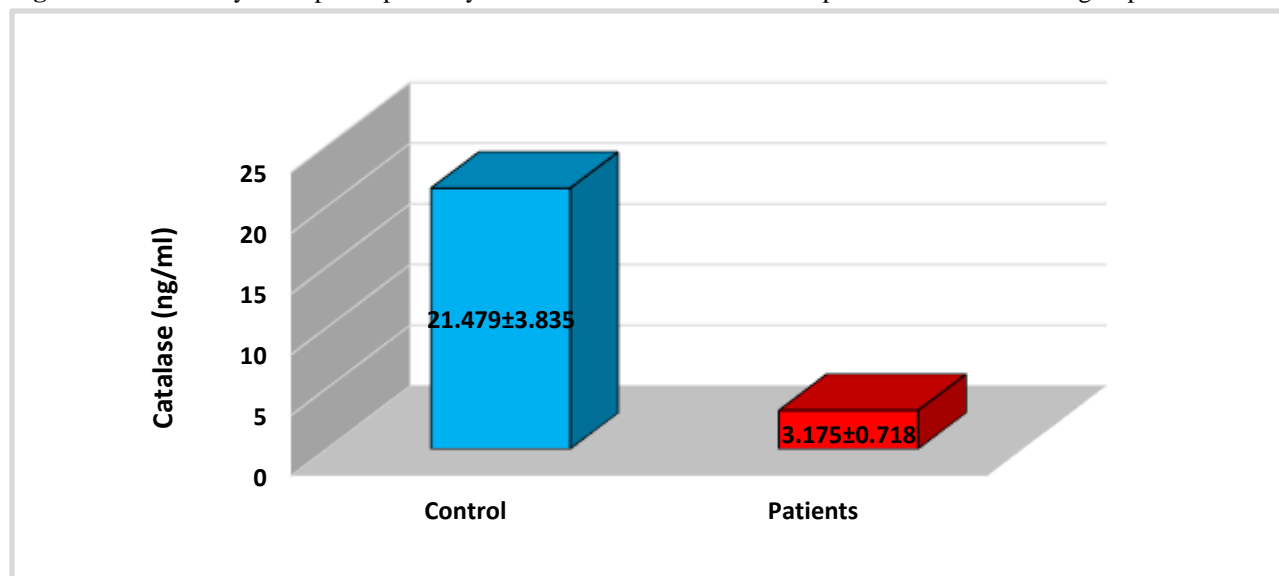
## Results

**Meprin alpha enzyme level assessment:** The activity and level of meprin alpha enzyme were estimated in the blood of healthy people and patients with colon cancer. The research results included the statistical values for colon cancer patients that were estimated in the blood of patients with colon cancer and the control group, where it was found when conducting a statistical comparison between the activity of meprin alpha enzyme in the blood of patients and controls,

it was found that there was a significant increase at a probability level ($P \leq 0.05$) in the activity of the enzyme in patients, where it was ($316.696 \pm 53.131$) and in the control, where it was ($223.385 \pm 40.820$) and shown in Figure 1.

**Catalase enzyme level assessment:** Catalase enzyme levels were investigated in the serum of patients with colon cancer compared to healthy controls. A statistically significant decrease was observed at the probability level of $P \leq 0.05$, where the values of Mean ± SD in healthy and infected patients were $21.479 \pm 3.835$ and $3.175 \pm 0.718$, respectively, as shown in Figure 2.
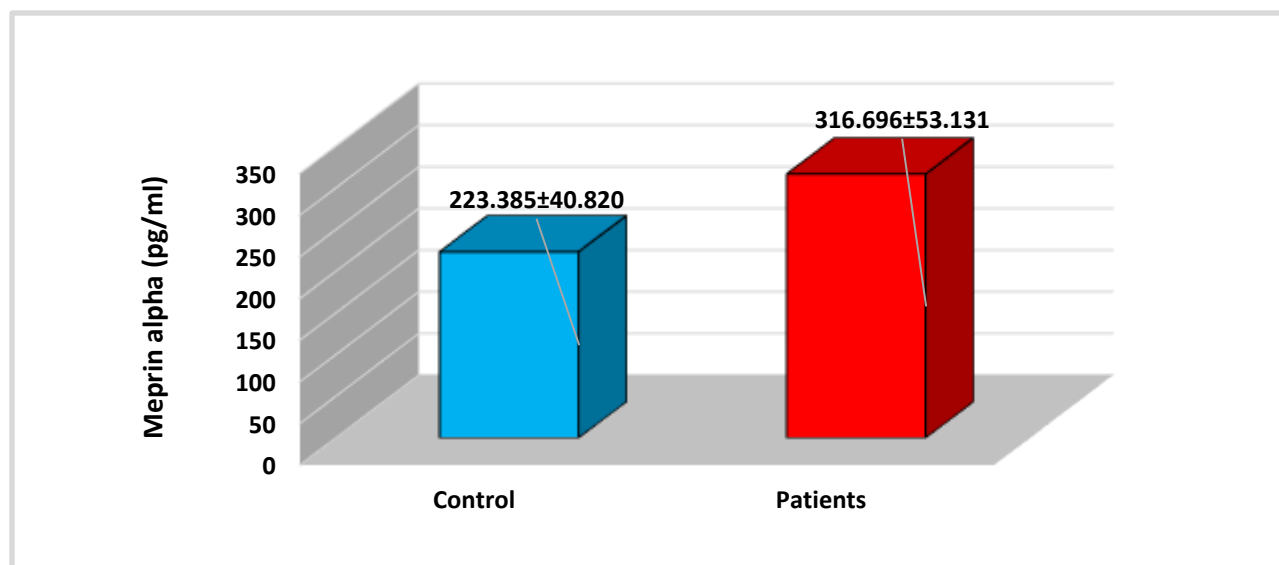


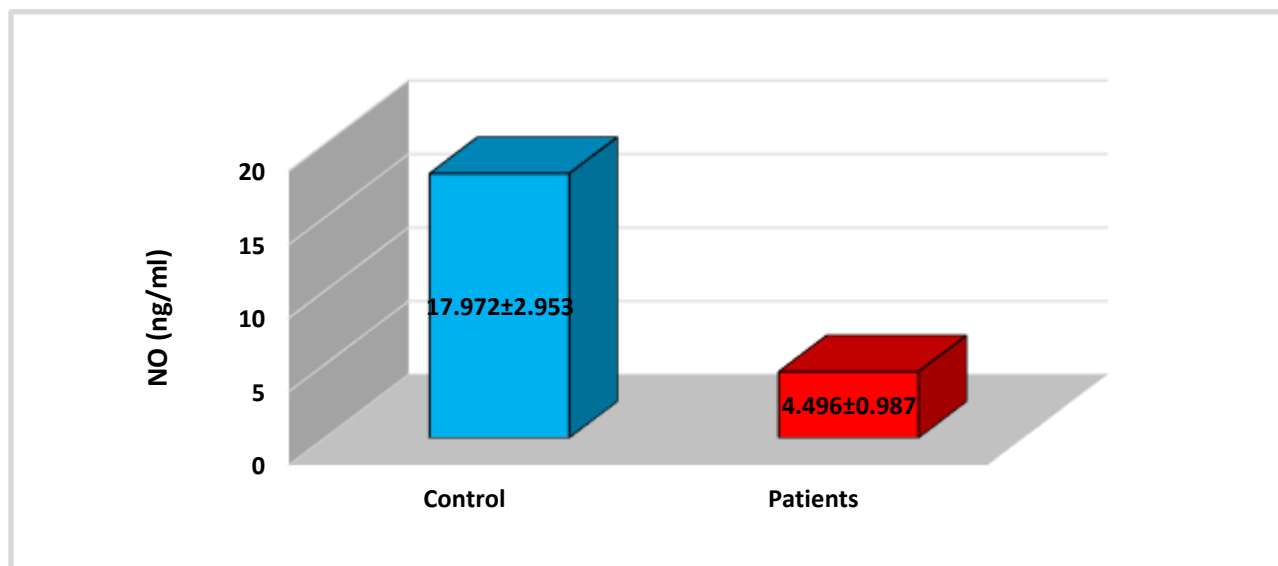**Figure 1.** The activity of meprin alpha enzyme in the blood of colon cancer patients and the control group.



**Figure 2.** shows the concentration of catalase enzyme in the blood of colon cancer patients and the control group.

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**Nitric oxide level assessment**: The results showed a statistically significant decrease at the probability level of P≤ 0.05 in the level of nitric oxide in the group of colon cancer patients, where the results in patients were (4.496±0.987) compared to the control group (17.972±2.953) as shown in Figure 3.

**Interleukin-12 level assessment:** By studying the levels of interleukin 12 in the serum of healthy people and those with colon cancer, the results showed a significant increase with statistical significance at the probability level of P≤0.05 in patients compared to healthy people, as the mean ± SD in patients was (377.891±80.119) and healthy people (136.927±33.929) as shown in Figure 4.



**Figure 3.** Shows the concentration of nitric oxide in the blood of colon cancer patients and the control group.
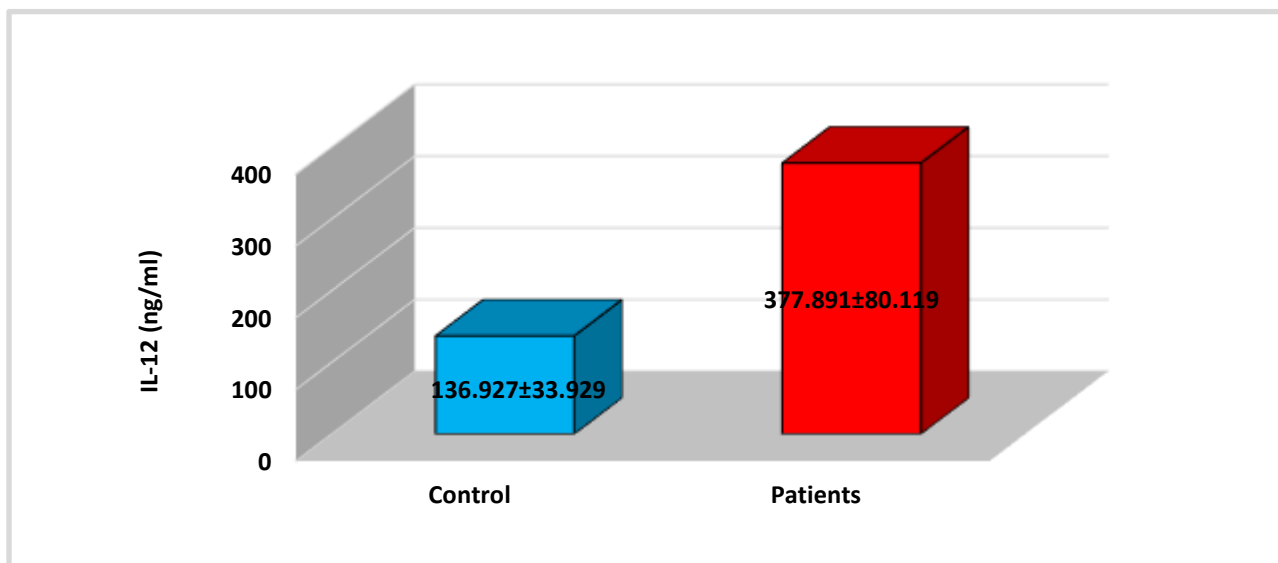


**Figure 4.** The concentration of interleukin-12 in the blood of colon cancer patients and the control group.

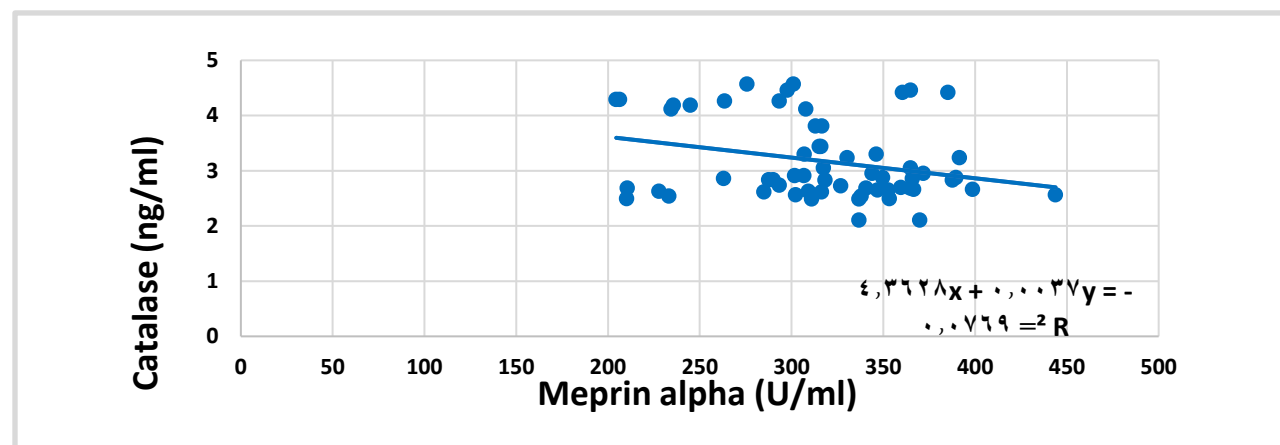**The correlation relationships of the enzyme meprin alpha with the variables under study:** To find the relationship between the enzyme meprin alpha and the clinical variables studied in the research for patients with colon cancer, it is necessary to find the correlation coefficient (linear correlation) between the enzyme and the variables, as the correlation coefficient r: is a measure of the degree of association or commitment between two independent variables and is used to describe the relationship and degree of association between the various measurements studied, as follows:

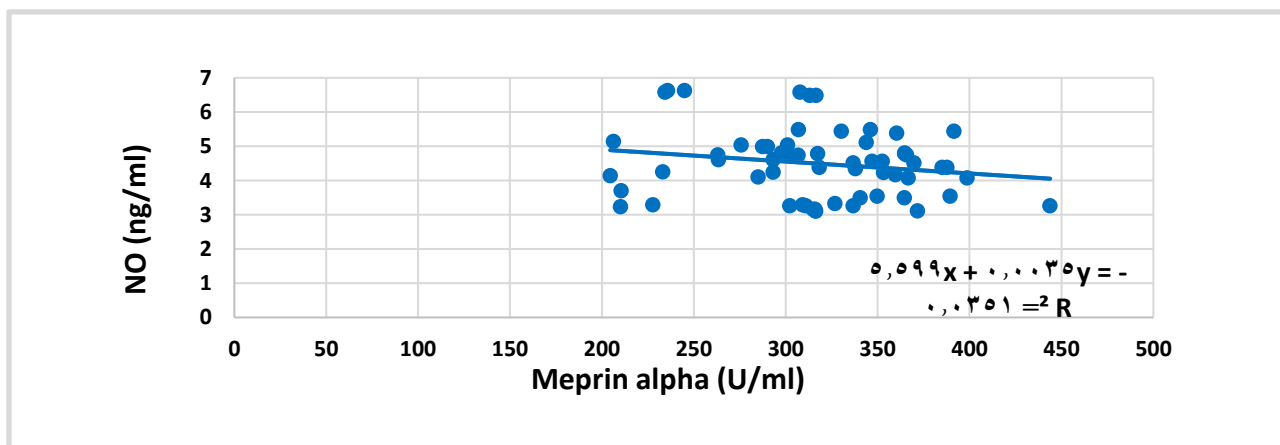**The binding relationship between meprin alpha and catalase:** The correlation between the enzyme meprin alpha and the enzyme catalase was studied in people with colon cancer and an inverse correlation was found as shown in Figure 5.

**The binding relationship between meprin alpha and nitric oxide:** The relationship between meprin alpha and nitric oxide was studied in people with colon cancer and an inverse relationship was found as shown in Figure 6.

**The binding relationship between Meprin alpha and IL-12:** The relationship between meprin alpha and interleukin-12 was studied in people with colon cancer and a direct correlation was found as shown in Figure 7.



**Figure 5.** The linear relationship between the enzyme meprin alpha and the enzyme catalase in the blood serum of patients with colon cancer.



**Figure 6.** The linear relationship between the enzyme meprin alpha and nitric oxide in the blood serum of patients with colon cancer.

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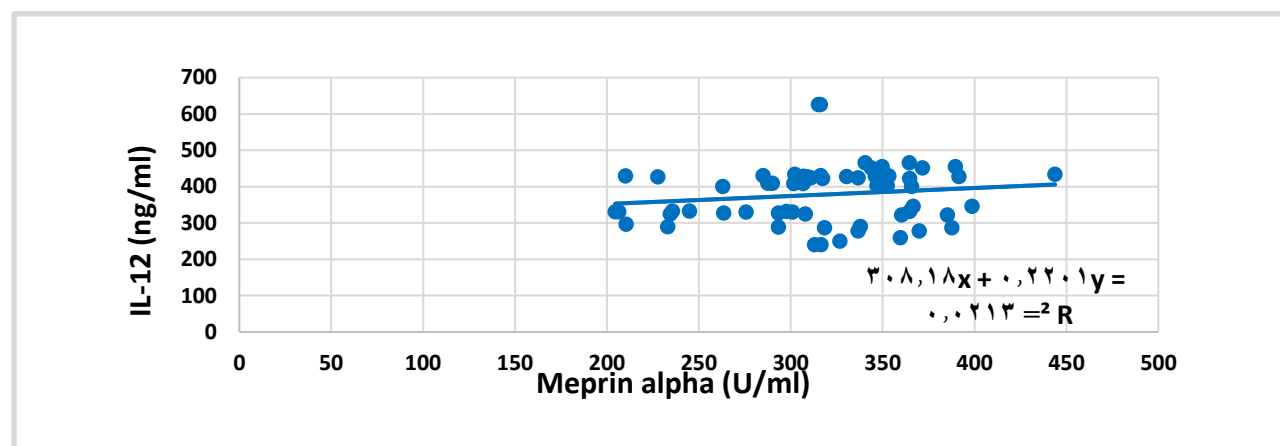


**Figure 7.** The linear relationship between the enzyme meprin alpha and interleukin-12 in the blood serum of patients with colon cancer.

## Discussion

Colon cancer is an uncontrolled growth of cells that begins in a part of the large intestine called the colon. The possible causes of colon cancer include genetic, environmental, and nutritional factors. This study was designed to help determine the causes and predict the occurrence of colon cancer. The results of our study showed lower levels of catalase and nitric oxide in people with colon cancer and higher levels of interleukin 12 and mebrin alpha in people with colon cancer. The study conducted by researchers Lutaz, Daniel, and their group was consistent with the results of our study, which indicated that mebrin alpha contributes to tumor progression by facilitating the migration of cancer cells and their entry into the blood vessels and their metastasis in patients compared to healthy individuals (15). Peters and colleagues observed that mebrin alpha levels were highly abundant in intestinal cells of both sexes, and their expression was associated with different stages of colon cancer. conditions (16). Anwar and colleagues noted that catalase is the major antioxidant enzyme that efficiently degrades hydrogen peroxide into water and oxygen, reducing oxidative stress and preventing cellular and molecular disintegration. This enzyme plays a crucial role in maintaining cellular redox balance. Its dysregulation is associated with the delicate balance between generating reactive oxygen species (ROS) and antioxidant defenses such as catalase, which is particularly vital in conditions such as cancer, where catalase can play a dual role in the survival of normal and cancer cells (17). Panneerselvan suggested that the reason for the lower levels of nitric oxide in patients with colon cancer compared to healthy individuals is due to the antioxidant and pro-oxidant properties of nitric oxide (18,19). Yin, X. L. demonstrated that IL-12 is a potent cytokine that plays a key role in the initiation of cell-mediated immunity by regulating biological groups as a growth factor for natural killer cells and activated T cells, favoring the formation of cytotoxic T cells, and stimulating the production of several cytokines (20,21).

## Conclusions

The change in immune response due to colon cancer resulted in high responsive concentrations of interleukin 12 and meprin alpha, with a strong association between them.

## Recommendations

It was recommended to study the relationship between meprin alpha enzyme and other cancer cases, such as lung and liver cancer. In addition, it was essential to

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study the effect of genes encoding the meprin alpha enzyme on patients with colon cancer and other cancers. Furthermore, it was recommended that the relationship between meprin alpha activity and oxidative stress be found, and the role of meprin alpha in remodeling the extracellular matrix be studied.

**Ethical clearance:** This study was conducted based on the ethical standards stipulated in the Declaration of Helsinki. Before taking the sample, the patient's informed written and verbal agreement was obtained, after the review and approval of the study protocol and subject's information by the local ethics committee according to the document number 12118 (including the number and the date in 9/11/2023) to get this approval by Task Facilitation Committee of Diyala Health Directorate in coordination with Tikrit University, College of Education for Pure Sciences, Department of Chemistry.

**Conflict of interest:** None.

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# محدد انزيم الفازيم ميبرين وعلاقته المضادة للاكسدة وانترلوكين ١٢ في مصل الدم لمرضى القولون العصبي

¹ حسين محمود خلف ، ² وسن نزهان حسين، ³ علاء حسن مصطاف

## الملخص

**الخلفية:** القولون العصبي: هو القولون غير المضبط للخلايا يبدأ في جزء من الامعاء الغليظة. القولون هو القسم الاول والاطول من الامعاء الغليظة. الاسباب المحتملة لسرطان القولون هي العوامل الضارة والبيئية والغذائي. وعدت هذه الدراسة للمساعدة في تحديد لاسباب والتنبؤ بحدوث سرطان القولون.

**الأهداف:** تحديد العامل المحتمل كمسببات لسرطان القولون في مجموعة من المرضى في محافظة ديالى ـ العراق.

**المرضى والطرق:** في هذه الدراسة تم جمع عينات من الدم من ٦٠ مريضا للكولسترول. تم جمع ٣٠ عينة من المرضى المصابين بالمرض والكولسترول والذين اعتبروا مجموعة المرضى. تم قياس مستويات انزيم الفا ميبرين وانزيم الكاتالاز واكسيد نتريك وانترلوكين ١٢ بواسطة المقايسة المناعية باستخدام جهاز الاليزا.

**النتائج:** أن مستويات الميبرين لسيارة فولفو والإنترلوكين ١٢ كانت ذات قيم ذات دلالة إحصائية، حيث كانت مستوياتهما أعلى من المجموعة الضابطة، في حين كانت مستويات الكاتالاز وأكسيد النيتريك دون وجودا بالمجموعة الضابطة. بالإضافة إلى: هناك أن هناك ارتباطاً إيجابياً يهم بين الميبرين فولكس فاجن والإنترلوكين ١٢، وهناك ارتباط عكسي بين الميبرين فولفو والكاتالاز وأكسيد النيتريك. أشارت دراستنا إلى سبب زيادة الميبرين لسيارة فول الصويا والإنترلوكين ١٢ المرتبط بالقولون العصبي.

**الاستنتاج:** وُجد ارتباطٌ إيجابيٌ كبيرٌ بين الميبرين ألفا والإنترلوكين ١٢، وارتباطٌ عكسيّ بين الميبرين ألفا والكاتالاز وأكسيد النيتريك. وأشارت نتائج دراستنا إلى أن سبب ارتفاع مستويات الميبرين ألفا والإنترلوكين ١٢ مرتبطٌ بسرطان القولون.

**الكلمات المفتاحية:** مضادات التغذية، الإنترلوكين ١٢، الميبرين فولفو، الكاتالاز، قرص نيتريك.

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