Antioxidant status and the in vitro effect of Trypsin and Chymotryps in enzymes on Iraqi pancreatic cancer patients.

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Abstract

cancer of the pancreas Pancreatic cancer has been identified as the 11th most prevalent cancer in the world. It is an incurable malignancy and the seventh major cause of cancer deaths globally in industrialized nations. 100 blood samples were obtained from AL-Anbar oncology cancer and the nearby Baghdad Hospital. Two groups were created out of them. The first group consists of 30 pancreatic cancer patients between the ages of 20 and 40, while the second group consists of 30 patients between the ages of 41 and 60, with 20 healthy individuals serving as the control group. Measurements were made to gather biological data to aid in the early diagnosis of the illness. The concentration of Trypsin enzyme in the serum of pancreatic cancer patients increased, while the concentration of Chymotrypsin enzyme and D3 decreased when compared to the control group, according to the results of the measurements of the two groups' levels of antioxidant vitamins (E and D3) and enzymes (Trypsin and chymotrypsin). Vitamin E level has no impact .

Materials and Methods the total number of blood samples collected was 80 samples. 40 blood samples of pancreatic cancer patients of different ages 20-60 years , including patients without chronic diseases Such as blood pressure, diabetic , heart disease, those with a family history of pancreatic cancer , and smokers (group A) of 80 samples Blood There are 40 samples of healthy controls (Group B). healthy controls has been selectedwithout chronic diseases or a family history of pancreatic cancer or other cancers.

Results: According to our research, people with Pancreatic cancer had significantly high concentration levels of trypsin while the concentration of Chymotrypsin enzyme and D3 decreased when compared to the control group, according to the results of the measurements of the two groups' levels of antioxidant vitamins (E and D3) and enzymes (Trypsin and chymotrypsin). Vitamin E level has no impact .

Conclusion: In our study, we can conclude that pancreatic cancer patients exhibited a significant reduction in the level chymotrypsin enzyme and antioxidant vitamin D3 ,while it showed an increase in the trypsin enzyme and vitamin E not effected.

Aims

1. Study the change in levels of enzymes (Trypsin and Chymotrypsin) in pancreatic cancer patient as compared with the control group .

2.evaluate the role of some antioxidants represented by vitamin D3and Vitamin E in development of pancreatic cancer

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Introduction

Despite decades of ongoing research, the five-year survival rate for pancreatic cancer is still less than 9 percent. It is one of the most aggressive cancers. According to recent data, pancreatic cancer is the 11th most frequent cancer worldwide and the seventh major cause of cancer deaths globally in industrialized nations (1) .

The pancreas is an organ in your abdomen that is located behind the bottom portion of your stomach. Pancreatic cancer starts in the tissues of the pancreas. Your pancreas generates hormones that help you control your blood sugar as well as enzymes that help with digestion.

The pancreas can develop both malignant and non-cancerous tumors, among other growths. The cells that lining the ducts that expel digestive enzymes from the pancreas are where the most prevalent type of pancreatic cancer first develops (pancreatic ductal adenocarcinoma).

Rarely is pancreatic cancer found in its earliest stages, when it is most treatable. This is due to the fact that symptoms frequently don't appear until the disease has spread to other organs.

Depending on how far advanced the illness is, many treatments are available for pancreatic cancer. Surgery, chemotherapy, radiation therapy, or a combination of these are all possible options(2). Among the dietary components with potential chemopreventive effects are antioxidant vitamins. Beta-carotene (pro-vitamin A), vitamin E, vitamin D3, and vitamin C are antioxidant vitamins that are thought to reduce the risk of cancer by limiting tissue damage by trapping organic free radicals and/or deactivating excited oxygen molecules, a byproduct of numerous metabolic processes(3). Trypsin and chymotrypsin level changes may serve as biomarkers for the early detection of pancreatic cancer(4).

Materials and Methods

Patients

In this study, the total number of blood samples collected was 80 samples. 40 blood samples were collected from patients during November of 2021 to july of 2022at the Baghdad Teaching Hospital andAL-Anbar oncology cancer,Iraq. 40 blood samples of pancreatic cancer patients of different ages 20-60 years. Blood samples were collected from people by intravenous withdrawal using A 5 ml syringes. After the samples were drawn, they were placed directly in tubes (gel tubes). The samples were left for 15 minutes at room temperature for coagulation, and sometimes an incubator was used to make the clotting process faster within 5-10 minutes At 37 °C, the tubes were centrifuged at 4000(xg) for 10 minutes, then the serum was transferred to other tubes (white tubes) using micropipette and stored at – 20 C until the analysis date.

Trypsin Enzyme

An Enzyme-Linked Immunosorbent Assay is included in this kit (ELISA)(Abcam). Porcine trypsin antibody has been pre-coated on the plate. The trypsin in the sample is introduced, and it binds to the antibodies on the wells. The biotinylated Porcine Trypsin Antibody is then added to the sample and binds to the trypsin. Streptavidin-HRP is next added, which binds to the biotinylated trypsin antibody. During the washing phase after incubation, all unbound Streptavidin-HRP is rinsed away. The color develops in response to the amount of Porcine trypsin added to the substrate solution. The reaction is stopped by adding an acidic stop solution and measuring the absorbance at 450 nm.

Human Chymotrypsin (CTR)

The CTR ELISA(AbcamUSA) kit uses a polyclonal anti-CTR antibody and a CTR-HRP conjugate to perform a competitive enzyme immunoassay. CTR-HRP conjugate is treated with the assay sample and buffer combined. for one hour on a pre-coated plate The wells are sterilized after the incubation period. five times decanted and washed After that, the wells are incubated with a HRP enzyme's substrate. The enzyme-substrate reaction's end product The result is a blue-colored complex. Finally, a stop solution is supplied to bring the process to a halt. .The solution will then turn yellow as a result of the reaction. In a micro plate reader, the color intensity is measure spectrophotometric ally at 450nm.

The 25(OH) Vitamin D Enzyme-Linked Immunosorbent

The 25(OH) Vitamin D Enzyme-Linked Immunosorbent Assay ELISA(Abcam UK) kit

This kit is based on a quantitative determination of 25(OH) Vitamin D3 and 25(OH) Vitamin D2 in human plasma and serum samples. UV rays from the sun act on 7-dehydrocholesterol to generate parent Vitamin D3, which is then converted to the active form of Vitamin D. Vitamin D can also be obtained as parent Vitamin D2 from a variety of food sources, whether natural or fortified.

These parent molecules are transferred to the liver,

where they are hydroxylated to form Vitamin D 25(OH). This metabolite is subsequently delivered to the kidney, where it is converted to 1,25(OH)2Vitamin D, which is the physiologically active form of Vitamin D**.**

Human Vitamin E (VE)

The Double Antibody Sandwich ELISA(mybiosurce USA) technique is used in this kit. The detection antibody is a biotinylated polyclonal antibody, while the pre-coated antibody is an anti-Human VE monoclonal antibody. Biotinylated antibodies and samples are added to the mix. After their respective additions to the ELISA plate wells, they were rinsed off with PBS or TBS. wells. Following that, Avidin-peroxidase conjugates are added to the wells Human Vitamin E (VE) ELISA Kit. After the enzyme conjugate has been properly rinsed out of the wells with PBS or TBS, the TMB substrate is utilized for coloring. TMB combines with peroxidase activity to produce a blue product, which then becomes yellow after the stop solution is added (Color Reagent C). The amount of target analyte in the sample and its color intensity are positively associated.

Statistical Analysis:

Statistical analysis was performed with Graph Pad Prism version 6(Graph Pad Software Inc., La Jolla, CA). Results were expressed as mean ± standard deviation (Mean ± SD). T-test was performed to analyze the statistical significance of the both groups. The P-values ≤0.05 were considered statistically significant .

Results and Discussion

Serum Trypsin enzyme level

The results present that the concentration of trypsin enzyme in pancreatic cancer patient were increased when competed with control .The group (20-40 year) it was (695.4±192.6ng/ml) comparing with control group(228.5±82.47 ng/ml) and the group (41-60 year) it was(673.1±176.1 ng/ml) with the control (243.3±69.83 ng/ml) both group was high and significant.

Fig. (1) depict the results of the serum trypsin enzyme test, which showed that patients with pancreatic cancer had significantly higher serum trypsin enzyme levels in the years 20 to 41 and 41 to 60 compared to controls, respectively.

a high serum There are numerous factors that contribute to the trypsin enzyme level in pancreatic cancer patients, including gene alterations. Cationic trypsinogen is an enzyme that can be produced using the PRSS1 gene(5). This enzyme is a serine peptidase, a type of enzyme that fragments (cleaves) different proteins. The pancreas produces cationic trypsinogen, which aids in food digestion. The pancreas secretes cationic trypsinogen, which is then carried to the small intestine where it is cleaved to create trypsinogen. This outcome could be caused by the trypsinogen being cleaved again into the active form known as trypsin. or, during the development of neoplasms, cellular stress and inflammation may cause the release of trypsin into compartments that would otherwise be protected from this enzyme. and R116C gene lead to altered cell cycle and activated to release enzymes that precipitate in blood that make trypsin in high levels(6).

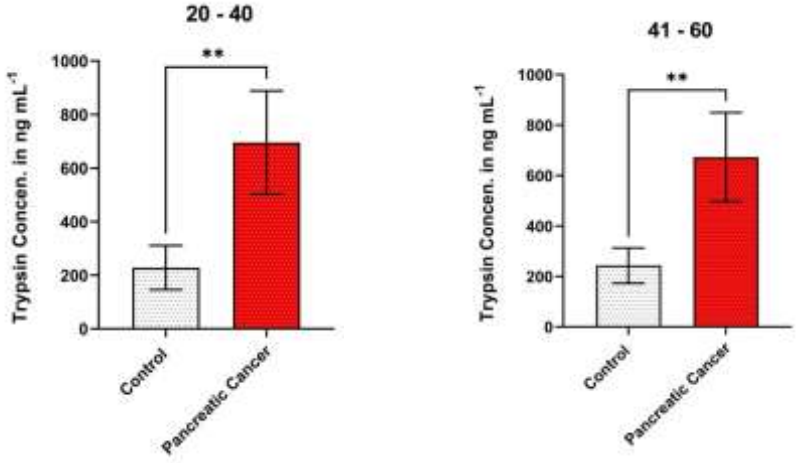

Fig. (1): Serum Trypsin concentration (ng/ml) in control group and patient pancreatic cancer age (20-40)(41-60).

Serum Chymotrypsin enzyme level

The results results that the concentration of chymotrypsin enzyme in pancreatic cancer. Have certain differences between groups of patient and control. In group of patient (20-40 year)there was significant decreasing in level of chymotrypsin enzyme and it was 184.8±67.13 (54.71±15.65 ng/ml) in comparing with control group(184.8±67.13 ng/ml) In the sometime group of patient (41-60 year) show same decreasing in comparing with control and it was 53.75±14.17 (ng/ml) and (216.0±52.76ng/ml) respectively.

The results of the serum chymotrypsin enzyme test are shown in **Fig.**(2)depicted in enzyme levels in years 20 to 41 and 41 to 60 than controls Patients with pancreatic cancer who have low serum chymotrypsin enzyme levels are thought to have obstructed pancreatic ducts or damaged or dead chymotrypsinogen-producing cells. Repeated episodes of acute pancreatitis or chronic pancreatitis, along with CFTR gene mutation, can cause duct obstruction and cell death. cause pancreatic insufficiency because insufficient amounts of digestive enzymes are delivered to the small intestine. Conditions like chronic pancreatitis and pancreatic cancer frequently exhibit this. This study attributes the activation of calcineurin by lysosomal chymotrypsin, which causes Drp1-mediated mitochondrial fission in apoptotic cells(7).

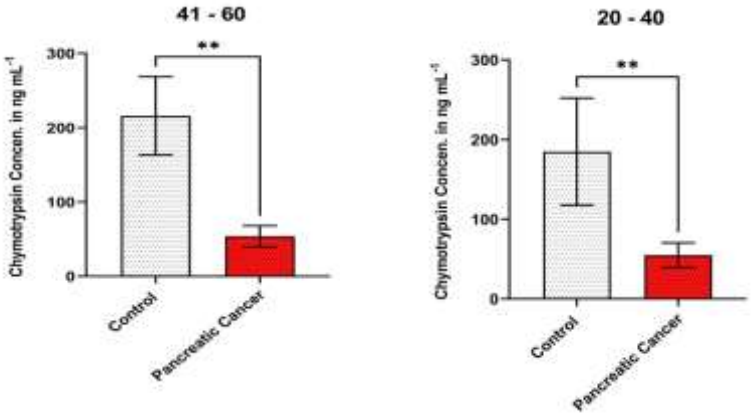

Fig.(2): Serum Chymotrypsin concentration (ng/ml) in control group and patient pancreatic cancer age (20-40)(41-60).

Serum D3 Vitamin level

The results revealed that the concentration of vitamin D3 in pancreatic cancer . Have certain differences between groups of patient and control. In group of patient (20-40 year)there was significant decreasing in level of vitamin D3 and it was (17.40±5.92 ng/ml) in comparing with control group(31.30±8.66ng/ml) In the sometime group of patient (41-60 year) show less significant differences in comparing with control and it was (27.91±9.33 ng/ml) and (37.27±9.03 ng/ml) respectively.

The results of serum vitamin D3 testing are depicted in **Fig**.(3), and they showed that patients with pancreatic cancer had considerably lower serum vitamin D3 levels in years 20 to 41 than in years 42 to 60, with a deficiency of less than 37% in years 43 to 60. Deficiency is most well-known for its link to bone disorders and fractures, as well as its more recent link to the likelihood of developing numerous cancer types.(8)

Patients' vitamin D3 insufficiency is caused by a variety of factors in addition to related Depending on the severity of the insufficiency, chronic pancreatitis may lead to exocrine pancreatic insufficiency. with the disease's development. The properties of pancreatic sterol-binding, which appear to be

important for vitamin D delivery, and inflammatory pancreas destruction (exocrine insufficiency) appear to be related.(9) impacts on the function of pancreatic beta cells and may have an impact on insulin production, which leads to diabetes. Beta cell malfunction and absolute or relative insulin shortage resulting from both hereditary and environmental factors are characteristics of vitamin D3 deficiency. immune cells that invade beta cells cause -cell damage, which causes a progressive decrease of beta cell function that finally results in apoptosis.(10) It was shown that patients in the early stages of the condition had lower levels of vitamin D3 deficiency than those in the later stages, which may be because those patients had undergone treatment plans and got vitamin D3-containing dietary supplements.(11)

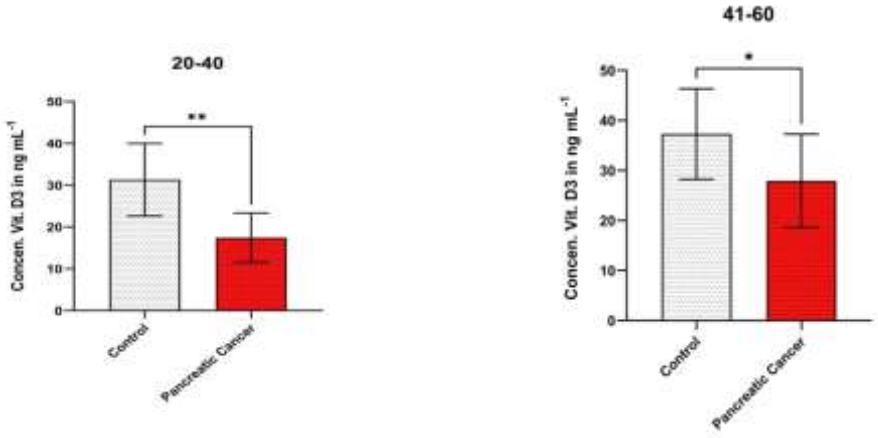

Fig.(3): Serum Vitamin D3 concentration (ng/ml) in control group and patient of pancreatic cancer (age20-40 and 41-60).

Serum E Vitamin level

The results present that the concentration of vitamin E in pancreatic cancer patient were no significance when competed with control .The group (20-40)year it was (12.81±3.513ng/ml) comparing with control group(12.77±3.899 ng/ml) and the group (41-60)year it was(13.14±2.544 ng/ml) with the control (12.85±3.167ng/ml) both group was no significant. The outcomes of serum vitamin E are shown in **Fig.**(4) and it revealed the same result in the serum vitamin E level in Patients of pancreatic cancer in(20-41)and(42-60) year the compare between patient and control .

The absence of a change in vitamin e concentrations is due to vitamin E no significant for pancreatic cancer or because the patients have undergone treatment programs and received nutritional supplements containing vitamin E(12).

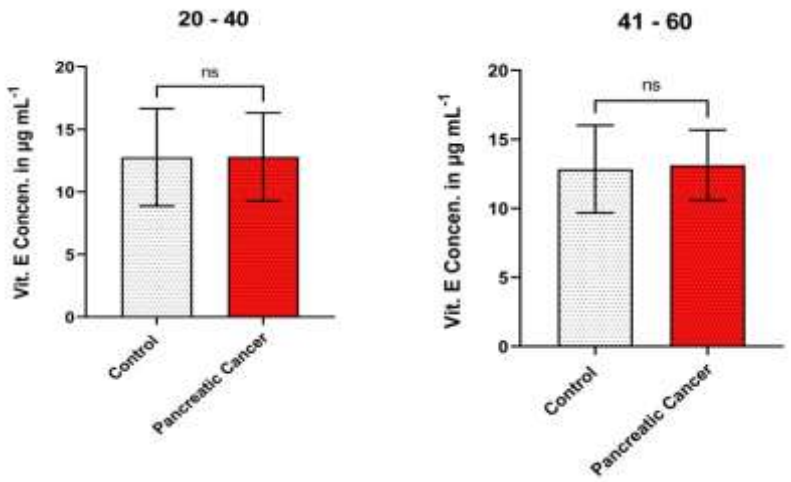

Fig.(4): Serum vitamin E concentration (ng/ml) in control group and patient of pancreatic cancer (age 20-40an 41-60).

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