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ORIGINAL RESEARCH ARTICLE

Oxidative enzymes and vitamin E in ovarian cancer: Insights from a case-control study

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ABSTRACT

Studying vitamin E's antioxidant capabilities and how they relate to oxidative enzymes in the context of ovarian cancer was the focus of this study. A case-control study was conducted, with 100 women with ovarian cancer serving as cases and 30 women in good health serving as controls. Enzyme-linked immunosorbent assay (ELISA) was used to assess serum levels of trypsin, chymotrypsin, pancreatic-type amylase, and vitamin E, while the dimercaptopropanol tributyrate (BALB) method was used to measure lipase levels. Patients with ovarian cancer were shown to have lower levels of chymotrypsin and lipase and higher levels of trypsin and amylase than controls. The two groups had almost the same vitamin E content. According to these findings, oxidative enzymes may have a role in the progression of ovarian cancer by increasing trypsin and amylase and decreasing chymotrypsin and lipase. Although vitamin E was thought to slow the development of gynecologic malignancies, the study found no such impact. Further research with larger study groups is necessary to obtain more robust results.

Keywords: ovarian cancer; oxidative enzymes; trypsin; chymotrypsin; lipase; amylase

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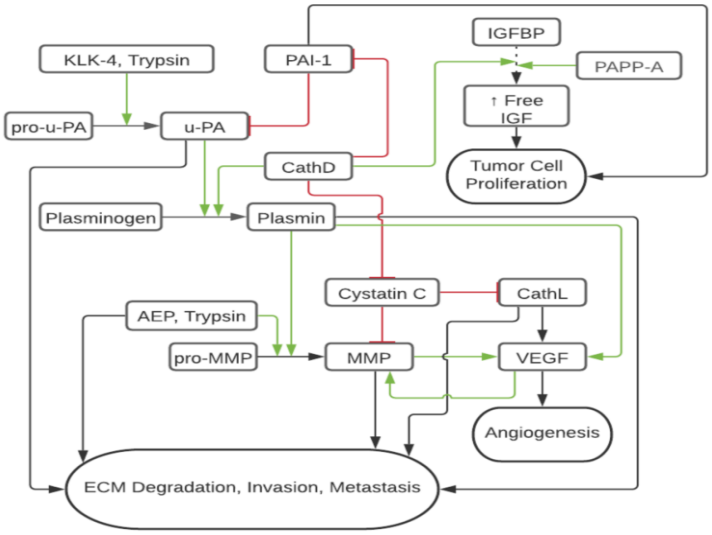
1. Introduction

Ovarian cancer has the highest mortality rates among gynecological malignancies, often diagnosed late at stages III or IV, resulting in a 5-year survival rate below 30%[1]. Despite being the seventh most common neoplasm in females worldwide, with over 200,000 new cases annually, symptoms are often absent, making early detection challenging[2]. Detecting ovarian cancer at stage I is associated with high cure rates, emphasizing the need for early diagnostic methods. Tumor markers like CA125 and CA19-9 are utilized for diagnosis, but the causes of ovarian cancer remain largely unknown, with family history and hormonal factors considered significant risks[3].

Proteases, including trypsin, play crucial roles in cancer growth and metastasis. Trypsin, produced by pancreatic acinar cells, is activated in the duodenum and is a digestive enzyme[4]. Detected in various tumors, including ovarian, trypsin's dysregulation contributes to harmful remodeling, tumor growth, chemo resistance, and metastasis[5,6]. In ovarian cancer, trypsinogen expression indicates aggressiveness, and trypsin activity can be observed in serum, ascites,

and cyst fluid. Trypsin degrades components of the extracellular matrix and activates other proteases, promoting tumor invasion[6]. **Scheme 1** illustrates the tumor protease cascade in ovarian cancer.



Scheme 1. Schematic of tumor protease cascade in ovarian cancer. The red flat line represents inhibition and decreased tumorigenic activity, while the green arrow represents cleavage or activation, increasing tumorigenic activity. The dashed line illustrates IGFBP binding of IGF, leading to decreased circulation of IG.

Chymotrypsin, a serine protease synthesized as the inactive proenzyme chymotrypsinogen in the pancreas, requires trypsin for activation. While sharing structural similarities, trypsin and chymotrypsin have distinct substrate specificities, with chymotrypsin constituting 10%–20% of total protein content in the exocrine pancreas[7]. Lipase, a key enzyme in triglyceride breakdown, is vital for fat digestion and metabolism, primarily found in pancreatic secretions[8]. As one of the major digestive enzymes, lipase, along with proteases and amylases, contributes to the breakdown of food components during digestion[9,10].

Amylase, crucial for breaking glycoside bonds in cellulose and glycogen, is synthesized by the pancreas and salivary glands but also present in various organs[11]. Hyperamylasemia is clinically relevant in conditions like acute pancreatitis and tumors producing amylase, including pancreatic, lung, stomach, uterine, and ovarian cancers[12]. Elevated amylase levels are commonly associated with pancreatitis but are rarely observed in ovarian cancer, with the cause remaining unclear[4]. Vitamin E, particularly alpha-tocopherol, acts as an antioxidant, inhibiting lipid peroxidation and protecting cell membranes and nucleic acids. In vitro studies suggest a protective effect against tumor development, but the relationship between vitamin E serum levels and neoplasm formation requires further research[12,13].

This analysis of ovarian cancer looked into the connection between oxidative enzymes and vitamin E, an antioxidant. Trypsin, chymotrypsin, amylase, lipase, and vitamin E levels in the serum were measured using the enzyme-linked immunosorbent assay (ELISA) and dimercaptopropanol tributyrate (BALB) techniques in both groups. We found that oxidative enzymes, including trypsin, amylase, and chymotrypsin, were altered in ovarian cancer, but chymotrypsin and lipase were found to be at normal levels. However, the study did not provide evidence that vitamin E plays a part in the development of gynecologic malignancies.

2. Materials and methods

The purpose of this research done in Iraq was to look into the link between ovarian cancer and oxidative enzymes and vitamin E. Biochemistry case-control study methodology was used, with 30 healthy women aged 30–45 serving as the study's control group and 100 women with histologically confirmed ovarian cancer serving as the experimental group. The women in the experimental group were split into two categories based on age: those between the ages of 20 and 40, and those between the ages of 41 and 60. Trypsin, chymotrypsin,

lipase, and amylase are all examples of oxidative enzymes that were tested in the serum alongside vitamin E levels in both the control and experimental groups.

2.1. Measuring trypsin serum levels

To quantify trypsin serum levels, the Porcine Trypsin ELISA kit from MyBioSource (Cat. No MBS1600900) was employed in this investigation. The sensitivity of the kit was 0.52 ng/mL, while the dynamic range of the standard curve was from 1 to 350 ng/mL. The intra-assay precision (CV8%) of the assay was evaluated by testing three samples of known concentrations on the same plate. Additionally, three samples were examined in separate assays to evaluate inter-assay precision (CV < 10%). The ELISA kit featured a pre-coated plate with Porcine trypsin antibody, enabling the connection between trypsin in the sample and the antibody. Subsequently, multiple substances were added sequentially, and the reaction was halted using an acidic stop solution. The absorbance (OD value) was measured at 450 nm. The proper functioning of the kit required an incubator maintained at 37 °C ± 0.5 °C. Serum samples from both the cases and controls were collected and allowed to clot for approximately 15 min at room temperature. Subsequently, the samples were centrifuged at 2000–3000 RPM for another 15 min, and the supernatant was carefully collected, discarding any sediment.

2.2. Measuring chymotrypsin serum levels

For measuring chymotrypsin serum levels, the Human Chymotrypsin (CTR) Elisa kit from MyBioSource (Cat. No MBS720164) was utilized. The kit exhibited a sensitivity of 1.0 ng/mL, with both intra-assay and inter-assay precision being less than 10%. The CTR ELISA kit employed a competitive enzyme immunoassay technique, utilizing a polyclonal anti-CTR antibody and a CTR-HRP conjugate. The sample and buffer were incubated with the CTR-HRP conjugate for one hour. Following that, the wells were washed and incubated with an HRP enzyme ligand. The reaction between the HRP enzyme and the ligand resulted in the formation of a blue complex, which was terminated by adding a stop solution that turned the solution yellow. The absorbance (OD value) was measured at 450 nm. Similar to the trypsin assay, a 37 °C incubator was required for this kit as well. Serum samples were collected and allowed to clot for approximately 2 h at room temperature, followed by centrifugation at 3000 RPM for 10–20 minutes. The serum was then collected and either assayed immediately or stored at −20 °C or −80 °C.

2.3. Measuring lipase serum levels

For measuring lipase serum levels, the QuantiChrom™ Lipase Assay Kit from BioAssay Systems was employed. This kit utilized an improved method called the dimercaptopropanol tributyrate (BALB) method. In this method, lipase breaks down BALB, resulting in the formation of SH groups. These SH groups interact with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to generate a yellow product. Absorbance was measured at 412 nm (OD_{412nm}) at two time points: 10 min (OD_{10min}) and 20 min (OD_{20min}). Lipase activity was determined using the Equation (1):

$$\text{Activity} = \frac{OD_{20min} - OD_{10min}}{OD_{calibrator} - OD_{H2O}} \times 735 \text{ (U/L)} \quad (1)$$

In this equation, OD_{20min} represents the OD_{412nm} sample value at 20 min, and OD_{10min} represents the value at 10 min. $OD_{Calibrator}$ and OD_{H_2O} refer to the OD_{412nm} measurements of the calibrator and water, respectively, at 20 min. The number 735 corresponds to the equivalent activity of the calibrator under the specific experimental conditions. For human serum, the lipase activity was found to be 75 ± 6 U/L. The linear detection range of this kit is 40 to 1600 U/L. It is important to note that lipase inhibitors such as mercaptoethanol and dithiothreitol can influence the results of this test and should be avoided during sample preparation.

2.4. Measuring pancreatic amylase serum levels

To measure pancreatic amylase serum levels, the Pancreatic Amylase Human ELISA Kit from Abcam (Cat. No ab137969) was utilized. This kit exhibited a sensitivity of approximately 0.053 mU/mL, with an intra-assay precision of 4% and an inter-assay precision of 9.3%. The ELISA kit contained 96-well plates pre-coated with a pancreatic amylase specific antibody and blocked. The wells were then incubated with serum samples before being probed with a biotinylated detection antibody specific for pancreatic amylase. A buffer solution was used to flush the wells. Sequentially, Streptavidin-Peroxidase Conjugate and Tetramethylbenzidine (TMB) were added, resulting in the formation of a blue complex. The reaction was stopped by adding an acidic stop solution, turning the solution yellow. Absorbance (OD value) was calculated at 450 nm. If wavelength adjustment capability is available, readings at 570 nm should be subtracted from those at 450 nm to correct for optical flaws. If wavelength adjustment is not available, the plate is read at 450 nm only.

For sample preparation, serum samples were collected and allowed to clot, followed by centrifugation at 3000 RPM for 10 min. The serum was then carefully removed and diluted 1:20 into 1X Diluent N before being assayed. Alternatively, the serum could be stored at ≤ 20 °C for up to 3 months before analysis.

2.5. Measuring vitamin E serum levels

The Human Vitamin E (VE) ELISA Kit by MyBioSource (Cat No. MBS269047) was utilized, which possesses a sensitivity of 0.5 μg/mL. This kit demonstrates an intra-assay precision of ≤8% and an inter-assay precision of ≤12%. The kit employs the Double Antibody Sandwich ELISA technique, utilizing an anti-Human VE monoclonal antibody as the pre-coated antibody and a biotinylated polyclonal antibody as the detection antibody. The wells are supplemented with Avidin-peroxidase complexes, while pigmentation is achieved using the TMB ligand. A blue product is formed, which turns yellow upon the addition of the stop solution. This kit necessitates a 37 °C incubator and a microplate reader that measures absorbance at 450 nm with 570 nm or 630 nm adjustment wavelength filters. Whole blood samples were obtained from both cases and controls, and they were allowed to cool at 4 °C overnight before being centrifuged at 1000–3000 RPM for 10 min. The supernatant was either immediately tested or stored at −20 °C/−80 °C for 1–3 months.

3. Results

This study was meticulously designed, segregating participants into two distinct groups for comprehensive analysis:

Group A:

- Age Range: 20–40 years
- Subdivisions:
- Women diagnosed with ovarian cancer
- Healthy women within the same age bracket

Group B:

- Age Range: 41–60 years
- Subdivisions:
- Women diagnosed with ovarian cancer
- Healthy women within the same age bracket

Rationale for Group Division:

The strategic division into two age-defined groups serves a pivotal purpose in understanding the diverse nature of ovarian malignancies across different age brackets. Specifically:

- Group A (20–40 years):

Tumor Types: Predominantly focuses on ovarian malignant tumors of germ cell and sex cord-stromal tumors, given their higher incidence within this age range.

- Group B (41–60 years):

Tumor Types: Shifts emphasis to malignant ovarian tumors more likely to be of epithelial origin. Epithelial ovarian cancers are notably more prevalent in women aged 41–60 years.

This meticulous age-based stratification enhances the precision of the study by accounting for the distinct histopathological characteristics of ovarian tumors within these age cohorts. Such granularity enables a nuanced exploration of ovarian cancer's varied origins and pathologies, offering valuable insights for tailored diagnostic and therapeutic strategies. **Table 1** shows a summary of our results.

Table 1. Summary of results.

	Group A			**Group B**		
Parameters	**Control 20–40**	**Patients 20–40**	***P* value**	**Control 20–40**	**Patients 20–40**	***P* value**
Trypsin	220.0 ± 80.0	500.0 ± 200.0	< 0.01	240.0 ± 80.0	680 ± 70.0	< 0.01
chymotrypsin	185.0 ± 65.0	53.0 ± 18.0	< 0.01	216 ± 51.0	53 ± 14.0	< 0.01
Lipase	85.0 ± 35.0	4.40 ± 2.20	< 0.01	94.0 ± 32.0	14.4 ± 2.20	< 0.01
Amylase	62.0 ± 13.0	425.0 ± 45.0	< 0.01	63.0 ± 15.0	385.0 ± 37.0	< 0.01
Vitamin E	12.7 ± 3.70	12.8 ± 3.40	> 0.01	12.9 ± 2.90	13.2 ± 2.40	> 0.01

3.1. Trypsin levels

The mean concentration of serum trypsin in the control group was 220 ± 80 ng/mL, whereas in women with ovarian cancer in the age group of 20–40 years old, it was 500 ± 200 ng/mL as shown in **Figure 1A**. For the age group of 41–60, the mean concentration of serum trypsin in the control group was 240 ± 80 ng/mL, while in women with ovarian cancer, it was 680 ± 170 ng/mL (**Figure 1B**).

Normal serum levels of trypsin typically fall within the range of 115–350 ng/mL. Elevated trypsin levels are usually associated with conditions such as acute pancreatitis, pancreatic cancer, cystic fibrosis, alcohol abuse, or bile duct obstruction. On the other hand, low trypsin serum levels are observed in cases of exocrine pancreatic insufficiency and chronic pancreatitis[14]. Recent studies have revealed that certain non-pancreatic cells and tissues are capable of producing trypsin or trypsin-like enzymes. The secretion of Trypsinogen-1 and -2 by various types of cancer cells has been reported[15]. Kim et al. and Paju et al. found high levels of trypsin in the serum of ovarian cancer patients[6,16]. However, Miyata et al. discovered overexpression of trypsinogen-1 by gastric carcinoma cells[15]. Additionally, studies have indicated overexpression of trypsin in colorectal[17] and cervical cancers[18].

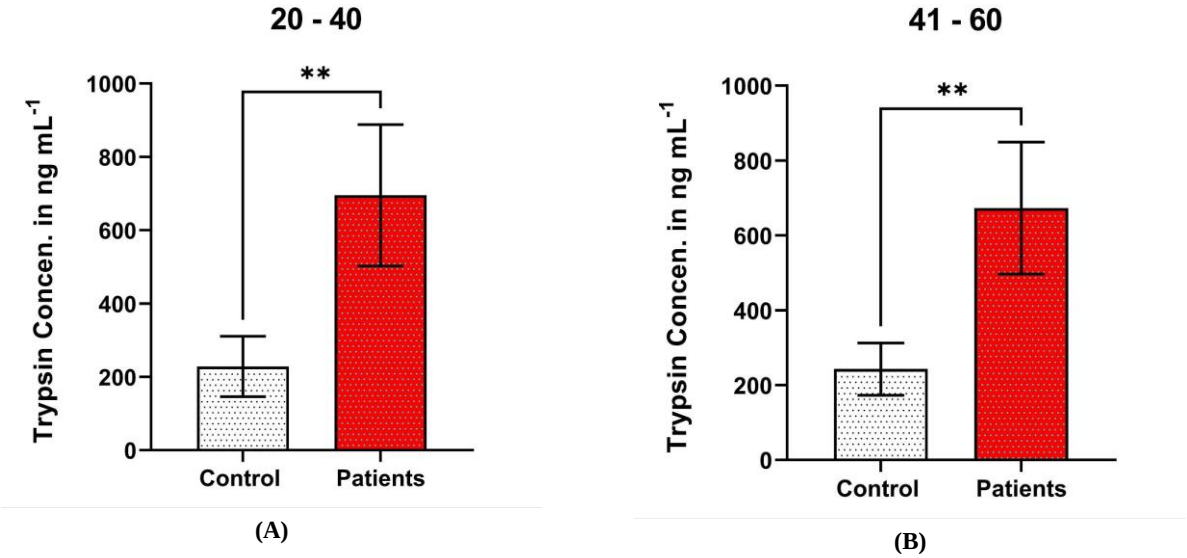


Figure 1. Trypsin concentrations in cases and controls of the age group **(A)** 20–40 and **(B)** 41–61.

3.2. Chymotrypsin levels

The mean concentration of serum chymotrypsin in the control group was 185 ± 65 ng/mL, whereas in cases within the age group of 20–40, it was 53 ± 18 ng/mL (**Figure 2A**). In the age group of 41–60, the mean concentration of serum chymotrypsin in the control group was 216 ± 51 ng/mL, while in cases, it was 53 ± 14 ng/mL as clarified in **Figure 2B**.

Chymotrypsin levels are typically measured in stool and fall within the range of 0.9–26.8 U/g. Low levels are associated with exocrine pancreatic insufficiency, while elevated levels are associated with diarrhea or excessive pancreatic enzyme supplementation[19,20]. Previous studies have examined chymotrypsin serum levels in patients with pancreatic cancer. It has been reported that chymotrypsin C expression decreases by over 75% after the onset of pancreatic cancer[21]. Rosendahl et al. discovered that individuals with inherited chronic pancreatitis and low chymotrypsin C mutants are at a higher risk of developing pancreatic cancer[22].

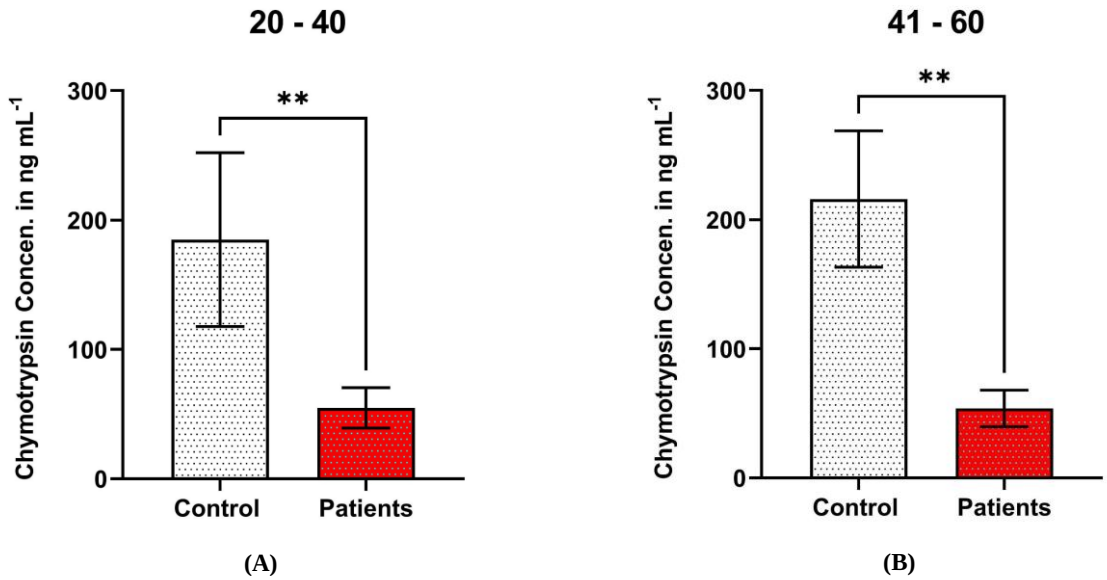


Figure 2. Chymotrypsin concentrations in cases and controls of the age group **(A)** 20–40 and **(B)** 41–60.

3.3. Lipase levels

The mean concentration of serum lipase in the control group of the age group 20–40 was 85 ± 35 U/L, whereas in the cases, it was 4.4 ± 2.2 U/L as shown in **Figure 3A**. Similarly, in the age group of 41–60, the mean concentration of serum lipase in the control group was 94 ± 32 U/L, while in the cases, it was 4.4 ± 2.2 U/L (**Figure 3B**).

Normal lipase serum levels typically range from 10 to 140 U/L in adults under 60 years old. Elevated lipase serum concentrations are commonly associated with pancreatic diseases, particularly acute pancreatitis. Increased serum lipase levels may also result from extra-pancreatic causes such as renal failure, cirrhosis, bowel problems[23], gallstones, cholecystitis, ulcers, or celiac disease. Conversely, markedly low lipase levels indicate permanent damage to the lipase-producing pancreatic cells, which is observed in chronic pancreatitis or cystic fibrosis[24].

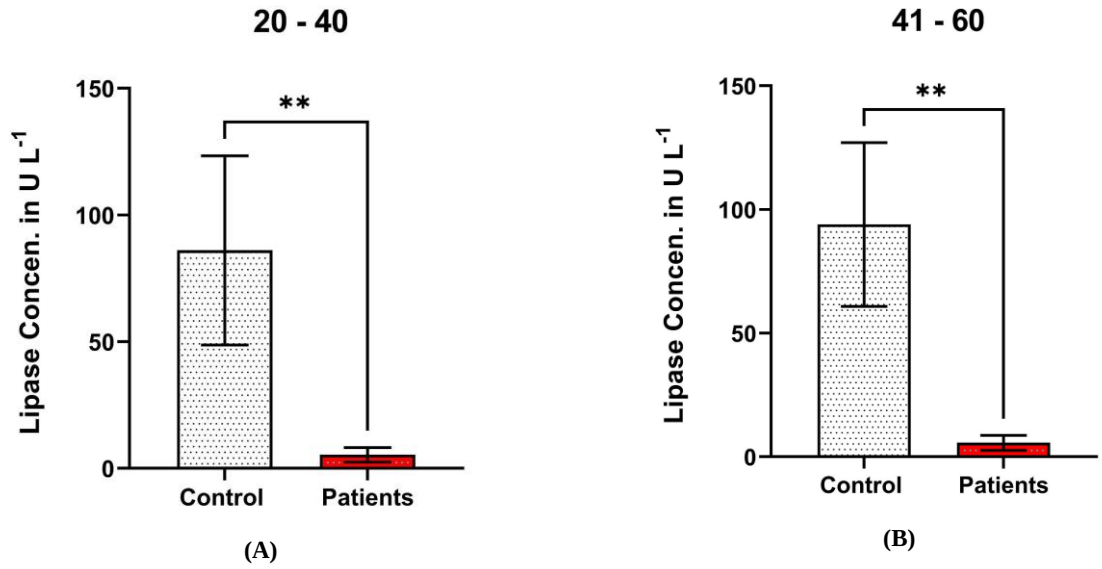


Figure 3. Lipase concentrations in cases and controls of the age group **(A)** 20–40 and **(B)** 41–60.

3.4. Amylase levels

The mean concentration of serum amylase in the control group of the age group 20–40 was 62 ± 13 U/L, whereas in the cases, it was 425 ± 45 U/L as presented in **Figure 4A**. In the age group of 41–60, the mean concentration of serum amylase in the control group was 63 ± 15 U/L, while in the cases, it was 385 ± 37 U/L as shown in **Figure 4B**.

The normal range for serum amylase levels in adults is typically 30–110 U/L[25]. Amylase can be categorized into two types: pancreatic amylase and salivary amylase, accounting for approximately 40%–45% and 55%–60% of total amylase, respectively[26]. Elevated amylase levels are associated with various benign and malignant conditions, including acute pancreatitis, pancreatic pseudocysts, perforated ulcer, intestinal infarctions, ascites, acute cholecystitis, ruptured ectopic pregnancy, peritonitis, diabetic ketoacidosis, salivary gland diseases, and multiple types of malignancies such as breast[27], lung[28], prostate[29], and ovarian cancers[4,30,31]. Low amylase levels can be attributed to chronic pancreatitis causing permanent cell damage, kidney disease, cystic fibrosis, liver disease, or pre-eclampsia[32].

To the best of our knowledge, our study is the first to investigate pancreatic amylase levels in relation to ovarian cancer, as previous studies primarily focused on the salivary type of amylase in other cancer types, such as gastric cancer[33,34]. Further studies with larger sample sizes are necessary to provide a more comprehensive evaluation of the association between pancreatic amylase elevation and ovarian malignancies.

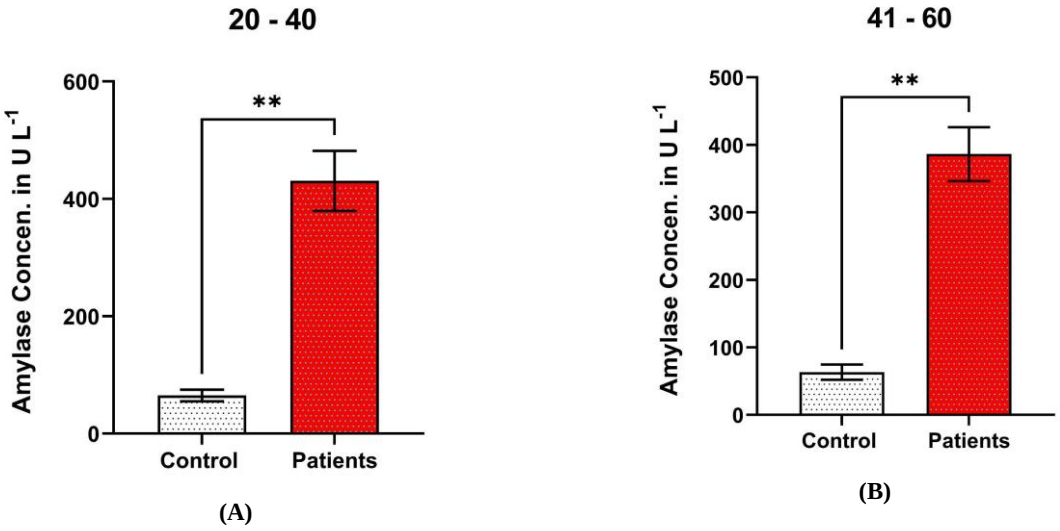


Figure 4. Amylase concentrations in cases and controls of the age group **(A)** 20–40 and **(B)** 41–60.

3.5. Vitamin E levels

The mean concentration of serum vitamin E in the control group of the age group 20–40 was 12.7 ± 3.7 µg/mL, while in the cases, it was 12.8 ± 3.4 µg/mL (**Figure 5A**). In the age group of 41–60, the mean concentration of serum vitamin E in the control group was 12.9 ± 2.9 µg/mL, and in the cases, it was 13.2 ± 2.4 µg/mL as illustrated in **Figure 5B**.

Normal serum levels of vitamin E (alpha-tocopherol) range from 6.8 to 31.7 µg/mL[35]. Low levels of alpha tocopherol are observed in conditions that hinder the body's absorption of vitamin E, including Crohn's disease, liver disorders, cystic fibrosis, celiac disease, pancreatitis, and certain genetic diseases. High levels of alpha tocopherol are rare and are usually associated with excessive vitamin E supplementation[36]. Several previous studies have investigated the relationship between serum vitamin E levels and various types of cancers. Torun et al. reported significantly lower vitamin E serum concentrations in females with breast neoplasms compared to cancer-free subjects[37]. Miyamoto et al. found significantly lower serum vitamin E levels in lung cancer patients compared to the control group[38]. Battisti et al.'s study failed to observe any significant differences in vitamin E levels between gastric cancer patients and healthy individuals[39]. Marco et al. studied vitamin E levels in patients with hematologic cancers and found no significant difference between the control and case groups[40].

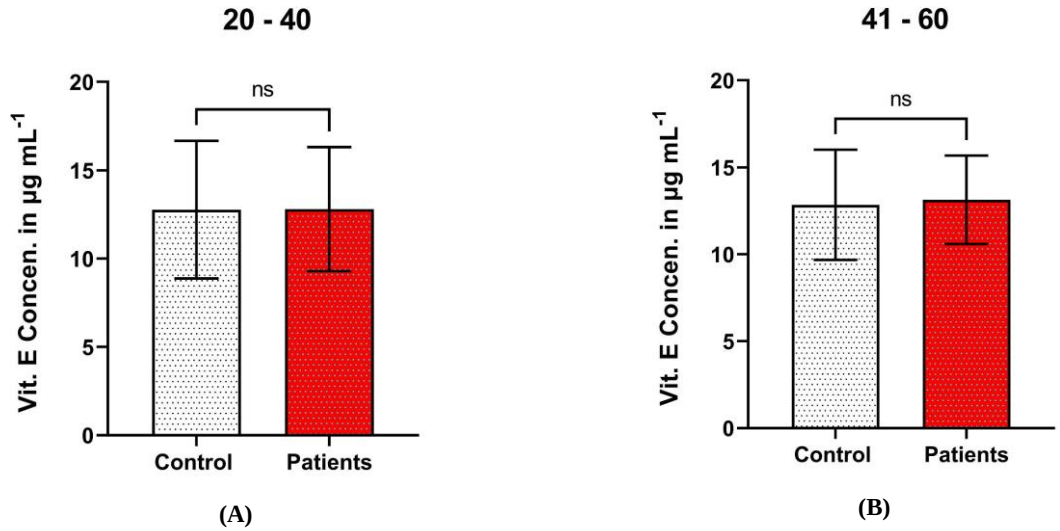


Figure 5. Vitamin E concentrations in cases and controls of the age group (A) 20–40 and (B) 41–60.

4. Discussions

4.1. Trypsin levels

In our study, trypsin serum levels were significantly elevated in ovarian cancer patients within both age groups, aligning with previous research[6,16]. High serum levels of trypsinogen-2 and trypsin-2-API have been observed in pancreatic and bile duct malignancies[19]. The elevation of these markers in such tumors may be attributed to increased secretion of pancreatic trypsin. However, this does not hold true for ovarian cancer[16]. Therefore, the results of our study suggest that ovarian malignant cells secrete trypsin, potentially contributing to aggressive tumor growth. Furthermore, the mean concentration of serum lipase was found to be significantly higher (680 ± 170 ng/mL) in older patients (age group 41–60) compared to younger patients (age group 20–40) (**Figure 1A,B**).

4.2. Chymotrypsin levels

To our knowledge, this is the first study to establish a relationship between ovarian cancer and chymotrypsin serum levels. We observed a significant decrease in serum chymotrypsin levels in cases from both age groups compared to the control group, which is consistent with findings in patients with pancreatic cancer from earlier studies (**Figure 2A,B**). Chymotrypsin serum levels have not been studied extensively in women with genital tract or ovarian-related diseases. Further research in this area is necessary to gain a better understanding of the role of chymotrypsin in ovarian malignancies.

4.3. Lipase levels

Our study revealed a significant decrease in lipase serum levels in women with ovarian cancer across both age groups. There was no substantial variation in lipase concentration between the two age groups (**Figure 3A,B**). Since this relationship has not been studied before, we were unable to compare these results with previous research. We speculate that this decrease in lipase levels may be attributed to pancreatic metastases, which can eventually result in permanent damage to the lipase-producing pancreatic cells, resembling the effects observed in chronic pancreatitis.

4.4. Amylase levels

The results of our study demonstrate a significant elevation in pancreatic amylase serum levels in ovarian cancer patients across both age groups as presented in **Figure 4A,B**. This finding is consistent with earlier studies. Guo et al. reported a case of ovarian carcinoma with hyperamylasemia in which amylase levels significantly decreased after treatment, although the specific type of amylase was not accurately confirmed in that case[4]. Kawakita et al. also presented a similar case where a woman with ovarian cancer exhibited very high amylase serum levels that returned to normal 24 h after surgery. The amylase elevation in that case was determined to be 99% of the salivary type[30]. Zakrezewska and Pietryńczak reported elevated amylase serum levels in over 35% of patients with ovarian cancer, with a dominance of the salivary type[31].

Here, the mean concentration of serum amylase was slightly higher (425 ± 45 U/L) in the younger patient group (age group 20–40) compared to the age group 41–60 (385 ± 37 U/L), while no significant difference was observed between the two control groups. This finding contradicts the results reported by Ueda et al., who found that amylase levels increase with age[33]. However, this discrepancy may be attributed to the small size of our study group.

4.5. Vitamin E levels

Several studies have also evaluated serum vitamin E levels in patients with ovarian malignancies. Heinonen et al.'s study in 1985 showed no significant difference in vitamin E levels between controls and ovarian cancer patients[12]. In another study by Heinonen et al., which included a larger study group and different types of gynecologic cancers, including ovarian cancer, the results were similar to the previous study,

demonstrating no differences in vitamin E levels between controls and cases[41]. In this study, as well as in previous studies, no significant differences in serum vitamin E levels were observed between the control group and ovarian cancer patients in both age groups. Furthermore, no differences were found between the two age groups (**Figure 5A,B**). These findings do not support the hypothesis that vitamin E has an effect on the progression of gynecologic tumors[42].

5. Conclusion

Our study revealed a significant association between oxidative enzymes and ovarian cancer. Ovarian cancer patients exhibited elevated levels of trypsin and amylase in their serum, while chymotrypsin and lipase levels were decreased. Interestingly, the rise in trypsin levels was more pronounced in older patients, whereas the increase in amylase levels was more prominent in younger patients. Age did not appear to have a significant impact on chymotrypsin and lipase levels. Importantly, this study is the first to investigate the correlation between chymotrypsin, pancreatic amylase serum levels, and ovarian cancer. However, our findings did not support the hypothesis that vitamin E may influence the progression of gynecologic tumors. Future studies with larger sample sizes are warranted to obtain more robust and conclusive results. The benefit of this research lies in establishing the connection between oxidative-type digestive enzymes and ovarian cancer. This finding holds potential for utilizing these enzymes as biomarkers in the future diagnosis of ovarian cancer.

Author contributions

Conceptualization, WYMA and MK; methodology, WYMA; formal analysis, WYMA and SFH; investigation, ESM; resources, WYMA and MK; data curation, ESM; writing—original draft preparation, WYMA; writing—review and editing, MK; supervision, WYMA and SFH; project administration, ESM. All authors have read and agreed to the published version of the manuscript.

Ethical approval

The research was granted ethical clearance from the Ethics Committee of the National Centre for Training and Human Development at the Iraqi Health's Baquba Teaching Hospital, as well as the Ethical Committee of the University of Diyala. Prior to their involvement, all participants furnished written and comprehensive consent.

Conflict of interest

The authors declare no conflict of interest.

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