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Evaluation of Glutathione Peroxidase and Glutathione Reductase Enzymes in Iraqi Patients with Colorectal Cancer

Noor E. Mahrose[1,*], Firas A. Hassan[1], Jamel Jebali[2], Salam Mohammed[3]

[1]Department of Chemistry, College of Science, Al-Nahrain University, Jadriya, Baghdad, Iraq.
[2]Higher Institute of Biotechnology of Monastir, University of Monastir, Monastir, Tunisia
[3]Department of Chemical and Petrochemical Engineering, College of Engineering and Architecture. University of Nizwa, Nizwa, Oman.

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Abstract

Glutathione peroxidase, and glutathione reductase Enzymes have a significant role in colorectal cancer (CRC) progression. The objective of this article is to measure glutathione peroxidase, and glutathione reductase enzymes in colorectal cancer patients and compare them with healthy subjects. Seventy newly diagnosed colorectal cancer patients were enrolled in the present study and divided as follows; Group A: (n=32) with an age range (of 20-40 years); Group B: (n=38) with age range (41-60 years). For comparison, total of (30) healthy subjects were also inserted in the current study divided into patients in the same manner. Serum levels of glutathione peroxidase and glutathione reductase enzymes were measured for patients and controlled by the ELISA technique. The current study shows that serum glutathione peroxidase levels varied significantly in (the 20-40 years) group when comparing the patient and control group (395.5 ng/ml vs. 71.61 ng/ml, p-value less than 0.0001) and also in (41-60 years) group with mean (469.4 ng/ml vs. 106.3 ng/ml, p-value less than 0.0001) for patients and control groups respectively. Antioxidant enzymes including glutathione peroxidase, and glutathione reductase were increased in colorectal cancer patients compared to healthy subjects, significantly. According to the current study, the evolution of these enzymes can be used to understand the pathophysiology of disease and also to diagnose the colorectal cancer.

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*Corresponding author email address: nooremad1441@gmail.com

1. Introduction

Colorectal cancer (CRC), often referred to as intestine and/or rectal carcinoma, is a major public health concern since it is the fourth most widely identified and second most lethal condition worldwide [1]. CRC was responsible for around 9.4% of cancer-related fatalities in 2020 [2]. Despite the significant rise in the total amount of recognized in the elderly population, the global rate of CRC is expected to more than double using 2035, with the poorest nations witnessing a major increase [3]. CRC is a disorder which impacts the colon or rectum. It is caused by an abnormal proliferation of glandular epithelial cells in the colon [4]. The global total of situations involving CRC is growing every day [5]. The likelihood of developing CRC is influenced by both environmental and hereditary factors.

Furthermore, in individuals with long-standing ulcerative colitis or Crohn's disease, the chance of getting CRC rises with age [6,7]. In normal cell metabolism, reactive oxygen species are formed. High amount of ROS causes oxidative stress (OS) that can lead to severe metabolic malfunctions and cell damage due to interaction of ROS with lipids, nucleic acids, proteins [8]. The general name for an enzyme subfamily with the function of peroxidase is glutathione peroxidase (GPX), whose main

biological purpose is to shield a living thing in opposition to oxidative damage [9]. Glutathione peroxidase's biological role is to convert lipid-converting free hydrogen peroxide to water and hydroperoxides to the corresponding alcoholic substances [10]. The main process that glutathione peroxidase catalyzes is:

$$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O \quad \ldots(1)$$

GSH stands for reduced monomeric glutathione, and GS-SG stands for glutathione disulfide. As a key mechanism of GSH breakdown, GSH is synthesized in the cell cytosol and exported into the plasma [11]. Glutathione can protect key cellular components from damage produced by reactive oxygen species, free radicals, peroxides, lipid peroxides, and heavy metals [12-15]. Glutathione reducer (GR), which is also referred to as glutathione-disulfide reductase (GSR), is a human protein expressed by the GSR gene [16]. Glutathione disulfide (GSSG) is catalyzed to glutathione sulfhydryl (GSH) by GR. GSH is essential for resisting oxidative stress and maintaining the cell's reducing state [17].

The GR acts as a dimeric disulfide oxido-reductase, converting one molar equivalent of GSSG to two molar equivalents of GSH using an FAD prosthetic group and NADPH [18]. Glutathione reductase from human erythrocytes is a homodimer composed of 52Kd monomers with three domains each. The architecture of GR is a single-sheet, double-layered structure with opposite directions beta-sheet revealed to the fluid on one face and random coils covering the other. A NADPH-binding field, one or more FAD-binding fields, and a dimerization field are all part of this. Each of the monomers has 478 sequences and one FAD molecule.

2. Material and Methods

2.1. Patients

About (70) newly diagnosed patients with colorectal cancer were enrolled in the current study. All patients were diagnosed by a physician in Al-Yarmouk Teaching Hospital in a period from September 2022 to January 2023. The age range of patients was (20-60 years). Patient subgroups were split into two. According to their ages: Group A: (n=32) with age range (20-40 years); Group B: (n=38) with age range (41-60 years). For comparison, thirty healthy subjects were inserted in the present study as the control group, also divided according to their ages into two groups (20-40 years) (n=20) and (41- 60 years) (n=10).

2.2. Assessment of serum proteolytic enzymes

Five milliliters of blood were withdrawn from each control and patient. transferred into a gel tube, allowed 15 minutes to clot, and centrifuged after that at 3000 rpm, the serum was then isolated for 10 minutes' centrifugation used for measurements of serum glutathione peroxidase and glutathione reductase enzyme levels which were assessed in blood serum by using ELISA technique and the supplier was Abcam Co., GSH-Px catalyzes the conversion of hydrogen peroxide and reduced glutathione into the water and oxidized glutathione. Its activity is measured by the rate of this reaction. To calculate GSH activity, the reduction not catalyzed by GSH-Px is subtracted. GSH quantity is determined by its reaction with dinitro benzoic acid, yielding a stable yellow compound measured at 412 nm. The Glutathione Reductase Fluorescent Activity Kit directly measures GR activity using a non-fluorescent compound that produces fluorescence. It can be an endpoint or kinetic assay, detecting GR activity in serum, plasma, erythrocytes, and cell lysates, validated for humans but expected to work in other species. GR, a ubiquitous flavoprotein, reduces oxidized glutathione (GSSG) to prevent cellular oxidative damage.

3. Results and Discussion

3.1. Results

The current study shows that there was a significant variation in serum glutathione peroxidase levels in (the 20-40 years) group when compared to the patient and control group (10.91±3.69 ng/ml vs. 7.10±1.8 ng/ml, p-value less than 0.0533), as shown in figure 2. There was a significant increment in serum glutathione peroxidase levels in (the 41-60 years) group when comparing the patient and control group (7.77±1.70 ng/ml vs 13.3±3.8 ng/ml, p-value less than 0.0001), as shown in figure 3. The current study shows that there was a significant variation in serum glutathione reductase levels in (20-40 years) group when comparing the patient and control group (1430±393ng/ml vs.251±49.2 ng/ml, p-value less than 0.0003), as shown in figure 4.

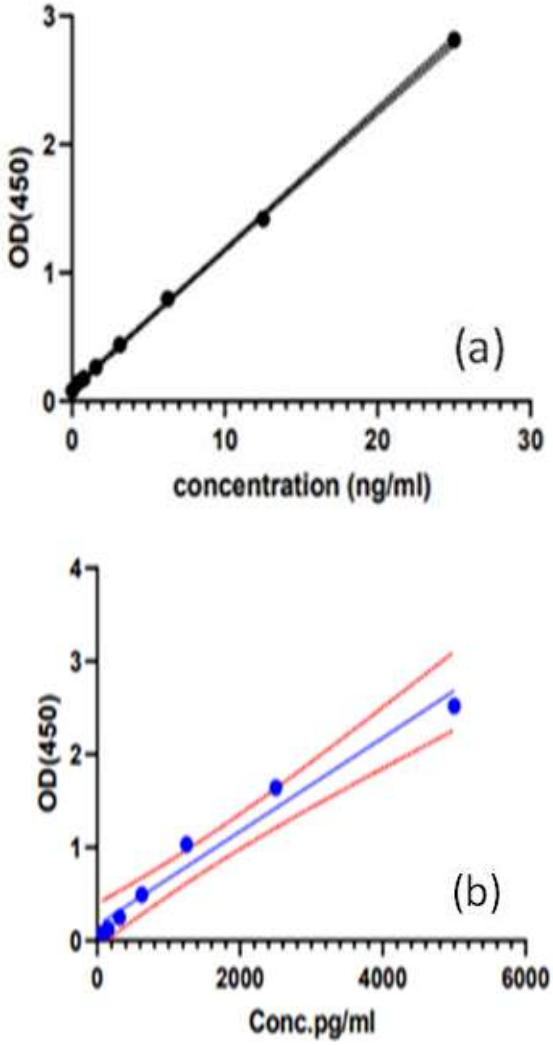

Figure 1. A. Standard curve for glutathione peroxidase, B. Standard curve for glutathione reductase.

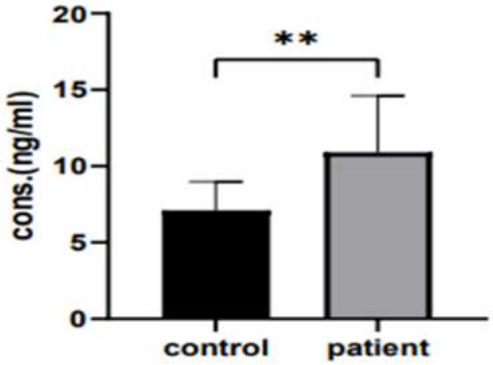

Figure 2. The mean of glutathione peroxidase levels in the 20-40 years group compared to the control group

There was a significant variation in serum cysteine protease levels in (the 40-60 years) group when comparing the patient and control group (1734±1120ng/ml vs.1075±960ng/ml, p-value less than 0.3414), as shown in figure 5.

3.2. Discussion:

The current study was designed to evaluate the serum levels of antioxidant enzymes in newly diagnosed patients with colorectal cancer patients Glutathione peroxidase, an antioxidant enzyme that reduces ROS, can prevent the formation of dangerous lipid peroxides. When glutathione peroxidase activity is inhibited, ferroptosis cell death occurs [20]. Mammalian cells feature a complex antioxidant enzyme system, similar to glutathione peroxidase systems [9].

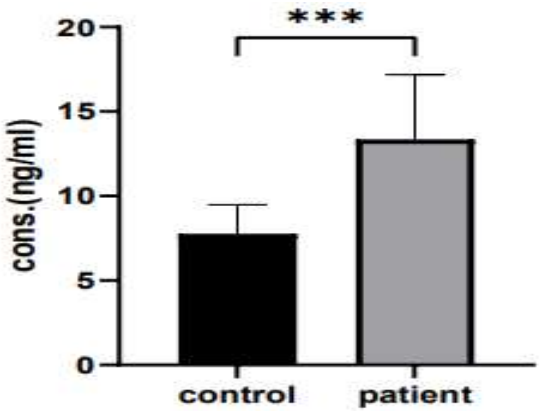

Figure 3. The mean of glutathione peroxidase levels in (the40-60 years) group compared to the control group

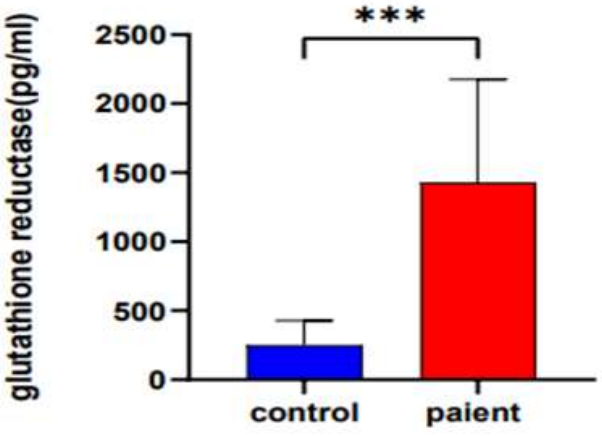

Figure 4. The mean of glutathione reductase levels in (the 20-40years) group compared to the control group

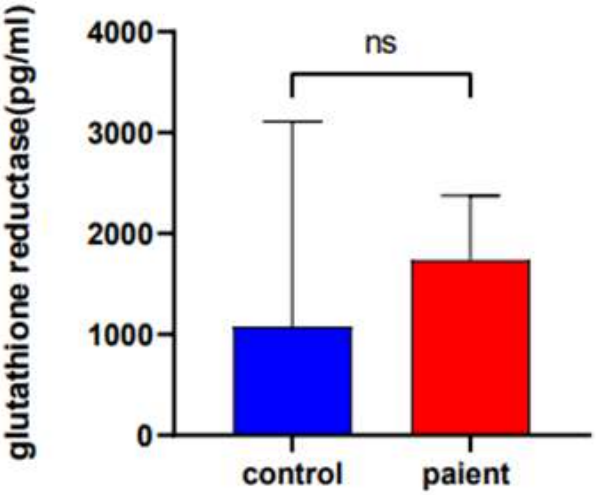

Figure 5. The mean of glutathione reductase levels in (the40-60 years) group compared to the control group

Small molecule antioxidants, pro-oxidants, and antioxidant enzyme targeting as cancer therapeutic possibilities are complicated by the dual involvement of reactive oxygen species and antioxidant enzymes in carcinogenesis and cancer formation [21]. It also emphasizes the significance of future studies into the role and regulation of these antioxidant enzymes in cancer [22]. In the current study the results of the current study in agreement with Gopčević, K.R., *et al.* (2013) who documented that Colorectal carcinoma is characterized by increased oxidative stress and antioxidant dis-balance. Progression of disease is followed by an increase in redox dis-balance and glutathione reductase one of them [23].

The involvement of oxidative stress in tumor tissues prompted researchers to look at the expression of genes and activity of this enzyme in colon cancer tumors and surrounding resection margins, one of the most frequent types of cancer in people. Increased glutathione reductase activity in tumor cells may give tumors with unique features. By creating lower GSH, glutathione reductase aids in cellular defense against ROS. Cancer cells' GSH level regulates potential growth-associated alterations such as mutagenesis processes, invasive cancer cells, remaining alive and dead, and susceptibility to treatment. Activation of several transcription factors may result in increased production of this enzyme, which would explain higher GR activity [24]. In conclusion, antioxidant enzymes significantly increase in newly diagnosed colorectal cancer so according to the present study they have a diagnostic importance. The glutathione reductase has increased concentration and high significant variation in (20-40) age and doesn't affect in (40-60) age so glutathione reductase is considered as a biomarker tool for colorectal cancer in people aged under 40 ages.

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Conflict of Interest:
All authors declare that there are no conflicts of interest.

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