A Biochemical Study for Evaluation of Some Cellular Oxidation parameters in Sera Samples of Gastrointestinal Tumors

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Abstract

During the period from the beginning of December 2018 to the end of May 2019, 108 resident of Babylon were included to participate in the current study, were divided into two main groups: 81 patients with GIT diseases, their age ranged from 12-80 years; and the controls group (27 individuals) with the age 17-71 years old. Furthermore, the patient group was divided into two subgroups, the first involved 51patients with GIT tumors and the other with non-tumoral diseases of GIT (30 individuals suffered non tumoral GIT illness were enrolled as a pathological control group).

Superoxide Dismutase (SOD), Nitric Oxide (NO), Oxidized Glutathione (GSSG), and Total Glutathione (T-GSH)) were evaluated in the sera samples of the study groups. The current study revealed statistical significant increase in the levels of SOD. Results of the present study illustrate that approximately 73% of malignant GIT tumors showed a statistically significant positive relationship ($p < 0.05$) when SOD and NO correlated together. On the other hand, a significant negative correlation ($r = - 0.582$ at $p < 0.05$, $r = - 0.681$ at $p <0.05$) when the levels of SOD with GSSG as well as T-GSH in the malignant GIT tumors patients were correlated, respectively. About 86% of patients with cancerous GIT tumors showed a negative correlation when the levels of NO and T-GSH were compared together (at $p = 0.002$).

SOD showed the highest (94%) single sensitivity among the assessed criteria. Moreover, the study exposed SOD as the most specific (78%) individual bio detector. The specificity% reached to 94% and 89%when T-GSH combined to SOD and NO; respectively.

Keywords: GIT, Cancers, Tumors, *SOD, N.O, GSSG, T-GSH*

1. Introduction

1.1Nitric oxide (N.O)

N.O was among the first discovered gases by Joseph Priestley in 1772 two years after his discovery of oxygen (O2). This colorless and odorless gas was considered highly toxic, until 1987 when it was shown to actually be produced naturally in the

body [1]. The average half-life of N.O in tissue is about 3–6 seconds and in blood 1–2 seconds; this short life makes in situ studies of N.O in living systems extremely challenging [2]. The reactivity of N.O depends upon its physical properties, such as its small size, high diffusion rate, and lipophilicity [3]. N.O's role in regulating blood pressure and relieving various heart ailments became well-established. Researches revealed that N.O is used by macrophages to kill tumor cells and bacteria [4].

NO is one of the 10 smallest molecules found in biological systems. It is essential to the everyday activities of many cells and tissues in the body [5]. Therefore, any pathology of NO production in the body can lead to many diseases, because of this, NO is also called a Vital-poison, the right amount of NO production is essential for life, but too much or too little can be deadly poisonous [6]. Many studies showed respected relationships between NO and several diseases, when hypertension, atherosclerosis, diabetes, ischemia, Alzheimer's disease, Parkinson's disease, fibrosis, and cancers are related to decrease in the NO levels. On the other hand septic shock, hypotension, excessive bleeding, meningitis, Rheumatoid arthritis were related to high NO level [7].

1.2Superoxide dismutase's (SODs)

SOD is a class of metalloenzymes was discovered about half a century ago [8]. It was subsequently well established that SODs are the first line of defense against oxygen free radicals [9].SODs are enzymes that function to catalyze the conversation of $O_2^{\bullet-}$ to O_2 and hydrogen peroxide (H_2O_2) [10]. Generally, SODs can be classified into four groups: Fe-SOD, Mn-SOD, CuZn-SOD, and Ni-SOD, and all the four groups can be found in Prokaryotic organisms, while in Eukaryotes Fe-SOD can be found in chloroplasts, Mn-SOD typically found in mitochondria and can be also found in peroxisomes, and CuZn-SOD can be found in the chloroplast, cytosol as well as in the extracellular space [11]. Chemically, the dismutase activity of SODs accelerates the reaction of the superoxide anion ($O_2^{\bullet-}$) with itself to form H_2O_2 and O_2 ($2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$). Superoxide is a negatively charged free radical formed through a single electron donation to oxygen [12]. The human body has a

complex antioxidant defense system (endogenous antioxidants) that includes: the antioxidant enzymes, i.e.; SOD, glutathione peroxidase (GSHPx), catalase (CAT), and peroxiredoxins (PRXs) [13]. These enzymes block the initiation of free radical chain reactions, while the non-enzymatic antioxidant components consist of molecules such as glutathione, α-tocopherol, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions [14].

1.3Glutathione

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is an essential tripeptid existing in all known organism, ranging from bacteria to humans [15]. It helps protect cells from free radical damage by acting as an antioxidant. Reduced Glutathione (GSH) is present in the free or bound form in the cytosol, nucleus, and mitochondria [16] which plays a key role in the control of signaling processes, detoxification and gene expression, DNA and protein synthesis and proteolysis. The majority (90-95%) of glutathione exist in reduced form (GSH) in healthy cells [17]. While, Glutathione Disulfide (GSSG) is the oxidized form of glutathione. It is reduced to GSH in presence of NADPH by the glutathione reductase (GR). The glutathione peroxidase (GP) converts hydrogen peroxide to water [15].

2. Materials and Methods

During the period from the beginning of December 2018 to the end of May 2019, 108 resident of Babylon were included to participate in the current study, were divided into two main groups: 81 patients with GIT diseases, and 27 individuals from the controls group. Furthermore, the patient group was divided into two subgroups, the first involved 51 patients with GIT tumors and the other with non-tumoral diseases of GIT (30 individuals suffered non-tumoral GIT illness were enrolled as a pathological control group). Samples of GIT cancers were collected from patients registered for treatment at Babel Cancer Center in Babylon. While the samples of benign GIT tumors

and non-tumoral diseases of the digestive system were collected from public hospitals (Morgan Hospital, Al-Imam Al-Sadiq Hospital) in Babylon. Cancerous GIT patients group were classified into five subgroups (Esophageal, gastric, hepatocellular, pancreatic, and colorectal carcinoma) according to the primary site of the tumor.

Five milliliters of intravenous blood samples were collected from patients and healthy subjects. The samples were left to coagulate at laboratory temperature, and then separated using a centrifuge at 5,000xg for 5 minutes. Sandwich-Enzyme-Linked Immune Sorbent Assay (Sandwich-ELISA) isolated sera were used to evaluate the current study parameters (N.O, SOD, and T-GSH, and GSSG).

3. Results and Discussion

3.1: Evaluation of SOD Concentration in the Serum Samples of GIT Cancer Patients (at Diagnosis), Benign GIT Tumors Patients, Pathological GIT Controls, and Healthy Control Groups

Levels of serum superoxide dismutase concentration were measured in the four study groups. Results show high SOD concentrations in the sera samples of malignant GIT tumors samples, while the concentrations of this enzyme stayed convergent in the benign GIT tumors to the pathological GIT control and healthy control groups. Significant variations were explained when superoxide dismutase of the malignant GIT tumors group was compared to benign GIT tumors ($p = 0.033$) pathological GIT controls ($p = 0.028$) and healthy controls ($p = 0.022$) groups; while no such results were observed when the two non-cancerous GIT diseases groups and healthy individuals group were compared together, as recorded in **Table 1.**

When the studied groups subdivided into eight subgroups according to their genders, the highest superoxide dismutase concentration (2603.375 pg/ml) was recorded in the group of male with malignant GIT tumors, while the lost concentration of this enzyme (1601.773 pg/ml) was noted in the subgroup of the female with non-tumoral GIT diseases. ANOVA test showed significant variations in superoxide dismutase levels when comparing the group of GIT carcinomas males ($P=0.040$, $P=0.039.$, $P= 0.029$) with both males with benign GIT tumors or those with non-

neoplastic gastrointestinal diseases and males in the healthy control subgroup, respectively.

By the same token, the results of the study indicated that there are significant statistical differences (p= 0.035, p= 0.027, p= 0.005) when comparing the group of females with cancerous tumors with both females with benign GIT tumors or those with non-neoplastic gastrointestinal diseases and females in the healthy control subgroup, respectively.

A recent study [18] showed indicated that meta-analysis of patients with advanced stages of gastric cancer had a lower SOD activity compared to healthy individuals, so, it is a biochemical sign that can be used to support the diagnosis and monitoring of stomach cancer. Several studies have assessed the role of SOD in various cancers. High levels of SOD expression were found in the ovarian cancer tissues compared normal tissues [19]. Increased SOD levels have been associated with poor prognosis and resistance to therapy of various tumors in the central nervous system, gastrointestinal tract, and head and neck [20].

3.2: *Evaluation of Nitric Oxide Concentration in the Serum Samples of GIT Cancer Patients (at Diagnosis), Benign GIT Tumors Patients, Pathological GIT Controls, and Healthy Control Groups*

The current study included the assessment of nitric oxide concentration. the statistical comparisons among the studied groups showed significant differences when the cancerous GIT group compared to benign GIT tumors (p = 0.048) and healthy individuals (p = 0.050) groups respectively, while no such results were noted when the cancerous GIT group compared to pathological control and with the same manner; the comparison among the benign GIT tumors and the two controls groups were statistically unacceptable, as showed in **Table 1**

ANOVA test didn't show significant variations when the two genders in each group compared together isolately, on the other side, significant variations were recorded when

male with malignant GIT tumors compared to their peers in the benign GIT tumors (p = 0.046), pathological controls (p = 0.050), and healthy controls (p = 0.042), respectively. Same results were observed when the females subgroup who suffered malignant GIT tumors compared to the females in the benign GIT tumors (p = 0.044), pathological GIT diseases (p = 0.047), and healthy persons (p = 0.050) respectively.

Table 1: *Levels of Superoxide Dismutase and Nitric Oxide (Mean ± S.D.) Concentration (pg / ml) in the Sera of Tumor Patients and Controls Subjects*

Study Groups (n)	***SOD Concentration (pg/ml) Mean ± S.D.***	***Min.- Max. Range***	***p-value***	***NO Concentration (µmol/L) Mean ± S.D.***	***Min.- Max. Range***	***p-value***
MGITT 33	**2217.273 ± 217.346**	**1665.875 - 2603.375 937.500**	***0.033 MGITT vs BGITT 0.028 MGITT vs PGITC 0.022 MGITT vs HC 0.982 BGITT vs PGITC 0.931 BGITT vs HC 0.892 PGITC vs HC***	**57.833 ± 12.954**	**17.576 - 72.424 54.848**	***0.048 MGITT vs BGITT 0.052 MGITT vs PGITC 0.050 MGITT vs HC 0.881 BGITT vs PGITC 0.889 BGITT vs HC 0.878 PGITC vs HC***
BGITT 18	**1781.452 ± 196.486**	**1601.773 - 2282.314 680.541**		**45.135 ± 21.934**	**17.516 - 103.030 85.514**	
PGITC 30	**1778.707 ± 197.222**	**1665.875 - 2314.024 648.149**		**46.013 ± 18.401**	**16.667 - 75.455 58.788**	
HC 27	**1733.754 ± 142.551**	**1717.422 - 2221.431 504.009**		**43.654 ± 22.808**	**20.909 - 98.182 77.273**	

Results of the present study were agreed with previous studies that referred to increase of nitric oxide levels in the malignant diseases, like colorectal, breast, endometrial, cervical, gastric, and tumors of the central nervous system [21-23].

***3.3**: Measurement of Oxidized Glutathione in the Sera Samples for Patients with Gastrointestinal Tract Diseases and Health Control Groups*

The statistical analysis using ANOVA test showed a respectable significant decrease in the oxidized glutathione levels in patients with malignant GIT tumors when compared with pathological GIT controls ($p = 0.009$) as well as healthy controls ($p = 0.042$) group, with the same way, benign GIT tumors group show significant decrease in the oxidized glutathione levels comparison to pathological controls ($p = 0.012$). On the other side; no significant variations were shown when benign GIT tumors compared to malignant GIT tumors as well as healthy controls groups together; as recorded in **Table 2.**

Significant variations were noted when male patients with malignant GIT tumors were compared to benign GIT tumors ($p = 0.048$), pathological GIT diseases ($p = 0.022$), and healthy controls ($p = 0.049$). As well as when female patients in the pathological controls subgroup were compared to their peer females in malignant ($p = 0.010$), benign ($p = 0.009$) GIT tumors, and healthy female controls ($p = 0.008$) respectively.

Statistically, non-significant differences were observed when the two genders in each one of the four basic groups compared together, *except those in the pathological GIT controls when the study illustrated significant deficiency in the oxidized form of glutathione in the sera samples of males comparing to females in the same group.*

***3.4:** Evaluation of Total Glutathione in the Sera Samples for Patients with Gastrointestinal Tract Diseases and Health Control Groups*

Total glutathione concentration was assessed in sera samples of the current study participants. Results showed significant decrease in the total glutathione concentration in the sera samples of malignant GIT tumors patients comparison to pathological

controls (p=0.032). Despite the absence of statistically significant difference from the relationship between the levels of the total glutathione when comparing it level in the two groups of malignant and benign GIT tumors (p = 0.642), the recorded results indicate the presence of statistically significant differences (p = 0.047) when comparing the levels of the total glutathione in samples of those with benign GIT tumors with their counterparts in the pathological control group, as shown in **Table 2.** Finally, the study proved an elevation of total glutathione levels in the non-tumoral GIT diseases.

Results of the present work indicate that there are significant differences between males with digestive system carcinomas when compared with their male peers in the pathological control subgroup (p=0.011), and by the same token the subgroup of women with carcinomas of the digestive tract tumors recorded results similar (p=0.008) to what was recorded in the male subgroup when comparing their levels of total glutathione with the pathological control subgroup of females.

The groups of males and females with benign GIT tumors recorded significant differences (p=0.007, p=0.004, respectively) when comparing each group of them with their counterparts in the control groups. As a result of the significant increase in the levels of total glutathione in the pathological group of both sexes, the study recorded a significant decrease in the levels of this criterion in the healthy control subgroups.

The reason for the decrease in the levels of glutathione may be due to a decrease or a deficiency in the effectiveness of the enzymes created for glutathione that is directly related to the abnormal changes of proteins produced by cancer cells.

The result of present study relatively constituent with other studies such as Michael's study that referred to the glutathione tends to be elevated in breast, ovarian, head and neck, and lung cancer while it was decreased in brain and liver tumors compared to disease-free tissue. On the other side, cervical, colorectal, gastric, and esophageal cancers show both higher and lower levels of tumor glutathione [24].

Table 1: *Levels of Oxidized Glutathione and Total Glutathione (Mean ± S.D.) Concentration (pg / ml) in the Sera of Tumor Patients and Controls Subjects*

Study Groups (n)	*GSSG Concentration (µmol/L) Mean ± S.D.*	*Min.-Max. Range*	*p-value*	*Total GSH Concentration (µmol/L) Mean ± S.D.*	*Min.-Max. Range*	*p-value*
MGITT 33	1.143±1.072	0.357 - 5.000 4.643	*0.052 MGITT vs BGITT 0.009 MGITT vs PGITC 0.042 MGITT vs HC 0.012 BGITT vs PGITC 0.071 BGITT vs HC 0.042 PGITC vs HC*	1.413 ± 0.803	0.441 - 4.853 4.412	*0.642 MGITT vs BGITT 0.032 MGITT vs PGITC 0.084 MGITT vs HC 0.047 BGITT vs PGITC 0.641 BGITT vs HC 0.047 PGITC vs HC*
BGITT 18	1.746 ± 0.987	0.177 - 2.143 1.966		1.708 ± 0.954	0.147 - 3.233 3.086	
PGITC 30	4.830 ± 2.645	0.179 - 9.821 9.642		4.801 ± 0.551	0.294 - 9.853 9.559	
HC 27	2.206 ± 1.997	0.179 - 6.786 6.607		2.179 ± 1.967	0.147 - 6.618 6.471	

3.5: Relationship of the Study Individuals Age and the Evaluated Parameters

3.5.1: The Relationship between the Age of the Study Participants and Serum Superoxide Dismutase Concentration

According to linear regression test that's applied on the four study groups, positive correlation in more than half of the patient cases (~ 58% at $p < 0.05$) was observed when SOD concentration was correlated to the age of cases in the group of patients with benign GIT tumors, and pathological GIT controls patients. While no such correlations were noted at this relation examined in the malignant GIT tumors and control group.

3.5.2: *The Relationship between the Age of the Study Participants and Serum Nitric Oxide Concentration*

Although the correlations between the concentration of N.O and age in the four study groups lacked statistical significance, these relationships were characterized by being positive in the group of patients with GIT carcinomas and also in the healthy control group, while the relationship was characterized as negative when linking the concentration of nitric oxide and age patients with non-neoplastic GIT disorders.

3.5.3: *The Relationship between the Age of the Study Participants and Serum Oxidized Glutathione Concentration*

The present study did not record any association between the age of patients with different GIT diseases as well as healthy individuals and serum oxidized glutathione levels.

3.5.4: *The Relationship between the Age of the Study Participants and Serum Total Glutathione Concentration*

Although the relations were seemed to be positive, but the results of the present work could not find statistically significant relationships when the age and total glutathione level were correlated together in the sera of the different GIT diseases' patients and healthy individuals groups.

3.6: *The Relationships among the Studied Parameters*

3.6.1: *Study the Relationship of Superoxide Dismutase to Nitric Oxide in the Sera Samples of GIT Tumors Patients, Pathological Controls, and Healthy Individuals Groups*

Results of the present study illustrate that approximately 73.416% of malignant GIT tumors showed a statistically significant positive relationship ($p < 0.05$) when SOD and

N.O correlated together. In the same manner about 50% of patients with non-tumoral GIT diseases showed a statistically positive correlation ($p < 0.05$) when SOD and N.O combined.

In contrast to these results, the present study recorded a moderate negative correlation ($r = -0.527$ at $p < 0.05$) between SOD and N.O in the group of patients with benign GIT tumors. The result was insignificant when the correlation between SOD and N.O was applied in the healthy individuals group.

The results of the current study were consistent with the results of the Sara study, which was performed on samples for women with cancerous breast tumors at diagnosis and through treated with chemotherapy and compared the results with patients with benign breast tumors and healthy controls group [Sara 2020], as well as with the Kawthar study, which was completed on samples for patients undergoing dialysis and those with chronic renal failure caused by the complications of type 2 diabetes [Kawthar 2020].

While the results of present work was disagreed with the findings of Ilham study which carried out on samples of patients with metabolic syndrome and patients suffered at least one of metabolic syndrome symptoms [Ilham 2020].

3.6.2: *Study the Relationship of Superoxide Dismutase to Oxidized Glutathione in the Sera of GIT Tumors Patients, Pathological Controls, and Healthy Individuals Groups*

A significant negative correlation ($r = -0.582$ at $p < 0.05$) between SOD and GSSG when their association in the samples of malignant GIT tumors samples was examined. While no such results were recorded in the other studied groups when the two parameters were combined.

3.6.3: *Evaluated the Relationship of Superoxide Dismutase to Total Glutathione in the Sera Samples of GIT Tumors Patients, Pathological Controls, and Healthy Individuals Groups*

A significant negative relationship ($r = -0.681$ at $p < 0.05$) was observed when the levels of SOD and T-GSH in the malignant GIT tumors patients were correlated. Non-significant relations were observed when the same criteria were correlated together in the samples of benign GIT tumors, pathological controls, and healthy individuals groups.

In samples with GIT carcinomas, correlational relationships clearly indicate to decrease in the ability of non-enzymatic antioxidants (glutathione in its various forms) to suppress the damage caused by cellular oxidative stress versus the ability of enzymatic antioxidants to capture free radicals formed by the abnormal activity of proliferating cancer cells.

3.6.4: *Study the Relationship of Nitric Oxide to Oxidized Glutathione in the Sera Samples of GIT Tumors Patients, Pathological Controls, and Healthy Individuals Groups*

The relationship between the levels of nitric oxide and oxidized glutathione concentration in the sera samples of malignant GIT tumors was characterized by a significant negative relationship **($r = -0.809$** at **$p = 0.001$)**.

On the other hand, the present study did not manage to find respectable relationship between nitric oxide and oxidized glutathione concentration in the other studied groups.

3.6.5: *Study the Relationship of Nitric Oxide to Total Glutathione in the Sera Samples of Patients with GIT Malignant and Benign Tumors, Pathological Controls and Healthy Individuals Groups*

About 86% of patients with cancerous GIT tumors showed a negative correlation when the levels of nitric oxide and total glutathione were compared together (at p =

0.002); while non- significant relationships were noted when the same parameters were correlated together in the other studied groups.

In the samples affected by GIT carcinomas, correlational relationships indicate increased oxidizing molecule production, indicating the emergence of a case of hyper oxidation coinciding with cancerous cellular transformations versus a decrease in the ability of non-enzymatic antioxidants (glutathione in its various forms) to suppress the damage caused by cellular oxidative stress, while a case of the relative equilibrium between oxidants and antioxidants in the rest of the study groups is an indication that oxidative stress is a feature inherent to cancerous cases only among the studied groups in the current work.

3.6.6: *Study the Relationship of Oxidized Glutathione to Total Glutathione in the Sera Samples of GIT Tumors Patients, Pathological Controls, and Healthy Individuals Groups*

The study indicated that the difference between the two studied bodies is very small in both the non-neoplastic injury group of the digestive system ($r = 0.999$ at $p = 0.000$) and the group of healthy individuals ($r = 0.997$ at $p = 0.000$), while the study showed the presence of differences in the two studied forms of the two neoplastic groups, when only 58% of malignant GIT tumors cases illustrated positively relationship at $p < 0.05$, and about 84% of benign GIT tumors cases demonstrated same response when the same criteria correlated together at $p < 0.005$.

The decrease in the correlation coefficient in the group of patients with malignant GIT tumors was due to a decrease in the oxidized form of glutathione versus the formation of the reduced form (GSH) of glutathione. This supposal indicates that the transformation between the two bodies is a sure indication of the absorption of oxidized glutathione to oxidant molecules such as hydrogen peroxide which generated by the accompanying cellular disorder during the tumor development process.

3.7: *Sensitivity of the Evaluated Parameters in Detection of Gastrointestinal Tract Cancer*

The individual and combined sensitivity of the four parameters (Superoxide Dismutase, Nitric Oxide, Oxidized Glutathione, and Total Glutathione) were evaluated in the group of malignant GIT tumors.

Superoxide Dismutase among the assessed criteria showed the highest (94%) single sensitivity when the levels of these parameters in all of the cases diagnosed with GIT cancers included in the current study were significantly higher than those of healthy peers. The Oxidized Glutathione allergic was estimated towards its ability to distinguish among patients with malignant digestive system tumors from healthy subjects (61%), followed by Nitric Oxide with a single differential sensitivity of 58%. Finally, Total Glutathione had the least individual sensitivity to GIT cancer with a sensitivity of 52%, when only 17 of the 33 patients diagnosed with GIT cancer had significantly lower levels of total glutathione than their control group counterparts.

These findings reinforced the objective of the present study to investigate the possibility of using parameters as diagnostic tools for GIT cancers.

Conclusion

Based on the results obtained from the current work, a number of conclusions can be reached, the most important of which are:

- Except glutathione, individual valuable criteria in the current work can be good diagnostic tools for digestive system tumors even in its early stages. The age and genetic factors were not the decisive factors in the GIT cancer occurrence, but they are enhanced malignancy progression.

- The anti-oxidant enzymatic system is influenced by the age in the pathological non-cancerous conditions of the gut as the ability of the body antioxidant rises in an attempt to prevent the production of excessive oxidative intermediates and products with age. The carcinogenicity progresses and symptoms of change in vital parameters in men appear faster and more clearly compared to women.
- The ability of non-enzymatic antioxidants to suppress the damage caused by cellular oxidative stress is decreased versus the ability of enzymatic antioxidants to capture free radicals formed by the abnormal activity of proliferating cancer cells.
- The age and genetic factors were not the decisive factors in the GIT cancer occurrence, but they are enhanced malignancy progression.
- The carcinogenicity progresses and symptoms of change in vital parameters in men appear faster and more clearly compared to women.
- Oxidized glutathione is decreased significantly in the sera samples of males compared to females in the same group.

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