RESEARCH ARTICLE

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Clinical Investigation of IL-31, TOS and GSH in the Sera of Gastric Cancer Females Patients In Iraq

Montadher Ali Mahdi*, Azal Hamoody Jumaa, Yasser Jassim Dawood

Abstract

Objective: the main objective of the study intends to assessing blood levels of interleukin-31 (IL-31), Total Oxidative Stress (TOS), and Glutathione (GSH) and Understanding the relationships between these factors and gastric cancer (GC), additionally studying receiving operation characteristic analysis to understand the effect of this parameters on GC that may reveal new information about the disease's pathogenesis and suggest possible diagnostic and therapeutic strategies. **Method:** The study sought to determine the concentrations of anthropometric factors (age and BMI), immunological parameters (IL-31) and parameters related to oxidative stress (TOS and GSH) in the sera of female gastric cancer females patients. The study included 80 people divided into two groups: 40 healthy participants and 40 gastric cancer patients' group, the samples were collected between January to March 2023. **Results:** There is a non-significant ($p>0.05$) difference in age and BMI between controls and patients. The study found a substantial increase ($P<0.001$) in IL-31 and TOS levels in GC females matched to controls. However, there was non-significant difference ($p>0.05$) in GSH levels between healthy controls and GC patients. From ROC analysis it is confirmed that TOS has the highest specificity and sensitivity among the studied parameters. **Conclusion:** The net result shows that there is an association among the inflammatory immunological parameter IL-31 and GC infection, and that the body's immunity system plays a significant role in fighting this disease, while also demonstrating that oxidative stress plays an important role in the regulation of the disease.

Keywords: GC- cancer- Gastric cancer- IL-31- TOS- GSH- oxidative stress- anti-oxidant

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Introduction

Gastric cancer (GC) regarding as one of the primary causes of mortality due to cancer worldwide, particularly in developing countries like Iraq [1]. This malignancy usually begins in the stomach mucosa and emerges as gastric adenocarcinoma, while other subtypes, including lymphoma and mesenchymal tumors, may occur. Despite improvements in medical care and diagnosis, the prognosis for GC frequently remains poor because of late-stage detection and fast disease development [2]. Early symptoms of GC, including heartburn, nausea, with abdominal discomfort, are frequently nonspecific, causing diagnostic delays. Systemic symptoms, such as excessive weight loss, dysphagia, jaundice, and hematochezia, occur as the disease spreads to distant organs such as the liver, lungs, and lymph nodes [3]. The development of GC is the result of a complex interaction of genetic, environmental, as well as lifestyle variables. Chronic inflammation, which is frequently caused by Helicobacter pylori infection, dietary choices, and oxidative stress, is critical in the development of stomach cancer [4]. Interleukin-31 (IL-31), a cytokine, has been identified as a potential contributor in the tumor microenvironment. Elevated levels of IL-31 have been linked to a variety of chronic inflammatory illnesses, including atopic dermatitis with autoimmune diseases, but its significance in cancer, specifically GC, remains unknown. Preliminary evidence suggests that IL-31 could be contributing to a pro-inflammatory milieu that promotes tumor growth and immune evasion [5]. The IL31 gene on chromosome 12 encodes IL-31, which is produced largely by activated T-helper type 2 (Th2) cells, mast cells, and other types of immune cells. It works by attaching to its receptor complex, IL-31RA, and activating downstream signaling pathways that control inflammation and immunity [6]. Among these variables, oxidative stress is becoming increasingly recognized as a major influence. It is caused by an imbalance in the generation of reactive oxygen species (ROS) and antioxidant defense mechanisms, resulting in damage to cellular components which include DNA, lipids, and proteins. This oxidative damage not only accelerates the onset and spread of cancer, but it also hinders the body's capacity to mount an efficient immunological response [7]. This difference

*Leading National Cancer Research Centre, University of Baghdad, Baghdad, Iraq. *For Correspondence: montadhermalky@yahoo.com, montadher.a@bccru.uobaghdad.edu.iq*

damages vital molecules and cells, possibly harming the entire body [8]. The oxidative cycle that takes place on occasion in cells is essential for their survival and death [9]. The coming are key points for comprehending oxidative stress. Molecular oxygen own the capability to dissociate and release unstable free radicals [10]. This unstable radical is particularly reactive, resulting in the formation of (ROS). Antioxidants play an substantial turn in the body fight against ROS [11]. Antioxidants, even in low concentrations, reduce oxidation and hence serve a range of physiological functions in the body [12-13]. Regular consumption of antioxidants, vegetables, and fruits have been related to a lower risk of chronic disease [14]. Given the scarcity of studies on the interaction of oxidative stress markers and cytokines associated with inflammation in GC, especially among Iraqi patients, this study intends to fill that gap so the aim of the study is to assessing blood levels of IL-31, Total Oxidative Stress (TOS), and Glutathione (GSH). Understanding the relationships between these factors and GC may reveal new information about the disease's pathogenesis and suggest possible diagnostic and therapeutic strategies.

Materials and Methods

Sample collection

This study included forty female patients with stomach cancer aged 31 to 52 years. The study was done at the Medical City, Baghdad, Iraq, through January to March 2023. Gastric cancer patients were evaluated by a doctor at the "Oncology Teaching Hospital." All trial participants gave informed written consent and filled out a questionnaire. All patients in the study had no history of smoking, alcohol consumption, or pregnancy. The study only included patients with stomach cancer and excluded those with other medical conditions including diabetes, hypertension, or hyperthyroidism. The patients groups in this study compared with 40 healthy participants.

Stage of Cancer

Patients have been diagnosed at stage IIA (T1, N2, M0), which means the tumor has penetrated the lamina propria or submucosa (T1) and implicated 3-6 regional lymph nodes (N2) but no distant metastases (M0) [15].

Serum collection

Blood samples were obtained and kept at room temperature for coagulation. After 15 minutes, the blood samples were ejected at 5000 x g and frozen at -20 ° C for use in biochemical analysis.

Biochemical parameters analyses
Determination of TOS

The Erel technique was utilized to measure the samples' overall oxidative state. The ferrous ion-O-dianisidine complex is transformed to the ferric ion by oxidants in the solution. Glycerol molecules, that are prevalent in the reaction medium, contribute to the oxidation process. In an acidic condition, the ferric ion forms a colorful complex with xylenol orange. The spectrophotometric evaluation of color intensity correlates to the amount of oxidant particles in the sample. The test was calibrated using H2O2, and the findings are provided in μmol H2O2 Eq./L [16].

Determination of GSH

The serum thiol content were evaluated utilizing the Ellman approach [17], which is based on the interaction of thiol groups and the color-less 5,5′- Dithio-bis-(2-nitrobenzoic acid), also referred to as DTNB, resulting in a yellow product. The absorption rate of the yellowish-colored solutions is proportional to serum GSH content.

IL-31 was assessed by (ELISA) method, the ELISA kit supplied by(Sun Long Biotech, China).
Statistical analysis

The data were analyzed by using T-test analysis, Pearson correlation, and ROC curve using the software "Graph-Pad Prism" version 10, (www.graphpad.com), for evaluating the mean ± standard deviation differences between the groups. The statistical tests are considered to be significant at ($p<0.05$) with a 95% Confidence Interval, and highly significant at ($p \leq 0.01$) with a 99% Confidence Interval [18].

Results

Results are analyzed using mean ± SD and calculated as significant at $P \leq 0.05$. There are non-significant differences ($P > 0.05$) in age and BMI between controls (40.43±8.96) and Gc (44.25±8.24) for age, and control (28.36±3.66) and Gc (29.90±4.14) for BMI.

The selection of patients and control has been intended to be the same among the collected categories to eliminate the impact of age and BMI on the variables being investigated (control and Gc) without overlaps. The findings of oxidative stress variables and interleukin (TOS, GSH, and IL-31) were done using the mean ± (SD).

The mean± SD of TOS value in gastric cancer patients was 9.28±2.56, whereas in the control group, it was 5.8±2.12. The p-value of <0.001 indicated a significant increase in TOS between the two groups.

The mean± SD of IL-31 value in gastric cancer patients was 72.2±23.5, whereas in the control group, it was 52.8±25.4. The p-value of <0.001 indicated a significant increase in IL-31 between the two groups. The mean± SD of GSH value shows that there was a non-significant change ($p>0.05$) in GSH levels among healthy controls 568±235 as compared with Gc female patients451±198 with The p-value 0.13 (Table 1). The results of ROC analysis for the studied parameters are shown in Table

Table 1. T Test Result for Oxidative Stress Parameters for Gc Diseases and Control

Parameters	Control (N=40) Mean± SD	Patients (N=40) Mean± SD	p-value
TOS	5.8±2.12	9.28±2.56	< 0.001
GSH	568±235	451±198	0.13
IL-31	52.8±25.4	72.2±23.5	< 0.001

* This symbol means different significant at $P \geq 0.05$; ** This symbol means different significant at $P \leq 0.001$

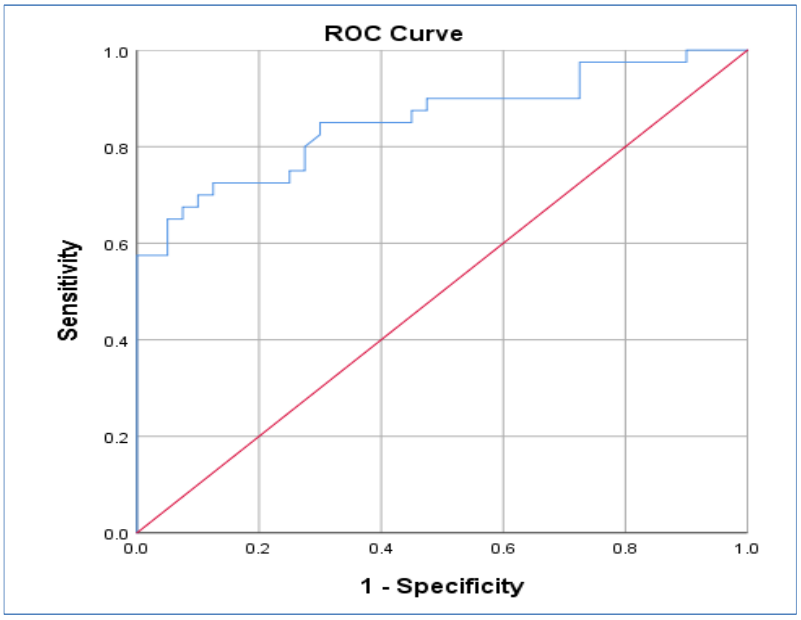

Figure 1. ROC Analysis for TOS

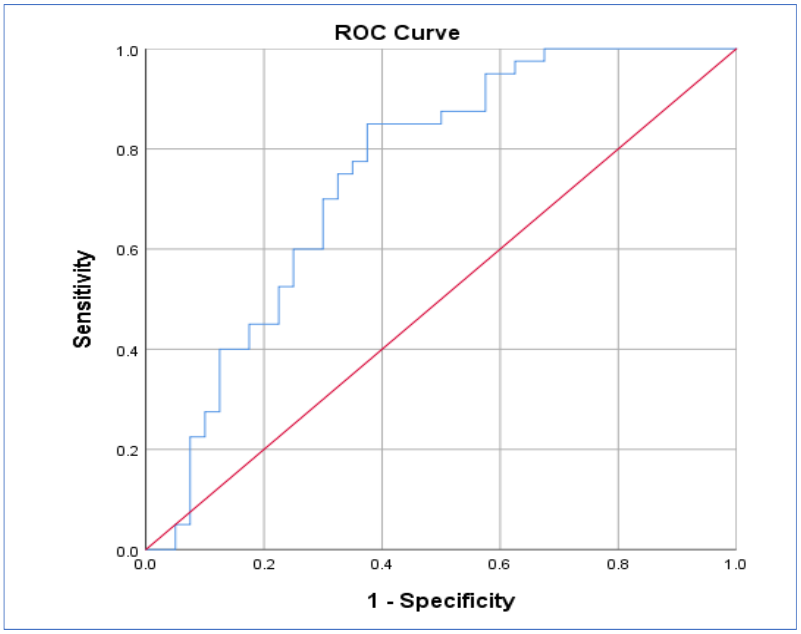

Figure 2. ROC Analysis for IL-31

2. The results show AUC with 0.854 for TOS and with 75% Sensitivity and 75% Specificity, AUC was 0.748 for IL-31 and with 70% Sensitivity and 70% Specificity, AUC was 0.58 for IL-31 and with 55% Sensitivity and 50% Specificity (Table 2, Figures 1,2).

Discussion

This work examines the relationship between oxidative stress markers and inflammation markers. It offers light on the underlying mechanisms of stomach carcinogenesis and suggests prospective therapeutic interventions and diagnostic refinements. Gastric cancer patients may have heightened oxidative stress and impaired antioxidant defense mechanisms, according to these findings. These data imply that abnormalities in metabolism, antioxidant and oxidative stress, and elevated inflammatory markers may contribute to the development or progression of

Table 2. ROC Analysis for the Studied Parameters

Parameter	AUC	SE	p-value	Cut-off value	Sensitivity	Specificity
IL-31	0.748	0.056	<0.001	50.97	70%	70%
TOS	0.854	0.043	<0.001	7.7	75%	75%
GSH	0.58	0.089	0.071	1.48	55%	50%

gastric cancer. The previous study's results demonstrate an increase in TOS levels, implying that oxidative stress plays an essential role in GC monitoring.

IL-31, which is typically released by injured and inflammatory tissues, particularly human mast cells, can also contribute to T helper type 2 (Th2) responses [19]. Although IL-31 serum levels were greater in GC patients than in controls, the internal relationships with clinical features were less evident than for IL-33. We hypothesized that mast cells would release IL-31 [20].

Oxidative stress proved more prevalent in GC patients, regardless of tumor stage than in first-degree relatives or controls. Notably, we established, to the best of our knowledge, that first-degree family members of GC patients had elevated lipid peroxidation, which was identical to that described in gastric cancer patients [21]. Because MDA is derived from polyunsaturated fatty acids and has been associated with mutagenesis and carcinogenesis, it may lead to a higher risk of GC in this population. Elevated oxidative stress may help identify those in greater danger of stomach cancer [22].

When creating therapeutic choices for GC patients, it is unifying that the inflammation reflects TOS quantities as an oxidative stress mirror can offer an image of the complicated phenomenon embodied in proteins/lipids damage, which are key contributors to illness outcome, and these biomarkers should be given more attention [23].

To understand the obtained data, it could be stated that the estimation of TOS in the serum is the fundamental measure of the amount of oxidative stress in the human body [24]. Oxidative stress interacts with GC. The elevated level of oxidative stress is a critical component in determining the severity and degree of disease development in patients with both disorders [25]. The mechanism of action in generating retching is in the GC.

There is a non-significant difference in GSH levels among control and GC patients. One reasonable explanation regarding this finding is that in GC patients, the Second layer of protection versus oxidative stress is increased due to oxidative changes in cell membranes, whereas the first line, like GSH and CAT, does not demonstrate any substantial alterations under enhanced oxidative stress [26]. The disparity between the acquired results and the researchers' results could be attributed to significant clinical aspects that could contribute to match results as disease length, disease activity, treatment, reduced immunity, and patient condition at the time of blood collection, Also, the variance is related to the varied races that have been investigated in the whole research dealt with, as well as the different eating techniques and lifestyle followed by the studied individuals [27].

The concentration of IL-31 is raised in GC as matched with control and it is agreed with another study, this result is in agreement with [28]. In humans, the IL31 gene on chromosome 12 encodes the protein interleukin-31 (IL-31). IL-31 is a cytokine that promotes cell-mediated immunity versus pathogens [29]. It was first reported to be generated by activating T helper cells, mast cells, specifically Th2 cells, dendritic cells, and macrophages [30]. However, a new study indicates that eosinophils additionally serve as a major generator of this cytokine, particularly in bullous pemphigoid. Basophils have even been found to express the cytokine, that strongly stimulates the generation of other Th2-kind cytokines from those cells via autocrine action. These data suggest that in Cancer diseases the dynamic migration of eosinophils and basophils could have a critical role in the intensity of IL-31[31]. Furthermore, these data indicate that IL-31 may have immunomodulatory effects by promoting Th2-type immunity, which frequently supports IgE-associated autoimmune disorders (like bullous pemphigoid and urticarial) and allergies [32]. Current research suggests that this cytokine plays a substantial role in blood pressure, chronic spontaneous urticarial, and dermatomycosis [33]. This suggests that pharmacological techniques that block the IL-31 receptor could be employed to treat IL-31-related illnesses [34]. The precise role of IL-31 is yet unclear, however, overexpression is associated with disease severity, and it has been proposed that IL-31 could be used in GC therapy [35]. There is currently a scarcity of information and studies on interleukin 31, and more detailed research is needed to comprehend its mechanism of action and secretion in connection with degenerative and immune illnesses [36].

In conclusion, the present study highlighted the increase in the level of IL-31 declares the instinctive turn of interleukin and the immune system in controlling the course and intensity of GC disease; the turn of this indicator could be clear in the control and monitoring of disease progression; and the increase in the level of TOS provided a good view of the effect of oxidative stress on the causing of GC because protein denaturation occurred and the immune system activated to respond to the damage. GSH levels indicate that in GC patients, the second line of defense versus oxidative stress, generated by oxidative alteration of cell membranes, is enhanced, although the first line, such as GSH and CAT, does not exhibit any significant changes in response to increasing oxidative stress. ROC study shows a very good ability for TOS and IL-31 to work as a diagnostic parameter for GC characterization. These findings deepen our understanding of the disease's etiology and could lead to novel therapy options that target oxidative stress and micronutrient availability. Further research is needed to understand the mechanisms and clinical use of these biomarkers in early and prognostic detection of gastric cancer.

Author Contribution Statement

Montadher Ali contributed to the study design and idea. Montadher, Yasser jassim, azal hamoody carried out the experimental work, data collection, and data analysis. Montadher and azal contributed to the results discussion, writing, drafting, and editing of the paper. All of the authors checked and approved the overall manuscript, and approved the final version of the manuscript.

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Ethical Approval

All procedures performed in studies involving human participants followed the ethical standards of the research committee of "Mustansiriyah University" and with the 1964 "Helsinki Declaration" and its later amendments or comparable ethical standards

Data Availability

The data will be available when asking by the editorial board

Conflicts of interest

The authors declare no conflicts of interest

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