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Measurements of enzymes (MDA, CAT, GST) Level in sera of lung cancer in Iraqi patients and the relation with taxol drugs

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Abstract:
Lung cancer remains a significant global health concern, characterized by uncontrolled cell proliferation and metastatic potential. This study delves into the intricate the correlation between oxidative stress and lung cancer., as well as the potential of paclitaxel as a treatment option. We explore the enzymatic activity of GST, CAT, and MDA in lung cancer patients and a control group, considering their smoking status. Additionally, we investigate the impact of paclitaxel on reactive oxygen species (ROS) production in a malignant cell line. The results demonstrate significant variations in enzymatic activity and ROS induction in lung cancer patients, emphasizing the importance of these factors in the disease's progression. The findings shed light on the potential of paclitaxel as an agent for ROS modulation in lung cancer therapy, providing valuable insights for improved treatment strategies and enhanced patient outcomes.
Key words : lung cancer, Oxidative stress, Paclitaxel, Enzymatic activity, Reactive oxygen species (ROS), glutathione-s-transferase (GST) , Catalase (CAT), malondialdehyde (MDA).

Introduction :
Lung cancer remains a leading cause of cancer-related deaths worldwide, presenting as a diverse group of disorders characterized by uncontrolled cell proliferation and the potential for metastasis. The disease originates from abnormal cell growth, leading to the formation of nodules within the lung tissues,[1]. eventually distinguishing a malignant lung tumor from lung cancer. If left untreated, the tumor can spread to other tissues and distant parts of the body, posing a significant threat to the patient's health[2]. Primary risk factors for lung cancer include exposure to smoke, especially inhalation of carcinogenic particles, affecting both active smokers and passive smokers exposed to secondhand smoke.[3].
One of the underlying mechanisms contributing to lung cancer progression is oxidative stress, which occurs due to direct exposure to factors promoting the production of free radicals. These elements include elevated oxygen levels, environmental irritants, and contaminants like oxidant gases, fine particulate matter, industrial pollution-generated nanoparticles, car emissions, and smoking[4], Oxidative stress is characterized by an imbalance between pro-oxidants' production and the body's ability to counteract their harmful effects.[5]. To combat the deleterious effects of oxidative stress, the body employs a complex antioxidant defense system, comprising both internal enzymatic antioxidants (e.g., glutathione, catalase, and superoxide dismutase) and dietary antioxidants (e.g., vitamins C and E). These antioxidants work together to maintain the equilibrium between reactive oxygen species (ROS) and antioxidants in the body. When this balance is disrupted, oxidative stress ensues, leading to cellular dysfunction. The potential therapeutic use of antioxidants, particularly in conditions like ischemic stroke, is under investigation as a strategy to reduce cellular injury caused by oxidative stress[6].
In the realm of lung cancer treatment, paclitaxel plays a prominent role, being used as both a monotherapy and in combination with other drugs for solid tumors, encompassing non-small-cell and small-cell types[7]. Paclitaxel, also known as taxol or TM, is a powerful anti-cancer drug derived from the Pacific yew tree. Over the course of three decades, extensive research has enhanced its efficacy against various cancers, thanks to a better understanding of its molecular structure and mode of action[8]. Paclitaxel is commonly used as a broad-spectrum anti-cancer drug for solid tumors. However, its utilization is restricted due to certain factors. These factors include poor solubility, which may result in recrystallization upon dilution, and potential toxicity when used with cosolvents. As a result, the practical application of paclitaxel is significantly constrained[9].

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To further explore the impact of oxidative stress and paclitaxel in lung cancer treatment, this study focuses on investigating the enzymatic activity of MDA, CAT, and GST in both a control group and lung cancer patients, considering their smoking status. Additionally, the study aims to assess the effect of paclitaxel on ROS production in lung cancer patients, utilizing a high-content screening technique. The findings from this comprehensive investigation will contribute valuable insights towards enhancing lung cancer treatment strategies and improving patient outcomes.

Materials and Method :

Patients:

The method entails taking 100 blood samples from normal individuals and patients, dividing the two groups into 30 smokers and 30 non-smokers in patients and 20 smokers and 20 nonsmokers in control group , which were received at Alamal National Hospital for cancer treatment and Baghdad Teaching Hospital between October 2022 and April 2023 . The participants in the control group were those who were clearly healthy, as seen by the absence of diabetes, hypertension, or any other sort of cancer in their family. In a heparinized tube, 5ml of blood from each subject was extracted and allowed to coagulate for 20 minutes at room temperature. After that, the tubes were centrifuged for 10 minutes. A separate tube was then used to transfer the serum, which was held at (-20 c) using a micropipette.

Enzymatic activity of GST,CAT ,MDA,: A cohort of lung cancer patients, comprising both smokers and nonsmokers, and a control group was recruited for this study. The enzymatic activity of GST, CAT, and MDA was assessed in each group. Additionally, the effect of Paclitaxel on ROS production in a malignant cell line was evaluated using high-content screening techniques.

Human catalase : The CAT activity was determined using a commercial ELISA reagent (No. MBS770782) from My Bio Source in Canada. The kit is founded on the technology of biotin double antibody sandwiching. On the provided coated plate, the standard and samples were pipetted, and the immobilized Ab surrounded the given CAT. Using a plate reader Power Wave STM (Bi oTek®, USA) at 450 nm Using a biotin-conjugated antibody that targets CAT (catalase), along with avidin-conjugated horseradish peroxidase and a stop solution, the degree of color development was assessed. Subsequently, the enzyme concentration was quantified in nanograms per milliliter (ng mL-1).

Statistical analysis: Standard deviation (SD) is used to express data as mean and SD. Graph Pad Prism, version 6 (Graph Pad Prism Software Inc., La Jolla, CA, USA) and one-tailed, unpaired t-test were used to examine significant variances (p 0.05) between different groups. Furthermore, IC50 was estimated using the Graph Pad Prism program.

Results:

Enzymatic activity of GST, CAT, and MDA in the control group and lung cancer patients were assessed and presented in Table 1 and Figure 1, respectively. Among the lung cancer patients, the activity of MDA was significantly higher in both smokers ($p \leq 0.0001$) and nonsmokers ($p \leq 0.0001$) when compared to the control group. Moreover, the enzymatic activity of GST and CAT was found to be lower in smoker patients compared to both nonsmoker control individuals and nonsmoker patients , as depicted in Table 1A and Figure 1A.

Further analysis of CAT activity, presented in Table 1B and Figure 1B, demonstrated significantly lower levels of CAT enzyme in both smoker patients and nonsmoker patients when compared to the control group. Notably, even among nonsmoker patients, there was a decrease in CAT enzyme levels compared to nonsmoker control individuals , Conversely, as shown in Table 1C, both smokers and nonsmokers diagnosed with lung cancer exhibited elevated levels of MDA when compared to the healthy control group.

The impact of Paclitaxel on ROS induction in a malignant cell line was assessed in Figure 2. At a drug concentration of 50 µg/ml, a moderate increase in ROS levels was observed (533.67±102.57), though not statistically significant compared to the control group. However, at concentrations of 100 µg/ml and 200 µg/ml, there was a significant elevation in ROS production (699.67±61.78, $p \leq 0.01$ and 799.67±39.21, $p \leq 0.001$, respectively) compared to the control group.

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Overall, the results suggest altered enzymatic activity and ROS induction in lung cancer patients, highlighting the potential significance of these factors in lung cancer pathogenesis and the impact of Paclitaxel as a therapeutic agent for ROS modulation in malignant cells.

Table (1) A: The mean ± standard deviation for serum enzyme glutathione-s-transferase(GST) level of control groups and lung cancer patients group.

GST level (ng/ml)	**Treatment ±SD**	**p-value**
Normal Vs. patients	Nonsmoker (15.57±3.2) Nonsmoker (12.20±4.93)	0.05
Normal Vs. patients	Nonsmoker (15.57±3.2) Smoker (10.80±3.64)	0.001

* P value ≤ 0.05

Table (1) B : The mean ± standard deviation for serum enzyme catalase (CAT) level of control groups and lung cancer patients group .

CAT level ()	**Treatments**	**p-value**
Control	Smoker (2032±624.9) Nonsmoker (2557±656.3)	0.05
Control Vs. patients	Nonsmoker (2557±656.3) Nonsmoker (1739±550.0)	0.0001
Control Vs. patients	Nonsmoker (2557±656.3) Smoker (1371±475.4)	0.0001
Control Vs. patients	Smoker (2032±624.9) Smoker (1371±475.4)	0.001

* P value ≤ 0.05

Table (1) C : The mean ± standard deviation for serum enzyme malondialdehyde (MDA) level of control groups and lung cancer patients group .

(MDA) level	**treatments**	**p-value**
Control Vs. patients	Smoker (152.1±58.3) Smoker (324.1±75.0)	0.0001
Control Vs. patients	Smoker (152.1±58.3) Nonsmoker (240.6±94.48)	0.001
Control Vs. patients	Nonsmoker (113.2±54.2) Smoker (324.1±75.0)	0.0001
Control Vs. patients	Nonsmoker (113.2±54.2) Nonsmoker (240.6±94.48)	0.0001
patients	Nonsmoker (240.6±94.48) Smoker (324.1±75.0)	0.001

* P value ≤ 0.05

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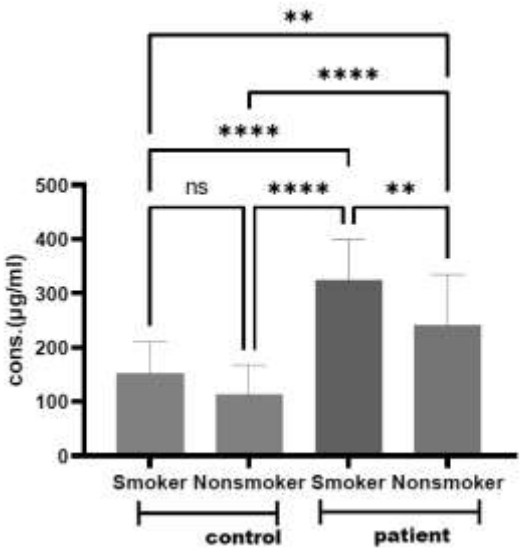
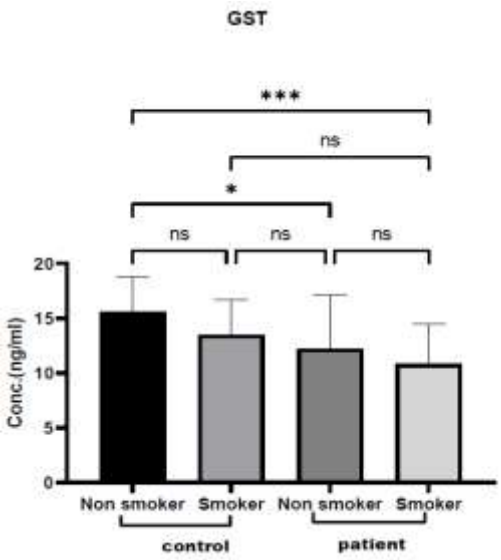


(A)



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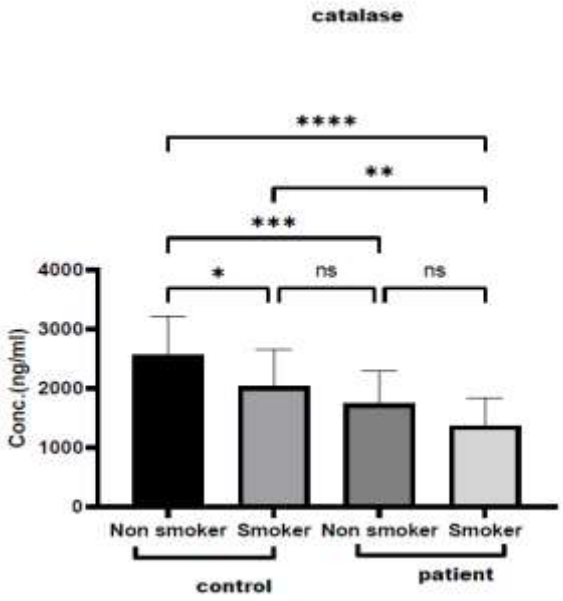

(B)

Figure(1): Graphical mean level (± SD) of A : Serum MDA concentration (ng /mL) B : Serum CAT concentration (ng /mL) C: Serum GST concentration (ng /mL) in healthy control, and lung cancer patients for smoker and nonsmoker in both group. NS: non-significant. ***: $p \leq 0.001$,**: $p \leq 0.01$, *: $p \leq 0.05$

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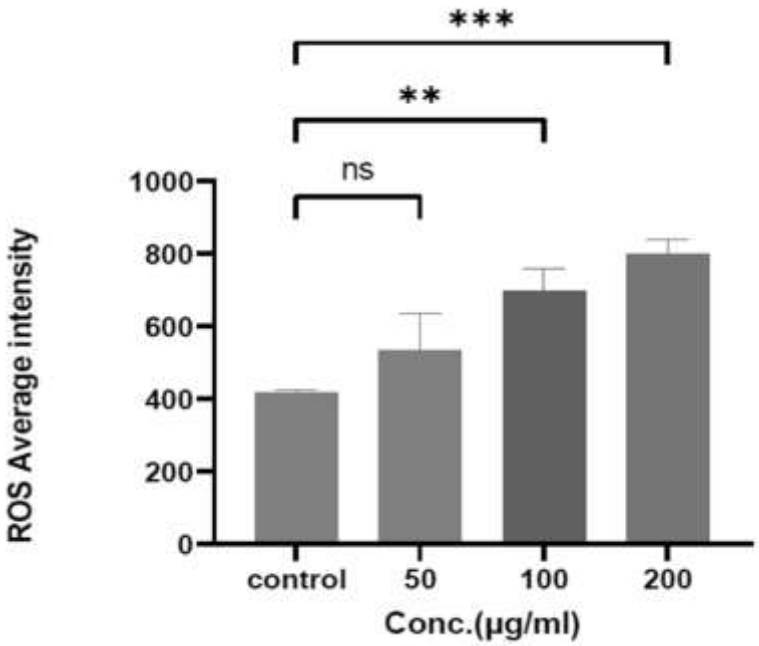

Figure(2): Paclitaxel's effect on ROS induction in a malignant cell line was studied after 24 hours at 37°C using a reader for thermal HCS(High-Content Screening) scientific matrix analysis. ***: $p \leq 0.001$,**: $p \leq 0.01$.

Discussion :

The results of the study revealed important insights into the enzymatic activity and reactive oxygen species (ROS) induction in both lung cancer patients and a control group. Enzymatic activity of GST, CAT, and MDA was assessed, and the findings were presented in Table 1 and Figure 1.

In lung cancer patients, the activity of MDA was significantly higher in both smokers and nonsmokers compared to the control group, indicating elevated levels of oxidative stress in these individuals. This observation suggests that oxidative damage and lipid peroxidation may play a critical role in lung cancer development.

MDA is a naturally occurring chemical compound that serves as a biomarker for oxidative stress when detected in blood plasma or tissues[10]. Several studies have provided evidence of elevated systemic MDA concentrations in individuals diagnosed with lung cancer[11] [12].

MDA is a significant byproduct of lipid peroxidation and has a considerable potential for mutagenesis. This is primarily due to its genotoxic effects arising from the formation of adducts with guanosine, cytidine, and adenosine. Regardless of the specific peroxidation product, MDA can induce point mutations in tumor suppressor genes, thereby significantly increasing the susceptibility to inflammation-related cancers, such as lung cancer[13].

Conversely, the enzymatic activity of GST and CAT was found to be lower in smoker patients compared to both nonsmoker control individuals and nonsmoker patients. This decrease in GST and CAT enzyme levels indicates a compromised antioxidant defense system in smoker patients, leading to reduced protection against oxidative damage. The decreased levels of GST enzyme observed in lung cancer patients who are smokers can be attributed to the cumulative exposure to tobacco smoke, which contains numerous carcinogens and toxic compounds[14].Chronic exposure to these substances can lead to oxidative stress[2].GSTs are enzymes responsible for detoxification processes, facilitating the binding of harmful electrophilic substances with glutathione, a protective molecule. This conjugation reaction acts as a shield for cellular macromolecules, safeguarding them against damage caused by oxidative stress and preventing their destruction[15].GSTs play a crucial role in both the initiation and survival of cancer cells. They act as protective agents during cancer development, but when this protection fails, they become vital for the survival of tumor cells[16].these findings align with the study conducted by (Carmichael J,)which concluded that GST activity is reduced in lung cancer patients[17].

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However, these results do not support the findings of the study conducted by(Cecerska-Heryć E), which reported an increase in glutathione S-transferase (GST) activity in cases of Non-small-cell lung cancer (NSCLC)[18].Furthermore, the research conducted by(Zalewska-Ziob M) revealed considerably higher GST enzyme activity in patients compared to the control group[4].

Further analysis of CAT activity showed significantly lower levels in both smoker and nonsmoker lung cancer patients when compared to the control group. Even among nonsmoker patients, there was a notable decrease in CAT enzyme levels compared to nonsmoker control individuals. This suggests that CAT's reduced activity may contribute to the accumulation of ROS, promoting lung cancer pathogenesis in both smoker and nonsmoker patients.

CAT, a critical antioxidant enzyme, plays a crucial role in converting hydrogen peroxide into oxygen and water through a two-step reaction[19].This enzyme, also known as catalase, acts as a protective mechanism for malignant cells, shielding them from damage caused by reactive oxygen species (ROS) in cancer. The ability to withstand the effects of ROS is vital for tumor development and formation[20].this study builds upon previous research conducted by(Mohan A, Poulose R, Gupta T, et al.) , which demonstrated that cancer patients have significantly lower levels and activity of the antioxidant enzyme catalase compared to healthy individuals[21].However, our investigation challenges the findings of another study conducted by (Kaynar H,) which suggests that patients with either non-small cell or small cell lung cancer (NSCLC or SCLC) exhibit much higher enzyme activity compared to control subjects[22].

The impact of Paclitaxel on ROS induction in a malignant cell line was evaluated, revealing that at a drug concentration of 50 µg/ml, a moderate increase in ROS levels was observed but was not statistically significant compared to the control group. However, at higher concentrations (100 µg/ml and 200 µg/ml), Paclitaxel significantly elevated ROS production compared to the control group. These findings indicate that Paclitaxel may effectively modulate ROS levels in malignant cells, potentially contributing to its efficacy as an anticancer agent. These findings suggest that paclitaxel treatment leads to an increase in ROS production. This elevation in ROS levels may be attributed to paclitaxel's ability to initiate the early generation of reactive oxygen species (ROS) within cancer cells. The primary source of hydrogen peroxide (H2O2) is the dismutation of superoxide (O2), a process catalyzed by superoxide dismutases (SODs). In most cells, oxygen is generated as a byproduct of aerobic metabolism and the activity of the mitochondrial respiratory chain. Additionally, NADPH oxidases (NOX), predominantly located in the plasma membrane, are responsible for generating extracellular O2[23].

Overall, the study highlights the altered enzymatic activity and ROS induction in lung cancer patients, suggesting their critical roles in the disease's pathogenesis. Additionally, the results demonstrate the potential of Paclitaxel as a therapeutic agent for ROS modulation in lung cancer treatment, further supporting its application as a valuable anticancer drug. The findings contribute valuable insights to the understanding of oxidative stress and the role of Paclitaxel in lung cancer therapy, paving the way for future research and improved treatment strategies for this deadly disease.

Conclusions:

This study revealed higher MDA levels, indicating increased oxidative stress in lung cancer patients, both smokers and nonsmokers. Smokers showed lower GST and CAT enzyme activity, compromising their antioxidant defense. Paclitaxel treatment increased ROS production in malignant cells, suggesting its potential as an anticancer agent. These findings highlight the importance of oxidative stress and Paclitaxel's role in lung cancer therapy, offering insights for future research and improved treatments.

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