# Evaluating the Levels of Oxidative DNA Damage, Antioxidant Profile and Pro-inflammatory Cytokines in Lung Cancer Patients

**Hazhar M. Balaky**
*Mergasor Technical Institute, Erbil Polytechnic University, Erbil, Iraq*

**Abstract**

   Eight-hydroxyguanosine  (8-OHdG) is considered as one of the principle forms of oxygen radicals that stimulated the oxidative stress and has been extensively utilized as a biomarker for oncogenesis. The primary goal of the present study was to investigate the alteration in the levels of 8-OHdG, antioxidant profile and proinflammatory cytokines levels in patients with lung carcinoma. Blood samples were collected from 40 cases with lung cancer (stage III) admitted before the treatment, for health examination at the Nanakaly Hospital in Erbil city and 45 healthy samples of controls with ages ranging between 38-69 years for both groups. Circulating concentration of 8-OHdG, tumor necrosis factor and interleukin-6 were evaluated by ELISA. Circulating levels of superoxide dismutase (SOD), peroxidase (POX) and ascorbic acid (vitamin C) levels were also analyzed by using ELISA. The current work proposes that (8-OHdG) can be used as a functional biological marker, considering oxidative stress among the patients with lung carcinoma. The obtained data also indicated a correlation between serum cytokine concentrations and the rate of survival in  lung carcinoma patients.

**Keywords:** 8-Hydroxyguanosine, Antioxidant profile, Pro-inflammatory cytokines, Lung cancer

تقييم مستويات تلف الدنا المؤكسد، ومضادات الأكسدة والسيتوكينات المسببة للالتهابات في مرضى سرطان الرئة

هژار محمد حمدامين

معهد ميرگەسور التقنية، جامعة اربيل التقنية، اربيل، العراق

الخلاصة

يعتبر ٨-هيدروكسي جوانوسين أحد الأشكال الرئيسية لجذور الأوكسجين  والذي يحفز على التمزق التأكسدي، وبالتالي تم استخدامه على نطاق واسع كمؤشر حيوي لتطور الأورام. الهدف الأساسي من الدراسة الحالية هو تحرى مستويات ٨-هيدروكسي جوانوسين ،ودوال مضادات الأكسدة الانزيمية  ومستويات السيتوكينات المسببة للالتهابات في مرضى سرطان الرئة. تم جمع عينات الدم من 40 حالة مصابة بسرطان القولون (المرحلة الثالثة) قبل بدء العلاج في مستشفى ناناكالي في مدينة أربيل مع 45 عينة اخذت من الاشخاص الاصحاء كمجموعة ضابطة ،تراوحت أعمار عينات الدراسة بين 38-69 سنة لكلا المجموعتين.

*Email: hazharbalaky86@yahoo.com

تم تقييم تركيز ٨-هيدروكسي جوانوسين ومستويات السيتوكينات المسببة للالتهابات بواسطة تقنية إليزا. تم تحليل المستويات المتداولة من مضادات الأكسدة الانزيمية وحامض الأسكوربيك باستخدام تقنية إليزا أيضًا. أظهرت النتائج ارتفاع معنوي في مستوى مصل ٨ هيدروكسي جوانوسين ومستويات السيتوكينات  للالتهابات وانخفاض معنوي في مستوى مصل دوال مضادات الأكسدة الانزيمية وفيتامين سي في مجموعة المرضى بالمقارنة بمجموعة الضوابط. تقترح الدراسة الحالية احتمالية استخدام ٨. هيدروكسي جوانوسين كمؤشر بيولوجي وظيفي بالنظر إلى الإجهاد التأكسدي بين مرضى سرطان الرئة، وتشير البيانات التي تم الحصول عليها إلى وجود علاقة بين تركيز السيتوكينات في الدم ومعدل البقاء على قيد الحياة في مرضى سرطان الرئة.

## Introduction

Lung carcinoma is the leading cause of cancer-related death globaly [1]. Small cell lung and non-small cell lung carcinoma are two principle histological kinds of lung cancer [2]. Oxygen radical (OR) contributed in genetic mutation, either directly or indirectly, and participated in the progression of cancer by damaging the DNA and the variation of cell signaling [3]. Oxidative destruction of DNA, by the action of oxygen and nitrogen radicals, results in the  generation of 8-hydroxyguanosine, which is a particular indicator of oxidative damage [1]. Elevated concentration of 8-hydroxyguanosine is linked with the cancer. Previous works recorded that the elevated 8-hydroxyguanosine expression in cancer was linked with a weak diagnosis. The practical importance of 8-hydroxyguanosine expression as a biological marker is still arguable [1]. Cytokines such as tumor necrosis factor (TNF-alpha), interleukin-6(IL-6) and chemokines are a broad category of peptides and glycoproteins released  by B or T cells, which play a vital pro-inflammatory role in tumor progression [2]. Recepters of tumor necrosis factor are recorded to be broadly expressed in lung carcinoma. Evidently tumor necrosis factor is known to be released  by cancer cells and there is an investigational confirmation from a various of models that tumor necrosis factor can stimulate the maturation of cancer cell [4].

Interleukin-6 acts as a principle cytokine for the inauguration and development of a cytokine commotion. Interleukin-6 promotes inflammatory process but it also has other significant physiological roles such as the invigoration of cell multiplication and cancer, where it enhances the spread of cancer cells [5]. Interleukin-6 acts as an acceptable biological marker to prognosticate the weak outcome and treatment of lung carcinoma [2]. As a vital antioxidant enzyme, superoxide dismutase 1 has been strictly associated with tumour. High oxygen radical content of cancer cells attributes to  the irregular metabolic pathway. Prevention of extravagant cellular damage and cell death programme during cancer development damage attributes to activate antioxidants (NRF2), which are cancer cell dependent antioxidants. Regularly, overexpression of superoxide dismutase 1  is reported in lung tumors. It has been  recorded that superoxide dismutase 1 is critical for the prgression of lung cancer and leukemia. as it greatly blocks lung cancer cell lines growth, operated by carcenogenic KRAS and EGFR، besides other tumour cell lines  [6]. Moreover, deficiency of superoxide dismutase 1 blunts ERBB2-operate mammary oncogenesis. Superoxide dismutase 1 has been characerized as a goal for anticancer drug with various recognized targeting agents. Despite  this development, the action of superoxide dismutase 1 in lung carcinoma has not been examined in animal models [7].

It has been shown that controlling  of peroxidase 3  is strictly correlated with the development  of various kinds of cancer. Additionally, methylation or deletion of peroxidase 3, which decline the degree of peroxidase expression are widel observed in many cancers [3]. It has been recorded that both circulating concentration of selenium and the rate of peroxidase activity are remarkably decreased in lung carcinoma patients in comparison with healthy

individuals. Furthermore, the National Center for Biotechnology Information Gene Expression Omnibus (GEO) profile research has demonstrated that the peroxidase mRNA activity remarkably decreased in lung carcinoma patients. Previous studies have also shown that methylation of peroxidase may have entangled in response to the chemotherapy in lung carcinoma patients [3].The most important roles of ascorbic acid is to act as an antioxidant to protect cellular components from free radical damage and can also contribute in chemopreventive impacts. Furthermore, ascorbic acid is involved in the protection cells from oxidative damage of DNA, via inhibition of the oncogenesis. Numerous studies have found the linkage between ascorbic acid consumption and lung carcinoma risk [8].

## Materials and Methods
### *Study Design*
In this research 85 samplesn ranging betweenthe ages of 38-69, were included. Two groups of samples were included in this study. Group (1) contained patients with lung cancer (n=45). Group (2) consisted of (healthy samples) (n=40) (control). Patients were clinically and histologically diagnosed with lung cancer. The samples were collected from Nanakaly hospital for blood diseases and cancer and Rizgary hospital (Oncology Unit) in Erbil city. Patients were assessed by their medical history in order to exclude any existing systemic disorder or if they were taking any drug.

### *Collection of Blood Samples*
About 5-6 ml of venous blood taken from each case was collected in gold-top serum separator tubes (SST) and was allowed to stand at room temperature for 10 minutes., The specimens were then centrifuged at (3000 rpm) for 15 minutes. The obtained serum samples were transferred immediately to pre-labeled and coded Eppendorf tubes. These samples were frozen at –20°C for subsequent assay.

### *Biochemical Assays*
The concentrations of 8-hydroxyguanosine (E-EL-0028), tumor necrosis factor-α (BMS223-4), Interleukine-6 (BMS213-2), total SOD (MBS2540401), POX (MBS1610102) and vitamin C (MBS726748) in the serum specimens were estimated by sandwich enzyme-linked immunosorbent assay (ELISA), technique using the kits manufactured by BioVision company. Concentrations of all biochemical parameters standards and their corresponding OD readings were plotted on the scale (x-axis) and (y-axis) respectively. The concentration of all biochemical parameters in samples were determined by plotting their OD values on the Y-axis of the calibration curve.

### *Statistical Analysis*
SPSS version 21 and GraphPad prism version 8 computer programs were used for statistical data analysis. Statistical test results and bar graphs were expressed as Mean ± SE. Unpaired T-test (Man-Whitney U) test was utilized for comparing the study parameter means between the two examined groups.

## Results and Discussion
### *Serum levels of 8-OHdG*
Figure 1 revealed a remarkable elevation (P=0.030) in serum concentration of 8-hydroxyguanosine in patients (28.85±13.94 pg/mL) as compared to controls (1.34±0.18 pg/mL).
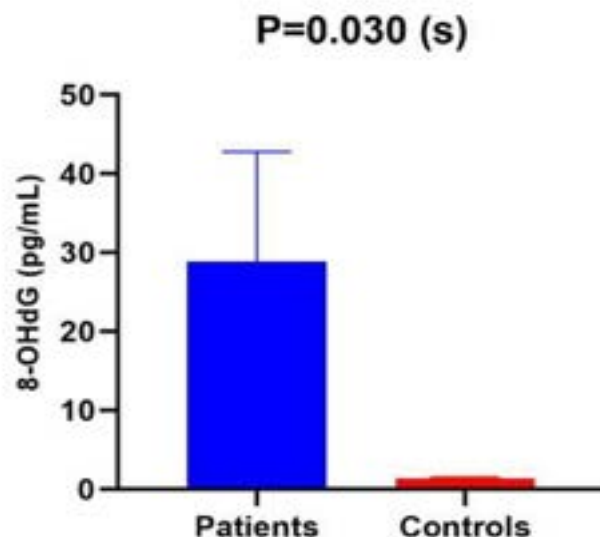
**Figure 1:** Comparison of 8-OHdG concentration between sera samples of control and LC patient groups

### Serum level of TNF-α and IL-6

Figure 2 showed significant increases of (P=0.047; P<0.0001) in serum concentrations of both tumor necrosis factors - alpha and interleukin-6, in patients (66.62±44.49 ng/mL and 16.48±2.64 ng/mL) in comparison to the control group (3.12±1.36 ng/mL and 8.67±5.96 ng/mL) respectively.
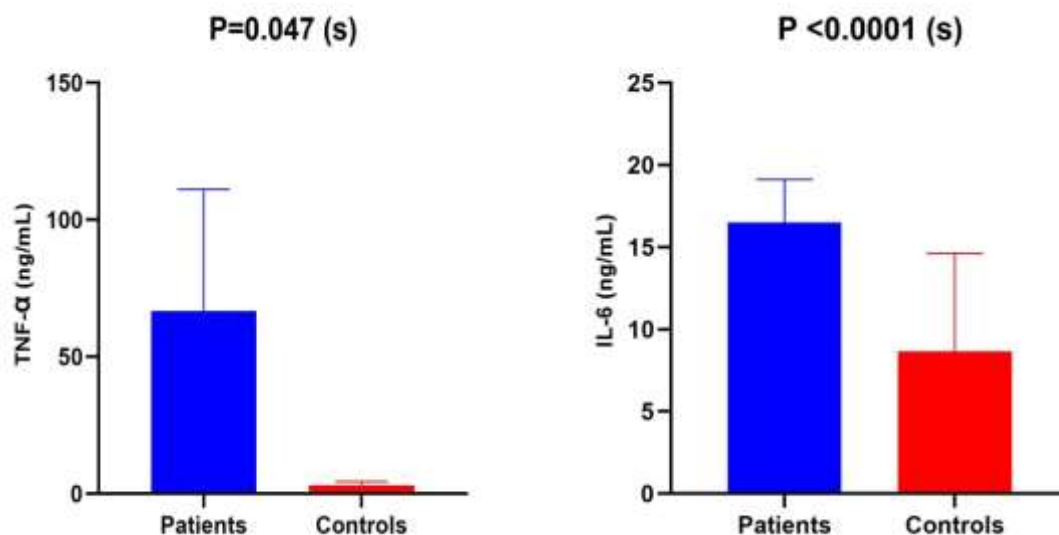


**Figure 2:** Comparison of TNF-α and IL-6 concentration between sera samples of control and LC patient groups

### Serum level of SOD and POX

Figure 3 showed that there was a significant reduction of (P<0.0001) in serum levels of both SOD and POX in patients (3.27±2.87 IU/mL and 1.05±0.25 IU/mL) as compared to controls (16.73±3.57 IU/mL and 6.71±1.16 IU/mL), respectively.
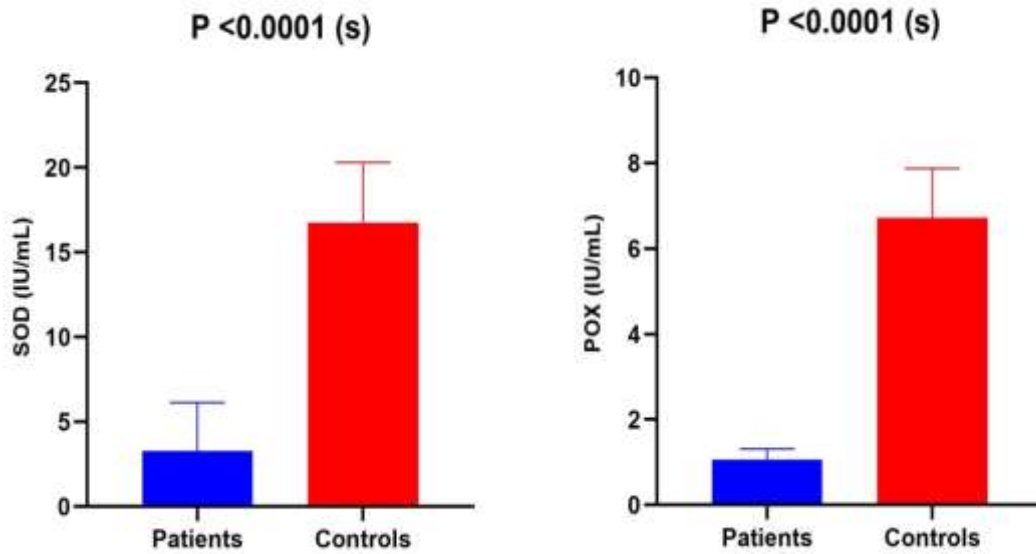
**Figure 3:** Comparison of SOD and POX concentration between sera samples of control and LC patient groups

### *Serum level of Vitamin C*

Figure 4 revealed significant decreases of (P=0.830) in serum level of vitamin C in patients (2.67±1.55 ng/mL) in comparison to control group (5.78±0.43 ng/mL).
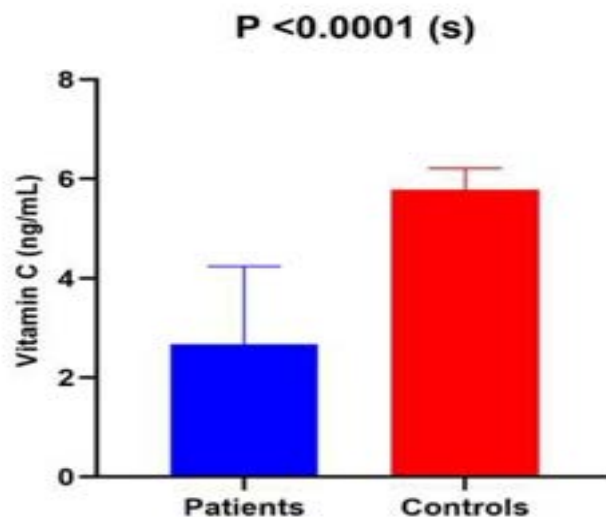


**Figure 4:** Comparison of Vitamin C concentration between sera samples of control and LC patient groups

### ROC curve

Based on the (Receiver Operating Characteristic) ROC curve, the area under the curve

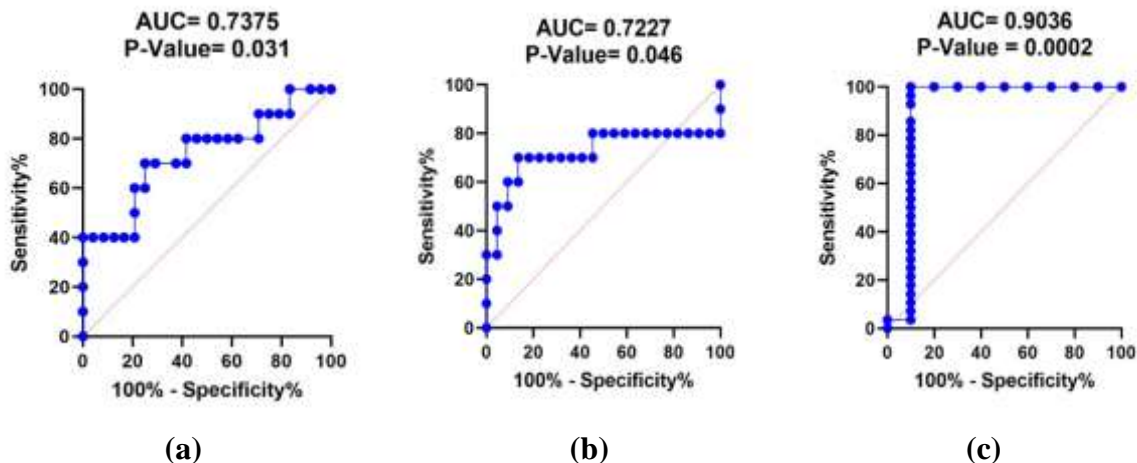(AUC) of 8-OHdG, TNF-α and IL-6 were (0.7375), (0.7227) and (0.9036) respectively. (Figure 5).



**Figure 5:** ROC curve of (a) 8-OHdG (b) TNF-alpha (c) IL-6.

## Discussion

### *Serum level of 8-OHdG*

The obtained results revealed a remarkable elevation in circulating concentration of 8-hydroxyguanosine in lung cancer patients in comparison to the healthy control. Oxygen radicals produced due to oxidative damage play a key role in the pathogensis of cancer [9]. The hugely unstable and reactive species create oxidative damage which eventually cause modifications of deoxyribonucleic acid (DNA) base and single damage, besides double-strand damage [10]. Oxygen radical and oxidative damage affect all stages of oncogenesis through altered pathways, interrelated with and destruct the biological matter, and were involved in the formation of tumors in these cells [11]. The association between degree of oxidative damage of DNA with the mutation rate and tumors prevalence, is based on the presence of sensative process for the investigation of inappreciable concentration of DNA damage [12]. 8-hydroxyguanosine a vital biological marker of oxidative damage generated by the oxidation of guanine base in DNA, play vital roles in lowering accumulation of 8-hydroxyguanosine in cellular DNAs [13]. Hence, the concentration of 8-hydroxyguanosine determined in cancer cell could be indicative of the oxidative DNA damage, improvement capacity of the cell and an intermediate biological marker of the degree of precipitated oxidative DNA damage [14,15]. The overexpression of 8-OHdG has been recorded in a several types of cancers, comprising breast, lung, bladder, colon, kidney, prostate, and stomach carcinomas [16]. A variety of potential mechanisms can support the opposite relationship of 8-hydroxyguanosine concentration and cancer combativeness.

### *Serum level of TNF-α*

The obtained results revealed a significant increase in circulating concentration of tumour necrosis factor-alpha in patients with lung carcinoma in comparison to the healthy individuals. Tumor necrosis factor-alpha is a major negotiator of inflammation with ambiguous impacts and has been identified in human ovarian, breast, pancreatic c, gastric, liver, prostate, bladder and colorectal cancers, besides lymphomas and leukemias [17].
Inflammation has been shown to have an impact in cancer inauguration and development and may thus be a principal contributor to oncogenesis. The oncogenic impact of inflammation may also widen cancer development. It has been recorded that chronic inflammation contributes in the evolution and advancement of tumour. Chronic inflammation can enhance an environment that leads to oncogenesis. In an inflammatory condition there is an increase in

the degree of turnover of cell. Microenvironment is frequently greatly oxidative and nitrosative stress, arising the chances for mutation and damage of DNA. Regulation of inflammation is achieved by cytokines, a group of molecules with signaling capability that can have autocrine, paracrine and endocrine impact [18]. Whereas earlier researches evaluated the association of circulating concentrations of cytokine with lung carcinoma [19].

It has been shown that the increased expression concentration of tumor necrosis factor-alpha in cancer tissue were linked to lung carcinoma diagnosis in a residents of patients with grade I adenocarcinoma. The existence of mediators of inflammation in the cancer microenvironment, produced either by the cancer cells or the cancer infiltrating cells has been extensively identified as one of the hallmarks of tumour [20].

### *Serum level of IL-6*

In a current study serum level of interleukin-6 was evaluated. Obtained data indicated a significantly elevated concentration of interleukin-6 in lung cancinoma patients. This data is in accordance with the findings of other studies, Yanagawa et al., Wojciechowska-Lacka et al. [21,22]. Interleukin-6 is generated by different cell types comprising cancer cells., Increased concentrations have been recorded in patients with various types of cancer such as lung, renal and ovarian carcinomas. Interleukin-6 is a multipotent cytokine exhibiting a variety of biological activities. Its circulationg concentration increases in different types of cancer. Investigation on Interleukin-6 has demonstrated its biological impacts in the pathogenesis and the diagnosis of tumour.s Interleukin-6 can also act as an autocrine growth factor, and it may stimulate cancer metastasis and invasiveness [23]. Interleukin-6 is a potent multidisciplinary protein, generated by many different cell kinds compricing T-cells, B-cells, monocytes, fibroblasts, chondrocytes, mesangial cells, glial cells, endothelial cells, keratinocytes and certain tumor cells [24]. However, monocytes/macrophages are predominantly responsible for Interleukin-6 synthesis. Interleukin-6 is considered to be a cytokine that plays a vital role in the host-defense mechanism. The biological importance of Interleukin-6 has been proved to be involved in the evocation of B-lymphocytes to biosynthesis of immunoglobulin. Contradictory data, regarding the action of Interleukin-6 in patients with various malignancies, has been produced. Antitumor capacities of Interleukin-6 were revealed in vitro and in vivo in patients with breast, lung carcinomas and some leukemias. On the other hand, Interleukin-6 supports the growth of myeloma. It has already been recorded that Interleukin-6 and Interleukin-6 - mediated systemic inflammation are linked to the high rate of metabolism and cachexia in lung cancer patients [22].

### *Serum level of SOD*

The present results show a remarkable diminish in serum level of SOD in lung cancer patients when compared to the controlled group. Superoxide dismutase is considered as a detoxificative enzyme that is responsible for the protection against oxygen radicals (ROS) and (SOD) [25]. According to the various researches, lung cancer is directly assoiated with oxidoreduction imbalance [26, 27, 28, 29]. Earlier studies observed that total superoxide dismutase activity remarkably elevated in lung carcinoma tissues, whereas an investigation done by [30] demonstrated that the total superoxide dismutase activity level in red blood cell lysate declined in nonsmall cell lung carcinoma patients in comparison to the healthy control subjects. Remarkable diminished activity was also linked with the existence of clinically advanced stage of cancer [30].

Meantime, in a study on colon carcinoma, total superoxide dismutase activities in red blood cells remarkably elevated as compared to the healthy groups. Likewise, in breast carcinoma, serum superoxide dismutase activity revealed a remarkable increase in cancer

patients, disregarding clinical grade. The mentioned variance in terms of alteration in total superoxide dismutase activity may arise from multifaceted mechanisms, including a condensation between oncogenesis and other parameters. Advancement stages of tumour can be correlated with the increased changes in the systemic profile of antioxidant. This alteration plays a vital role in conserving stability of DNA and solidity by removing anions of superoxide to inhibit oncogenesis. As previously shown, lung carcinoma is strongly linked with oxidative stress, hence , altering the systemic oxidative homeostasis [31]. Disturbance of the antioxidant system makes cells vulnerable to the damaging impacts of reactive oxygen species, comprising DNA damage and modifications of protein which is involved in oncogenesis. Consequently diminished superoxide dismutase activities are linked with elevated concentration of DNA base modifications in lung carcinoma than in cancer-free lung [32].

### Serum level of POX

The present study showed a significant reduction in serum level of POX in lung carcinoma patients as compared to healthy individuals. The observed decreased serum peroxidase activity (SPO) activity in the serum samples seemed to agree with the findings of Bahar et al. [9] who found that all salivary antioxidants including salivary peroxidase were substantially reduced in the patients with malignant epithelial neoplasm of oral cavity. They reported that this decrease was due to the depletion of salivary antioxidant systems as a result of the increase in oxygen and nitrogen radicals. Such a state explains the oxidative damage of deoxyribonucleic acid and proteins molecule and the progression of tumour [33].

Increased oxygen radicals [34] are well known stimulator of cellular and tissue pathogenesis resulting in tumor creation. Such reactive oxygen species are normally removed by antioxidant enzymes among which salivary peroxidase is one of the most critical one. This antioxidant system acts to eliminate the negative impact of these reactive species contributing in the appearance of tumour [35]. It was reported that at the time of malignant tumor setting, the reduction in serum peroxidase activity was the highest and such a reduction may result from an increase in oxidant level and can be of paramount importance, since serum peroxidase activity has a binary role. First, it regulates the concentration of $H_2O_2$ released by bacteria and white blood cell from the salivary glands into the oral cavity mouth by a mechanism that safeguards oral mucosa from cellular disruption prompted by $H_2O_2$. Meanwhile, $H_2O_2$ is considered as ROS which plays a vital role in human tumour progression, as this compound together with other free radicals, contributes in the DNA base variation, breaks into single and double DNA strand, destructs anti-oncogene, and an augmented expression of mutated gene. Oxygen radical instigated mutation could also cause protein damage [36].

### Serum level of Vitamin C

The present result demonstrated a remarkable decline in circulating concentration of ascorbic acid in lung carcinoma patients in comparison to a healthy individual. Ascorbic acid acts as a species with antioxidant activity at low levels, but as a species with pro-oxidant activity at high levels. Ascorbic acid can block glygolytic pathway of cancer cells, apprehend cancer cell cycle and instigate cancer cell death programme., Hence, it acts as an anti-cancer vitamin. A study in Canada observed that antioxidants compound such as ascorbic acid and vitamin A could decrease the hazard of lung carcinoma in females with a moderate degree of smoking [37]. Ascorbic acid is supposed to diminish the hazard of cancer by reason of its function in extinguishing free reactive species and decreasing degree of DNA damage by oxygen radical [38]. Earlier systematic review has proposed that ascorbic acid consumption diminished the risk of colon cancercinoma and gastric cancer., Ascorbic acid was linked with

a decrease in risk of cancer. Systematic review has also proposed that the hazard of uterus carcinoma as determined in dosage-reaction sample, decreases with every elevate in the consumption of ascorbic acid [39]. It has been recorded  that the consumption of  ascorbic acid and vitamin B9 was found to be linked with a diminished risk of lung carcinoma [40].

**Diagnostic Performance of Serum (8-OHdG, TNF-alpha and IL6)**
    ROC investigation was achieved. As illustrated in Figure 5, value of the area under the curve (AUC) is 0.7375 & p =0.031. The ROC curve designated a good fitting impact of this logistic regression model and a sizeable AUC value which indicated its more efficacious identification.. As demonstrated in Figure 5, 8-OHdG was hugely efficacious in the identification of lung carcinoma with AUC being 0.7375 & p =0.031. The results of  receiver operator characteristic (ROC) curve investigation showed that serum 8-OHdG could be used in the prognosis of lung carcinoma risk and is hugely efficacious in its identification.All of these findings propose that oxidative DNA damage induced mutations play vital roles in the progression of lung carcinoma and the noticeable elevate of serum 8-OHdG can serve  as a probable non-invasive liquid biological marker for its risk determination, untimely caution and detection.

    Receiver operator characteristic curve analysis was performed to investigate the diagnostic utility of proinflammatory cytokines (TNF-alpha and IL6) measurements for the diagnosis of lung cancer patients. was investigated using (Fig. 5). The area under the curve that gives the best prognostic precision values for TNF-alpha and IL-6 determined in this analysis were (AUC: 0.7227 & p=0.046); (AUC: 0.9036 & p<P=0.0002) respectively. Utilizing these cut-off values, the vulnerability, specificity and the prognostic accuracy observed for each cytokine, are demonstrated in (Figure 5: b & c). High accuracy, specificity and sensitivity of oxidative stress markers including serum 8-OHdG and proinflammatory cytokines (TNF-alpha and IL6) can be used as discriminatory markers for efficient diagnosis of lung cancer.

**Conclusions**
    The data of current article underpin the opinion that oxidative damage caused by the cumulative damage done by free radicals, plays a vital role in the progression of lung carcinoma. Consequently, serum 8-OHdG levels may be applied as a biological markers reflecting oxidative damage among lung canr patients. Depending on the current results, it appears that an increase in proinflammatory cytokines concentration in lung carcinoma patients can provide a predictive factor. Remarkable oxidoreduction imbalance in carcinoma patients proposes their cardinal significance in the progression of lung carcinoma sensitivity and a specific marker in the diagnosis of lung cancer patients, suggesting that serum 8-OHdG and proinflammatory cytokines concentrations can be used as non-invasive method for lung cancer screening.

**References**
[1]   A. R. An, K. M. Kim, H. S. Park, K. Y. Jang, W. S. Moon, M. J. Kang, Y. C. Lee, J. H. Kim, H. J. Chae, and M. J. Chung, "Association between Expression of 8-OHdG and Cigarette Smoking in Non-small Cell Lung Cancer," *Journal of pathology and translational medicine,* vol. 53, no. 4, pp. 217, 2019.
[2]   W. Ke, L. Zhang, and Y. Dai, "The role of IL-6 in immunotherapy of non-small cell lung cancer (NSCLC) with immune-related adverse events (irAEs)," *Thoracic cancer,* vol. 11, no. 4, pp. 835-839, 2020.
[3]   I. J. Oh, H. E. Kim, S. Y. Song, K. J. Na, K. S. Kim, Y. C. Kim, and S. W. Lee, "Diagnostic value of serum glutathione peroxidase 3 levels in patients with lung cancer," *Thoracic cancer,* vol. 5, no. 5, pp. 425-430, 2014.

[4]   K. Gong, G. Guo, N. Beckley, Y. Zhang, X. Yang, M. Sharma, and A. A. Habib, "Tumor necrosis factor in lung cancer: Complex roles in biology and resistance to treatment," *Neoplasia,* vol. 23, no. 2, pp. 189-196, 2021.

[5]   K. Smetana, and J. Brábek, "Role of interleukin-6 in lung complications in patients with COVID-19: Therapeutic implications," *in vivo,* vol. 34, no. 3 suppl, pp. 1589-1592, 2020.

[6]   A. Glasauer, L. A. Sena, L. P. Diebold, A. P. Mazar, and N. S. Chandel, "Targeting SOD1 reduces experimental non–small-cell lung cancer," *The Journal of clinical investigation,* vol. 124, no. 1, pp. 117-128, 2014.

[7]   X. Wang, H. Zhang, R. Sapio, J. Yang, J. Wong, X. Zhang, J. Y. Guo, S. Pine, H. Van Remmen, and H. Li, "SOD1 regulates ribosome biogenesis in KRAS mutant non-small cell lung cancer," *Nature communications,* vol. 12, no. 1, pp. 1-15, 2021.

[8]   J. Luo, L. Shen, and D. Zheng, "Association between vitamin C intake and lung cancer: a dose-response meta-analysis," *Scientific reports,* vol. 4, no. 1, pp. 1-7, 2014.

[9]   G. Bahar, R. Feinmesser, T. Shpitzer, A. Popovtzer, and R. M. Nagler, "Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile," *Cancer,* vol. 109, no. 1, pp. 54-59, 2007.

[10]  R. K. Gupta, A. K. Patel, N. Shah, A. K. Choudhary, U. K. Jha, U. C. Yadav, P. K. Gupta, and U. Pakuwal, "Oxidative stress and antioxidants in disease and cancer: a review," *Asian Pacific journal of cancer prevention,* vol. 15, no. 11, pp. 4405-4409, 2014.

[11]  J. Kruk, H. Y. Aboul-Enein, A. Kładna, and J. E. Bowser, "Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis," *Free radical research,* vol. 53, no. 5, pp. 497-521, 2019.

[12]  M. Sanchez, R. Roussel, S. Hadjadj, A. Moutairou, M. Marre, G. Velho, and K. Mohammedi, "Plasma concentrations of 8-hydroxy-2′-deoxyguanosine and risk of kidney disease and death in individuals with type 1 diabetes," *Diabetologia,* vol. 61, no. 4, pp. 977-984, 2018.

[13]  Y. Nakabeppu, "Cellular levels of 8-oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells," *International journal of molecular sciences,* vol. 15, no. 7, pp. 12543-12557, 2014.

[14]  P. Karihtala, Y. Soini, L. Vaskivuo, R. Bloigu, and U. Puistola, "DNA adduct 8-hydroxydeoxyguanosine, a novel putative marker of prognostic significance in ovarian carcinoma," *International Journal of Gynecologic Cancer,* vol. 19, no. 6, 2009.

[15]  M. Pylväs-Eerola, P. Karihtala, and U. Puistola, "Preoperative serum 8-hydroxydeoxyguanosine is associated with chemoresistance and is a powerful prognostic factor in endometrioid-type epithelial ovarian cancer," *BMC cancer,* vol. 15, no. 1, pp. 1-7, 2015.

[16]  H. Sova, A. Jukkola-Vuorinen, U. Puistola, S. Kauppila, and P. Karihtala, "8-Hydroxydeoxyguanosine: a new potential independent prognostic factor in breast cancer," *British journal of cancer,* vol. 102, no. 6, pp. 1018-1023, 2010.

[17]  M. F. Mercogliano, S. Bruni, P. V. Elizalde, and R. Schillaci, "Tumor necrosis factor α blockade: an opportunity to tackle breast Cancer," *Frontiers in oncology,* vol. 10, no. 584, 2020.

[18]  S. Ballaz, and J. L. Mulshine, "The potential contributions of chronic inflammation to lung carcinogenesis," *Clinical lung cancer,* vol. 5, no. 1, pp. 46-62, 2003.

[19]  L. Enewold, L. E. Mechanic, E. D. Bowman, Y.-L. Zheng, Z. Yu, G. Trivers, A. J. Alberg, and C. C. Harris, "Serum concentrations of cytokines and lung cancer survival in African Americans and Caucasians," *Cancer Epidemiology and Prevention Biomarkers,* vol. 18, no. 1, pp. 215-222, 2009.

[20]  L. M. Coussens, L. Zitvogel, and A. K. Palucka, "Neutralizing tumor-promoting chronic inflammation: a magic bullet?," *Science,* vol. 339, no. 6117, pp. 286-291, 2013.

[21]  H. Yanagawa, S. Sone, Y. Takahashi, T. Haku, S. Yano, T. Shinohara, and T. Ogura, "Serum levels of interleukin 6 in patients with lung cancer," *British journal of cancer,* vol. 71, no. 5, pp. 1095-1098, 1995.

[22]  A. Wojciechowska-Lacka, M. Matecka-Nowak, E. Adamiak, J. Lacki, and B. Cerkaska-Gluszak, "Serum levels of interleukin-10 and interleukin-6 in patients with lung cancer," *Neoplasma,* vol. 43, no. 3, pp. 155-158, 1996.

[23] S. Okada, T. Okusaka, H. Ishii, A. Kyogoku, M. Yoshimori, N. Kajimura, K. Yamaguchi, and T. Kakizoe, "Elevated serum interleukin-6 levels in patients with pancreatic cancer," *Japanese journal of clinical oncology,* vol. 28, no. 1, pp. 12-15, 1998.

[24] S. Akira, and T. Kishimoto, "IL-6 and NF-IL6 in acute-phase response and viral infection," *Immunological reviews,* vol. 127, pp. 25-50, 1992.

[25] O. Ighodaro, and O. Akinloye, "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid," *Alexandria journal of medicine,* vol. 54, no. 4, pp. 287-293, 2018.

[26] J. D. Hayes, A. T. Dinkova-Kostova, and K. D. Tew, "Oxidative stress in cancer," *Cancer cell*, 2020.

[27] A. Otsmane, G. Kacimi, S. Adane, F. Cherbal, and S. A. Bouguerra, "Clinico-epidemiological profile and redox imbalance of lung cancer patients in Algeria," *Journal of medicine and life,* vol. 11, no. 3, pp. 210-217, 2018.

[28] M. Mateu-Jiménez, A. Sánchez-Font, A. Rodríguez-Fuster, R. Aguiló, L. Pijuan, C. Fermoselle, J. Gea, V. Curull, and E. Barreiro, "Redox imbalance in lung cancer of patients with underlying chronic respiratory conditions," *Molecular Medicine,* vol. 22, no. 1, pp. 85-98, 2016.

[29] K. Zabłocka-Słowińska, I. Porębska, M. Gołecki, M. Kosacka, K. Pawełczyk, L. Pawlik-Sobecka, K. Zarębska, and H. Grajeta, "Total antioxidant status in lung cancer is associated with levels of endogenous antioxidants and disease stage rather than lifestyle factors–preliminary study," *Contemporary Oncology,* vol. 20, no. 4, pp. 302-307, 2016.

[30] J. C. Ho, M. Chan-Yeung, S. Ho, J. Mak, M. Ip, G. Ooi, M. Wong, K. Tsang, and W. Lam, "Disturbance of systemic antioxidant profile in nonsmall cell lung carcinoma," *European Respiratory Journal,* vol. 29, no. 2, pp. 273-278, 2007.

[31] K. B. Skórska, S. Płaczkowska, A. Prescha, I. Porębska, M. Kosacka, K. Pawełczyk, and K. Zabłocka-Słowińska, "Serum Total SOD Activity and SOD1/2 Concentrations in Predicting All-Cause Mortality in Lung Cancer Patients," *Pharmaceuticals,* vol. 14, no. 11, no. 1067, 2021.

[32] J. C.-m. Ho, S. Zheng, S. A. Comhair, C. Farver, and S. C. Erzurum, "Differential expression of manganese superoxide dismutase and catalase in lung cancer," *Cancer research,* vol. 61, no. 23, pp. 8578-8585, 2001.

[33] H. Wang, Y. Liu, R. He, D. Xu, J. Zang, N. Weeranoppanant, H. Dong, and Y. Li, "Cell membrane biomimetic nanoparticles for inflammation and cancer targeting in drug delivery," *Biomaterials science,* vol. 8, no. 2, pp. 552-568, 2020.

[34] N. Sharma, "Free Radicals, Antioxidants and Disease," *Biology and Medicine,* vol. 6, pp. 2-6, 10/01, 2014.

[35] R. Filip, T. Sebastian, G. Ferraro, and C. Anesini, "Effect of ILex extracts and isolated compounds upon peroxidase secretion in rat submandibular glands," *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association,* vol. 45, pp. 649-55, 05/01, 2007.

[36] H. R. Hasan, and N. N. Aburahma, "The Variations in Saliva and Serum Total Peroxidases System's Activity in Patients with Different Oral Tumors," *Biological,* vol. 2307, pp. 615-620, 2020.

[37] L. liang, w. yu, b. Cai, q. Wang, x. Huang, j. Chen, m. Zeng, y. Wang, B. Qu, and f. liu, "Determination of multiple vitamins in 178 patients undergoing chemotherapy for lung cancer," *Archives of Medical Science*, 2021.

[38] R. J. Sram, B. Binkova, and P. Rossner Jr, "Vitamin C for DNA damage prevention," *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* vol. 733, no. 1-2, pp. 39-49, 2012.

[39] M. R. Munafò, and J. Flint, "Meta-analysis of genetic association studies," *Trends in Genetics,* vol. 20, no. 9, pp. 439-444, 2004.

[40] C. G. Slatore, A. J. Littman, D. H. Au, J. A. Satia, and E. White, "Long-Term Use of Supplemental Multivitamins, Vitamin C, Vitamin E, and Folate Does Not Reduce the Risk of Lung Cancer," *American Journal of Respiratory and Critical Care Medicine,* vol. 177, no. 5, pp. 524-530, 2008.