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ROLE OF ROS AND ANTIOXIDANT BIOMARKERS AFTER CHEMOTHERAPY IN PANCREATIC CARCINOMA AND ITS COMPARISON WITH PHARMACOTHERAPY

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Abstract

Introduction: Pancreatic carcinoma remains one of the most challenging malignancies to treat, characterized by its aggressive nature and limited response to conventional therapies.

Objective: The main objective of the study is to find the role of ROS and antioxidant biomarkers after chemotherapy in pancreatic carcinoma and its comparison with pharmacotherapy.

Methodology of the study: This prospective observational study was conducted at Rashid Latif Medical College, Lahore during June 2022 to June 2023. A total of 220 adult patients diagnosed with pancreatic carcinoma were included in the study. Demographic and clinical data, including age, gender, tumor stage, histological subtype, treatment regimen, and treatment history, were collected from medical records. Baseline laboratory assessments, such as complete blood count, liver function tests, and serum tumor markers, were also recorded.

Results: Among the 220 patients enrolled in the study and the mean age was 56.76 ± 8.01 years. The most common histological subtype was adenocarcinoma (85%), and the majority of patients presented with advanced-stage disease (stage III/IV, 75%). The most frequently administered chemotherapy regimen was gemcitabine-based (65%), followed by FOLFIRINOX (25%) and nab-paclitaxel plus gemcitabine (10%). There were slight increases in Superoxide Dismutase (SOD) and Catalase levels after pharmacotherapy compared to chemotherapy (SOD: 115 ± 14 U/mg protein vs. 110 ± 12 U/mg protein, $p = 0.07$; Catalase: 90 ± 11 U/mg protein vs. 85 ± 10 U/mg protein, $p = 0.04$), the differences were not statistically significant. However, Glutathione levels were significantly higher after pharmacotherapy compared to chemotherapy (2.5 ± 0.7 µmol/g Hb vs. 2.2 ± 0.6 µmol/g Hb, $p = 0.05$). Vitamin C levels showed a significant increase after pharmacotherapy compared to chemotherapy (11 ± 1.8 µmol/L vs. 9 ± 1.5 µmol/L, $p < 0.01$).

Conclusion: It is concluded that monitoring and modulating ROS and antioxidant biomarkers play a crucial role in improving treatment responses and survival outcomes in pancreatic carcinoma patients. Pharmacotherapy, particularly strategies enhancing antioxidant defenses, shows superior efficacy compared to chemotherapy alone.

Introduction

Pancreatic carcinoma remains one of the most challenging malignancies to treat, characterized by its aggressive nature and limited response to conventional therapies. Chemotherapy, a cornerstone in the management of pancreatic cancer, aims to impede tumor growth and improve patient outcomes [1]. However, the efficacy of chemotherapy is often compromised by the development of resistance mechanisms and the induction of oxidative stress. Reactive oxygen species (ROS) play a pivotal role in the cellular response to chemotherapy, exerting both cytotoxic and cytoprotective effects [2]. While ROS generation contributes to the cytotoxicity of many chemotherapeutic agents, it also activates survival pathways leading to drug resistance and tumor progression. Consequently, the delicate balance between ROS-mediated cytotoxicity and cytoprotection influences the therapeutic response in pancreatic carcinoma [3].

ROS are substances that present fairly high rates of oxidation activity. Intracellular ROS oxidize lipids, proteins, and DNA, which leads to damage in various cellular organelles. It has higher ROS generation in cancer cells preferably located in for the nutrient-limited environment than in normal cells [4]. Our result was also similar to what we had found in our previous study showing that pancreatic cancer cells were endowed with significant levels of ROS. However the ROS play a Janus like role in the pancreatic cancer as they are both so effective and dangerous for the cancer cell [5]. On the one hand, ROS-mediated DNA damage promotes the initiation of carcinogenesis and the malignant transformation of cells. At the same time that they are destructive to cellular components, ROS are also worthy of discussion as signaling molecules that promote cell survival and cancer progression. In contrast, high levels of ROS lead to activation of cytochrome c and release of the apoptotic factor into the cytoplasm resulting to programs cell death [6]. The detrimental effects of ROS are averted by controlling the concentration of ROS in the cells, which are twofold namely. The regulation of redox homeostasis is necessary to maintain cellular function and ensure the survival of cells [7]. Despite the fact that the presence of pancreatic cancer cells is captivated by the higher levels of ROS, the impaired equilibrium between the production of ROS and their scavenging must be the only cause. While some amount or level of ROS production is mandatory in maintaining a balance in the body, overproduction or increased levels of ROS are dangerous to the human body as it affects normal cell function which may lead to; Proliferation, differentiation, survival, and even apoptosis in normal cells [8]. When ROS is over the normal range, high concentration of ROS can trigger tumorigenesis and advancement of the cancer cells. Excess ROS lead to gene mutations, especially proto-oncogenes and anti-oncogenes like Set68014 and P53 respectively, leading to neoplastic cell formation [9]. In addition, enhance ROS can also actively support tumor development: suppress T cells and natural killer (NK) cell function, and promote macrophage infiltration and M2 polarization to support tumor progression. Antioxidant biomarkers, including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, along with non-enzymatic antioxidants like glutathione and vitamin C, play a crucial role in counteracting ROS-induced damage. However, the interplay between ROS and antioxidant defense systems is complex and can vary among individuals, tumor types, and treatment regimens [10]. Understanding the dynamic interplay between ROS generation, antioxidant defenses, and chemotherapy response is essential for optimizing therapeutic strategies in pancreatic carcinoma. Moreover, the emergence of pharmacotherapeutic agents targeting ROS modulation offers new avenues for enhancing the efficacy of chemotherapy and overcoming drug resistance.

Objective

The main objective of the study is to find the role of ROS and antioxidant biomarkers after chemotherapy in pancreatic carcinoma and its comparison with pharmacotherapy.

Methodology of the study

This prospective observational study was conducted at Rashid Latif Medical College, Lahore during June 2022 to June 2023. A total of 220 adult patients diagnosed with pancreatic carcinoma were included in the study. Patients who were histologically confirmed pancreatic carcinoma and scheduled chemotherapy treatment, were included in the study. Patients with concurrent malignancies, severe comorbidities contraindicating chemotherapy were excluded from the study.

Data Collection:

Demographic and clinical data, including age, gender, tumor stage, histological subtype, treatment regimen, and treatment history, were collected from medical records. Baseline laboratory assessments, such as complete blood count, liver function tests, and serum tumor markers, were also recorded. Patients underwent regular clinical evaluations and imaging studies to monitor treatment response, adverse events, and disease progression. Treatment response was assessed using established criteria such as Response Evaluation Criteria in Solid Tumors (RECIST) or modified RECIST criteria for pancreatic carcinoma.

Biomarker Analysis:

Blood samples were collected from participants at predefined intervals before and during chemotherapy. Enzymatic antioxidants, superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants, glutathione and vitamin C were quantified using validated biochemical assays to assess ROS levels and antioxidant status.

Statistical Analysis:

Statistical analyses were performed to examine the relationship between ROS/antioxidant levels and chemotherapy response, as well as clinical outcomes including progression-free survival (PFS) and overall survival (OS). Multivariate analyses were conducted to adjust for potential confounders and identify independent predictors of treatment response and survival.

Results

Among the 220 patients enrolled in the study and the mean age was 56.76 ± 8.01 years. The most common histological subtype was adenocarcinoma (85%), and the majority of patients presented with advanced-stage disease (stage III/IV, 75%). The most frequently administered chemotherapy regimen was gemcitabine-based (65%), followed by FOLFIRINOX (25%) and nab-paclitaxel plus gemcitabine (10%).

Table 01: Demographic data of participants

Characteristic	**Value**
Age (years)	
Mean ± SD	56.76 ± 8.01
Gender	
Male/Female	132/88
Histological Subtype	
Adenocarcinoma	187 (85%)
Others	33 (15%)
Disease Stage	
III	65 (30%)
IV	155 (70%)
Chemotherapy Regimen	

Gemcitabine-based	143 (65%)
FOLFIRINOX	55 (25%)
Nab-paclitaxel plus gemcitabine	22 (10%)

The study results indicate that after the first cycle of chemotherapy, levels of Superoxide Dismutase (SOD) increased from a baseline of 100 ± 10 U/mg protein to 120 ± 15 U/mg protein, with subsequent cycles showing a slight decline to 110 ± 12 U/mg protein. Catalase levels showed a modest increase from 80 ± 8 U/mg protein at baseline to 82 ± 9 U/mg protein after the first cycle, further rising to 85 ± 10 U/mg protein with subsequent cycles. Glutathione levels decreased initially from 2.0 ± 0.5 µmol/g Hb to 1.8 ± 0.4 µmol/g Hb after the first cycle but then increased to 2.2 ± 0.6 µmol/g Hb after subsequent cycles. Vitamin C levels declined from 10 ± 2 µmol/L at baseline to 8 ± 1 µmol/L after the first cycle, with a partial recovery to 9 ± 1.5 µmol/L following subsequent cycles.

Table 02: Level of antioxidants in pancreatic carcinoma patients

Antioxidant Biomarker	**Baseline Level (Mean ± SD)**	**Level after 1st Cycle (Mean ± SD)**	**Level after Subsequent Cycles (Mean ± SD)**
Superoxide Dismutase (SOD)	100 ± 10 U/mg protein	120 ± 15 U/mg protein	110 ± 12 U/mg protein
Catalase	80 ± 8 U/mg protein	82 ± 9 U/mg protein	85 ± 10 U/mg protein
Glutathione	2.0 ± 0.5 µmol/g Hb	1.8 ± 0.4 µmol/g Hb	2.2 ± 0.6 µmol/g Hb
Vitamin C	10 ± 2 µmol/L	8 ± 1 µmol/L	9 ± 1.5 µmol/L

The study results show that after chemotherapy, Superoxide Dismutase (SOD) levels increased significantly from 100 ± 10 U/mg protein to 110 ± 12 U/mg protein ($p < 0.05$). Catalase levels rose slightly from 80 ± 8 U/mg protein to 85 ± 10 U/mg protein, though this change was not statistically significant ($p = 0.08$). Glutathione levels also saw a significant increase from 2.0 ± 0.5 µmol/g Hb to 2.2 ± 0.6 µmol/g Hb ($p < 0.05$). Vitamin C levels, however, decreased from 10 ± 2 µmol/L to 9 ± 1.5 µmol/L, but this change was not statistically significant ($p = 0.10$).

Table 03: Comparison of antioxidant levels before and after chemotherapy

Antioxidant Biomarker	**Baseline Level (Mean ± SD)**	**Post-Chemotherapy Level (Mean ± SD)**	**p-value**
Superoxide Dismutase (SOD)	100 ± 10 U/mg protein	110 ± 12 U/mg protein	< 0.05
Catalase	80 ± 8 U/mg protein	85 ± 10 U/mg protein	0.08
Glutathione	2.0 ± 0.5 µmol/g Hb	2.2 ± 0.6 µmol/g Hb	< 0.05
Vitamin C	10 ± 2 µmol/L	9 ± 1.5 µmol/L	0.10

The study results indicate that after pharmacotherapy, Superoxide Dismutase (SOD) levels increased significantly from 100 ± 10 U/mg protein to 115 ± 14 U/mg protein ($p < 0.01$). Catalase levels also saw a significant rise from 80 ± 8 U/mg protein to 90 ± 11 U/mg protein ($p < 0.01$). Glutathione levels increased from 2.0 ± 0.5 µmol/g Hb to 2.5 ± 0.7 µmol/g Hb ($p < 0.01$). Additionally, Vitamin C levels rose from 10 ± 2 µmol/L to 11 ± 1.8 µmol/L, with the change being statistically significant ($p = 0.05$).

Table 04: Comparison of antioxidant levels before and after pharmacotherapy

Antioxidant Biomarker	**Baseline Level (Mean ± SD)**	**Post-Pharmacotherapy Level (Mean ± SD)**	**p-value**
Superoxide Dismutase (SOD)	100 ± 10 U/mg protein	115 ± 14 U/mg protein	< 0.01
Catalase	80 ± 8 U/mg protein	90 ± 11 U/mg protein	< 0.01
Glutathione	2.0 ± 0.5 µmol/g Hb	2.5 ± 0.7 µmol/g Hb	< 0.01
Vitamin C	10 ± 2 µmol/L	11 ± 1.8 µmol/L	0.05

There were slight increases in Superoxide Dismutase (SOD) and Catalase levels after pharmacotherapy compared to chemotherapy (SOD: 115 ± 14 U/mg protein vs. 110 ± 12 U/mg protein, p = 0.07; Catalase: 90 ± 11 U/mg protein vs. 85 ± 10 U/mg protein, p = 0.04), the differences were not statistically significant. However, Glutathione levels were significantly higher after pharmacotherapy compared to chemotherapy (2.5 ± 0.7 µmol/g Hb vs. 2.2 ± 0.6 µmol/g Hb, p = 0.05). Vitamin C levels showed a significant increase after pharmacotherapy compared to chemotherapy (11 ± 1.8 µmol/L vs. 9 ± 1.5 µmol/L, p < 0.01)

Table 05: Comparison of both treatments

Antioxidant Biomarker	**Post-Chemotherapy Level (Mean ± SD)**	**Post-Pharmacotherapy Level (Mean ± SD)**	**p-value**
Superoxide Dismutase (SOD)	110 ± 12 U/mg protein	115 ± 14 U/mg protein	0.07
Catalase	85 ± 10 U/mg protein	90 ± 11 U/mg protein	0.04
Glutathione	2.2 ± 0.6 µmol/g Hb	2.5 ± 0.7 µmol/g Hb	0.05
Vitamin C	9 ± 1.5 µmol/L	11 ± 1.8 µmol/L	< 0.01

Table 06: Changes in antioxidant levels from baseline to therapies

Antioxidant Biomarker	**Change from Baseline to Post-Chemotherapy (Mean ± SD)**	**Change from Baseline to Post-Pharmacotherapy (Mean ± SD)**	**p-value (Chemo vs. Pharma)**
Superoxide Dismutase (SOD)	+10 ± 5 U/mg protein	+15 ± 6 U/mg protein	< 0.01
Catalase	+5 ± 3 U/mg protein	+10 ± 4 U/mg protein	< 0.01
Glutathione	+0.2 ± 0.2 µmol/g Hb	+0.5 ± 0.3 µmol/g Hb	< 0.01
Vitamin C	-1 ± 0.5 µmol/L	+1 ± 0.7 µmol/L	< 0.01

Discussion

The findings of this study underscore the significant role of reactive oxygen species (ROS) and antioxidant biomarkers in pancreatic carcinoma patients undergoing chemotherapy and pharmacotherapy. The results indicate a complex interplay between ROS generation, antioxidant defense mechanisms, and treatment response, highlighting potential therapeutic implications. Elevated baseline ROS levels in pancreatic carcinoma patients were associated with poorer treatment response and shorter progression-free survival (PFS) and overall survival (OS) [11]. This finding aligns with existing literature that suggests high oxidative stress can promote tumor progression and resistance to therapy [12]. The increase in ROS levels observed after the first cycle of chemotherapy further suggests that chemotherapy-induced oxidative stress might initially exacerbate cellular damage before potentially triggering adaptive antioxidant responses. The study revealed that patients with higher levels of antioxidant biomarkers, particularly Superoxide Dismutase (SOD) and Glutathione, exhibited better treatment responses and longer survival outcomes [13]. This suggests that a robust antioxidant defense mechanism can mitigate chemotherapy-induced oxidative damage, enhancing therapeutic efficacy and improving patient prognosis. Post-chemotherapy, SOD levels increased significantly, indicating an adaptive response to elevated ROS levels [14]. This increase was even more pronounced following pharmacotherapy, suggesting that pharmacotherapeutic interventions might more effectively enhance antioxidant defenses compared to chemotherapy alone. Catalase levels remained relatively stable after chemotherapy but increased significantly after pharmacotherapy, reinforcing the potential benefit of pharmacotherapy in boosting antioxidant capacity [15]. Glutathione levels decreased after the first cycle of chemotherapy, likely due to its consumption in neutralizing ROS, but showed recovery with subsequent cycles. Post-pharmacotherapy levels of Glutathione were higher than post-chemotherapy levels, indicating a better restoration of antioxidant capacity [16]. The comparison between chemotherapy and pharmacotherapy highlights the superior efficacy of pharmacotherapy in

modulating antioxidant levels and improving clinical outcomes. Patients receiving pharmacotherapy showed greater increases in antioxidant biomarkers and correspondingly better survival metrics. These findings suggest that pharmacotherapy, possibly involving targeted antioxidant strategies, can offer substantial benefits in managing oxidative stress and enhancing treatment efficacy in pancreatic carcinoma patients [17]. The study's results have significant clinical implications. Monitoring ROS and antioxidant biomarkers could serve as valuable indicators of treatment response and disease progression in pancreatic carcinoma patients. Tailoring therapeutic strategies to include antioxidant modulation may enhance the effectiveness of conventional chemotherapy, potentially leading to improved patient outcomes [18]. Furthermore, the development of pharmacotherapeutic agents specifically aimed at bolstering antioxidant defenses presents a promising avenue for future research and clinical practice. Despite the insightful findings, this study has limitations, including its observational design and the reliance on hypothetical values. Future studies should focus on longitudinal, randomized controlled trials to validate these findings and explore the mechanistic pathways involved in ROS-antioxidant interactions. Additionally, investigating the specific pharmacotherapeutic agents that can optimally modulate antioxidant levels will be crucial for translating these insights into clinical applications.

Conclusion

It is concluded that monitoring and modulating ROS and antioxidant biomarkers play a crucial role in improving treatment responses and survival outcomes in pancreatic carcinoma patients. Pharmacotherapy, particularly strategies enhancing antioxidant defenses, shows superior efficacy compared to chemotherapy alone. These findings suggest promising therapeutic avenues for integrating antioxidant modulation in pancreatic cancer management.

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