

To Estimate the Levels Serum MDA and Glutathione in Patients with Cervical Cancer

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Abstract

Background

Healthy cells are transformed into tumour cells during the course of a complex, multi-step process called cervical cancer. Several internal and external cues regulate this transition. These triggers include reactive oxygen species (ROS), which are important in the development of tumours. Cervical cancer is the second most common cancer in women worldwide. Cervical cancer is more prevalent in rural areas than in urban areas. Malondialdehyde and glutathione were measured as indicators of lipid peroxidation and endogenous antioxidant, respectively

Aim and Objectives

- To measure serum glutathione and malondialdehyde levels in cervical cancer patients and healthy controls.
- To compare the serum levels of glutathione and malondialdehyde in cervical cancer patients and healthy controls.

Materials and Methods

This hospital-based cross-sectional study was carried out in departments of biochemistry, pathology, and gynaecology at SGT Hospital in Budhera, Gurugram's Faculty of Medicine and Health Sciences. As the control group, we used 50 age-matched, healthy volunteers from the general community. Each participant in both groups were fully informed of the study's aims before providing a signed and informed consent. The clearance of the institutional ethics committee was taken before starting the sample collection. The serum is separated from the clot in the plain tube by centrifuging the sample at 3000 rpm for 10 minutes. After the blood has been centrifuged at 3000 rpm in the lithium heparin tube, the plasma is decanted. A commercially available ELISA kit (MyBioSource) is used to quantify malondialdehyde and glutathione levels

Results

Patients with cervical cancer had a significantly higher level of serum MDA and lower value of serum Glutathione when compare with controls having mean and standard deviation for MDA (5.5 ± 1.48 nmol/ml and 3.4 ± 0.87 nmol/ml) and Glutathione (4.5 ± 1.5 Mm and 11.9 ± 2.7 Mm) .

Conclusion

In conclusion, decreased antioxidative enzyme activity and enhanced lipid peroxidation are signs of oxidative stress in cervical cancer, which is more obvious in advanced stages because of the increased tumour burden. Antioxidant supplements may slow the disease's progression in cervical cancer sufferers.

Key Words: *Cervical cancer, Malondialdehyde (MDA), Glutathione, reactive oxygen species (ROS), Oxidative Stress.*

Introduction:

Healthy cells are transformed into tumour cells during the course of a complex, multi-step process called cervical cancer. Several internal and external cues regulate this transition. These triggers include reactive oxygen species (ROS), which are important in the development of tumours [1]. Eukaryotic cells will always produce ROS since it is a consequence of mitochondrial metabolic processes. Typically, the cell has little ROS present. It is necessary for various subcellular functions, including apoptosis, disulfide bond formation, gene expression, metabolism, and intracellular signal transduction (2,3). Cervical cancer is the second most common cancer in women worldwide. Cervical cancer is more prevalent in rural areas than in urban areas. It rapidly rises between the ages of 45 and 55 before peaking. (4) Women in low-resource environments have a lifetime risk of cervical cancer of 2-4%. Despite being a condition that can be avoided, it is a problem in the majority of developing countries due to factors like illiteracy, poverty, poor health seeking behaviour, late presentation, a delay in starting the appropriate treatment based on histology, and insufficient treatment due to loss to follow-up. 80–90% of cases manifest in the final stages of the illness in women between the ages of four and six. (7) A dialdehyde with a high level of reactivity is malondialdehyde. MDA occurs in the body as an enolate anion ($\text{O}=\text{CH}-\text{CH}=\text{CHO}$), a form that is only moderately reactive and forms Schiff base with molecules that have a free amine group. One of the most prevalent indicators of oxidative stress is malondialdehyde (MDA), which is also an oxidant-antioxidant adduct in cancer patients. Lipid peroxidation is started by reactive oxygen species (ROS), which are generated by several mechanisms that result in excessive MDA formation, which alters normal cell function and results in cancer (8). The elevated quantity of MDA can be attributed to an increase in ROS generation as a result of oxidative damage in uterine cancer patients. The creation of oxygen radical also rises as the disease progresses, which in turn boosts lipid peroxidation. DNA and cell membranes are harmed or degenerate as a result of this process (9). A spike in serum MDA is a sign of excessive lipid peroxidation caused by an increase in free radical production. Free radicals can manifestly alter how cell membranes work and how DNA is structured, which can result in mutations. Therefore, it can be said that a probable cause of cancer growth could be a byproduct of lipid peroxidation (9,11). One of the primary detoxifying agents is assumed to be glutathione (GSH). It is well established that the level of GSH in a cell affects its toxicity and sensitivity to anticancer therapy (a decline in GSH enhances drug toxicity). The level of GSH must therefore be determined in order to predict whether malignant cells will be sensitive to the effects of the treatment or if the drug will have no effect on normal cells. It is established that cervical cancer patients who experience a complete response to treatment have much less GSH in their blood and tumours than those who get a partial response (12,13). Enzymes involved in metabolism that employ tripeptide as a substrate can have an impact on GSH alterations. The enzyme glutathione S-transferase (GST) must be active enough for cancer chemotherapy to be effective because when it reacts with GSH during an enzyme-catalyzed conjugation, the solubility of drugs and other toxic materials in the water increases and they are eliminated more effectively. As a result, the effect on the organism is reduced, which can result in a worse response to the treatment and a shorter survival time (14) Therefore, the goal of this study was to determine whether antioxidant level and cervical cancer were related, as well as to look into how oxidative indicators affected the severity of cervical cancer in patients at SGT Hospital. Malondialdehyde and glutathione were measured as indicators of lipid peroxidation and endogenous antioxidant, respectively.

Materials and Methods:

Statement of ethics

Before beginning the study, ethical approval was acquired from our institution's institutional ethical committee. All patients and healthy people are asked to sign written informed consent forms.

Subjects

Between March 2021 and March 2023, the study was done. Based on data discovered in the hospital's MRD (medical records department), 50 individuals with cervical cancer who were receiving care at SGT Hospital's Obs & Gyne OPD and were between the ages of 18 and 65 were identified. The basis for case definition will be histopathological analysis. The control group in the study will consist of 50 females who frequently attend the gynaecology outpatient department and have pap smear findings that are NILM (negative for intra epithelial lesion or malignancy). Both sets of study participants read and agreed to the study's objectives before signing written consent forms. The participants in the trial are non-pregnant women, cervical cancer cases with a histological diagnosis, controls with a Papanicolaou smear result of NILM (negative for intra epithelial lesion/malignancy), and patients without a history of prior tumour treatment. Women who were pregnant, those who had previously had a hysterectomy, and anyone who had previously undergone any type of decisive therapy for a tumour were all disqualified from the trial.

Gathering and processing of samples

In an aseptic setting, participants will be venepunctured with a 5 ml sterile disposable syringe and needle to collect a sample of venous blood. The first 2.5 ml aliquot will be put into a plain tube, and the second 2.5 ml aliquot will be put into lithium heparin tubes. Each tube will have the patient's code inscribed on it. The serum will be removed from the clot in the plain tube by centrifuging the sample at 3000 rpm for 10 minutes. After the blood has been centrifuged at 3000 rpm in the lithium heparin tube, the plasma will be decanted.

Laboratory evaluation

A commercially available ELISA kit (MyBioSource) will be used to quantify malondialdehyde and glutathione levels. The samples will be kept at -20°C until the test is finished, at which point they will be discarded.

Statistical analysis

The generated results must go via SPSS statistical analysis. Statistical significance is defined as a P-value of ≤ 0.05 . The chi square test will be used to non-parametric variables. A student t-test will be used to compare the average between two groups. The Pearson's correlation coefficient will be utilised to ascertain the link between the variables.

Results:

The goal of this experiment was to measure the serum levels of MDA and glutathione.

Parameters	Cases(mean+STD) n=50	control(mean+STD) n=50	t value	p value	Significance
MDA	5.5±1.48	3.4±0.87	8.659	$p < .05$	Significant
Glutathione	0.36±0.1	7.67±1.81	-28.428	$p < .05$	Significant

Table 1 showing the mean and Standard deviation of serum MDA and Glutathione

Demographic Parameters:

This study comprised 50 cases of cervical cancer in females between the ages of 18 and 65, as well as 50 participants in the control group.

Figure 1 from our study shows that the mean and standard deviation of serum MDA levels in the patients were 5.5 ± 1.48 nmol/ml and 3.4 ± 0.87 nmol/ml (shown in table 1), respectively, with a p value of <0.05 which are higher when compared with controls.

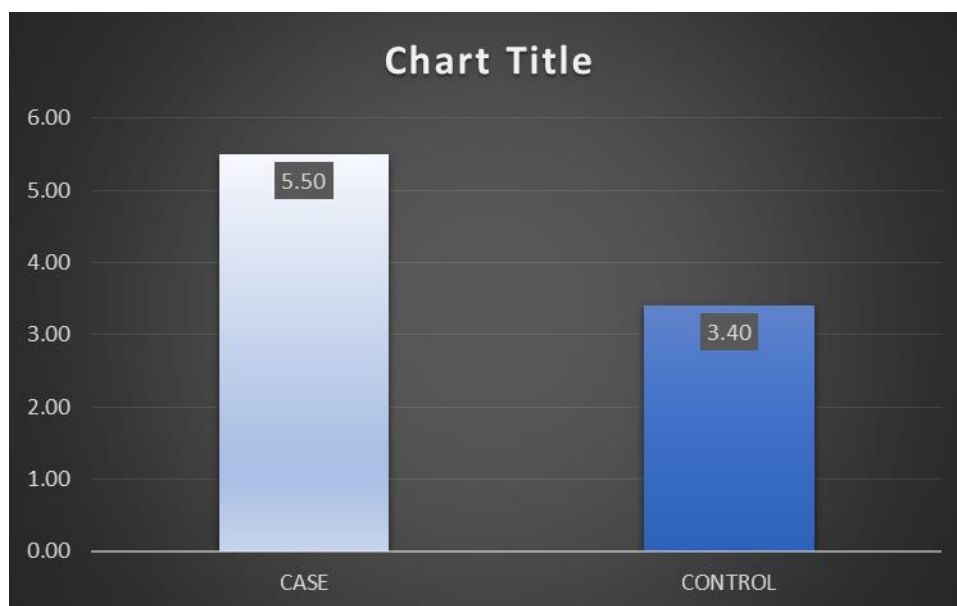


Figure 1: Graphical representation of serum MDA in the cases and controls.

Figure 2 shown that Glutathione levels were decreased in study group (4.5 ± 1.5 Mm) than controls (11.9 ± 2.7 Mm) having p value <0.05 (table 1).

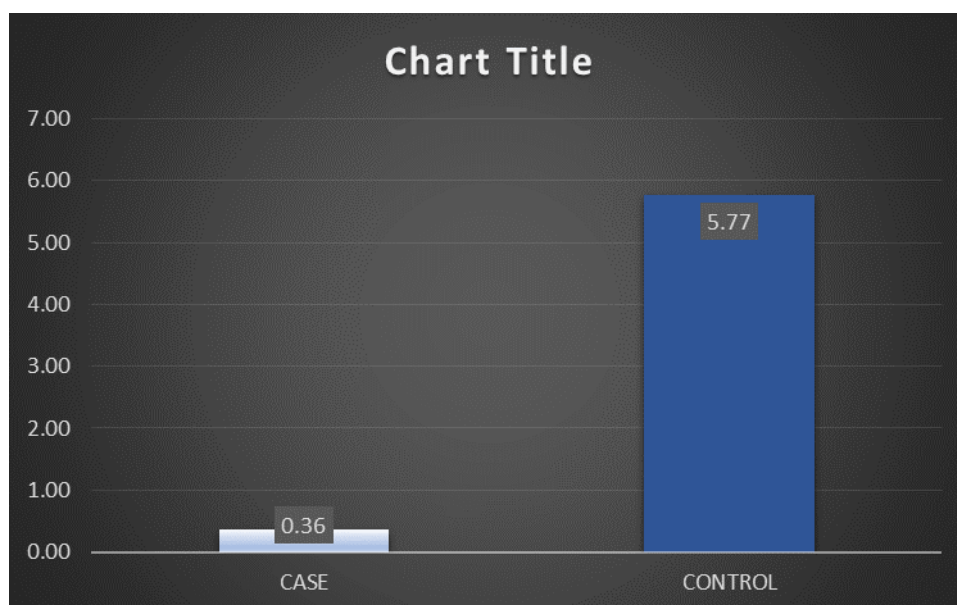


Figure 2: Graphical representation of serum Glutathione in the cases and controls.

Discussion

Free radicals are crucial for controlling the majority of cellular processes, but they can also be a sign of oxidative stress in large quantities, especially in the form of active oxygen radicals. The antioxidative system protects the organism from oxidative stress damage. Numerous malignancies, including cervical cancer, have been the subject of extensive research on oxidative stress changes. However, there is a dearth of information that demonstrates the significance of oxidative stress in terms of prognostic and predictive characteristics.(15) Malondialdehyde is a

highly cytotoxic byproduct of lipid peroxidation and a tumour promoter. According to our study's mean and standard deviation, the level of MDA in cervical cancer patients has dramatically increased in cases when compared with controls (5.5 ± 1.48 nmol/ml and 3.4 ± 0.87 nmol/ml). Moreover, previous studies MDA levels in the blood of various cancer patients were shown by Hristozov et al., D. Skrzydlewska et al., Kumaraguruparan R. et al., to be significantly greater than those of healthy controls (16, 17, 18). Likely higher levels of lipid peroxidation were found in the serum of all four groups at the FIGO stages than in the healthy control group, according to the findings of this investigation. Patients with cervical intraepithelial neoplasia (CIN) showed a similar trend in plasma MDA (19). Therefore, even in the early stages of carcinogenesis, oxidative stress is thought to be a major influence. Even in the initial stages of carcinogenesis, we believe that oxidative damage is a significant influence. Since cancer cells themselves produce oxidants and deplete antioxidants, creating a vicious cycle, this stress multiplies in malignant cells. Due to the increased tumour burden in advanced stages, this becomes more obvious. This study thus supports the theory that an imbalance in oxidant-antioxidant levels causes lipid oxidative damage, which serves as a molecular basis for the onset and development of cancer. By scavenging reactive oxygen species, glutathione functions as a secondary antioxidant and defends the cell against a variety of cytotoxic and cancer-causing substances. To restore an adequate amount of antioxidants and to activate the scavenger enzymes required to combat free radical damage, a high GSH level is required (20). In our study the level of glutathione was significantly decreased when compared with healthy controls having mean and standard deviation in cases (4.5 ± 1.5 Mm) and controls (11.9 ± 2.7 Mm). A drop in serum GSH may be a predictor of how well radiation therapy would work for cervical cancer patients, according to research by Vidyasagar et al. (21). The research demonstrates that, in contrast to patients who obtain no response at all, the GSH concentration dramatically drops after chemoradiation, especially for patients who achieve CR. Our investigation showed the same outcomes.

Conclusion

When compared to healthy controls, our findings indicated an imbalance between the oxidant-antioxidant status of patients in groups of cases. Even if the involvement of these factors in oxidative stress changes, this imbalance is crucial to the pathogenesis and development of cervical cancer. In conclusion, decreased antioxidative enzyme activity and enhanced lipid peroxidation are signs of oxidative stress in cervical cancer, which is more obvious in advanced stages because of the increased tumour burden. Antioxidant supplements may slow the disease's progression in cervical cancer sufferers.

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