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Research Paper

Malondialdehyde Levels in Blood Serum of Wistar Rats After Exposure to Panoramic and Cephalometric Radiation (Experimental Laboratory Research)

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Abstract

Background: Panoramic and cephalometric radiographs are used in dentistry, especially orthodontics, sometimes accompanied by periapical examination. Behind the benefits of using radiography, there are disadvantages for the body, which cause changes in the normal cell arrangement, detected by increased levels of Malondialdehyde (MDA) in blood serum. **Purpose**: This study was to determine differences in blood serum MDA levels of wistar rats a few days after exposure to panoramic and cephalometric radiation. **Materials and Methods:** This study used 35 male Wistar strain Rattus norvegicus, weighing 200-250 grams, aged 3-4 months, in good physical health. Animals are divided into five groups. Group K did not do fixation and was not given radiation exposure; groups K1, K2, K3, and K4 underwent fixation were given radiation exposure, MDA levels were measured, groups K1, K2, K3, K4, day 1, 3, 5 and 7 after radiation exposure. MDA levels were measured by the thiobarbituric acid method using a spectrophotometer with a wavelength of 532nm. **Results**: There was a significant increase MDA levels in K1 (45.2704 + 2.08684). Significant decrease of MDA levels occurred in K2 (34.7747 + 7.90103), K3 (18.1266 + 5.33797), and K4 (12.0494 + 1.91399). **Conclusion**: There was an increase in MDA levels after radiation exposure, and MDA decreased significantly from the third to the seventh day after radiation exposure. However, MDA levels have not returned to normal.

Keywords *Panoramic Radiograph; Cephalometri Radiograph; Malondialdehyde; Blood Serum*

INTRODUCTION

Radioactive material and ionizing radiation are crucial in medical diagnostics and therapy, but high-dose radiation exposure can cause damage to biological tissue at atomic and molecular levels (Raidha et al., 2018). Even low-dose exposure from medical imaging can contribute to up to 2% of cancer worldwide. This issue affects not only medical professionals and patients but also the environment and global people's welfare. Modern medical imaging facilitates diagnosing and treating human diseases, but significant side effects emerge with negligence. Radiation exposure quietly harms the health and welfare of people globally. The increase in natural radiation of the earth has been largely ascribed to medical imaging, including X-ray (Luan et al., 2021).

Radiation must prioritize safety, and the losses incurred must be less than the benefits generated. Ionizing radiation exposure can have both somatic and stochastic impacts on the body, as well as stochastic consequences on the genome. Gene mutations, the replication of altered cells (such as in leukemia, thyroid cancer, and salivary gland tumours), and congenital anomalies are examples of stochastic effects that might occur. Genetic impacts are less common than somatic effects, although they can nonetheless happen. On the other hand, non-stochastic consequences that may occur include xerostomia, osteoradionecrosis, cataracts, cell death, and developmental abnormalities in the developing foetus (Mallaya & Lam, 2018). Radiation from panoramic and cephalometric radiographs falls into the category of ionizing radiation, which should be reevaluated due to its potential to cause harm to normal cells as well as gene and cell mutations

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(Berawi & Agverianti, 2017).

The principal risk of dentomaxillofacial radiography is the unlikely chance of radiation-induced cancer. Cancer is a common disease, affecting approximately 40% of people at some time during their lives and accounting for approximately 20% of all deaths. This hypothesis considers no threshold or "safe dose" below which there is no added cancer risk (White & Pharoah, 2018). Damage from exposure to ionizing radiation is the impact of oxidative stress conditions. Oxidative stress can occur when free radicals and antioxidants are in an unbalanced condition where the number of free radicals is greater than the number of antioxidants (Berawi & Agverianti, 2017).

Stress oxidative is a physiological process that occurs when free radicals and antioxidants are not closely related (Yuslianti, 2018). Oxidative stress can cause necrosis, which is biochemical damage to tissues.

Additionally, oxidative stress can impair biological processes, including ion homeostasis, enzyme activity, membrane integration, and cell function, causing cell damage or death. Numerous plasma proteins, including albumin, globulin, and blood clotting factors, are present in the plasma. Colloid osmotic pressure, or oncotic pressure, is preserved by albumin. Immunoglobulin, a protein that is essential for the body's defence against microbes and other invaders, is formed from the protein globin. Blood clotting is greatly aided by coagulation factors found in blood plasma. Blood plasma contributes to the body's buffer system, crucial for maintaining acid-base balance. In addition to playing an important role in the blood clotting process, blood plasma coagulation factors also function in the body's buffer system, also known as the buffer system, which functions to maintain acid-base conditions in the body (Khonsary, 2017).

Serum is a component of blood that resembles plasma in composition but is devoid of clotting proteins, such as fibrinogen and other blood clotting factors. Inorganic ions, minerals, hormones, vitamins, enzymes, intermediates, and metabolic end products are all found in blood serum. These elements can form potent complexes with many components found in blood plasma. Due to its easy, mostly non-invasive, and economical collection process, blood serum is frequently utilized as a clinical specimen (Byrne et al., 2020). Along with carbs, lipids, amino acids, and over 300 different forms of protein, it also includes up to 114,000 identified metabolites in a range of quantities. In addition to providing a wealth of indicators for the diagnosis of diseases, abnormalities in endogenous (Byrne et al., 2020; Situmorang & Zulham, 2020)

MDA is the end product of the lipid peroxidation process. MDA can be used as an indicator of injury to cell membranes (Harliansyah & Rozaan, 2019). MDA is found in circulation, which is a toxic compound for cells. MDA can be found in plasma, serum, and urine. An increase in antioxidant levels usually accompanies a decrease in MDA levels. In order to quantify the lipid peroxidation of plasma proteins, which represent systemic oxidative stress, malondialdehyde (MDA) levels were ascertained (de Wolde et al., 2022). Based on the above background, it is necessary to study the levels of malondialdehyde in the blood serum of Wistar rats a few days after radiation exposure combined with panoramic radiography and lateral cephalometric radiography, namely on days 1, 3, 5 and 7.

RESEARCH METHOD

The research sample consists of a control group and an experimental group drawn randomly from the population, as is typical in experimental laboratory research. The study was classified as actual experimental laboratory research, controlling all variables not included in the sample and drawing both groups at random from the population 2020, according to Ahyar et al. The experimental unit is the part of the investigation that will be treated and observed. The experimental unit in this study was a male Wistar strain Rattus novergicus 3-4 months old, weighed between 200 and 250 grams, and was in good health. It was characterized by clear eyes, swift movements, lustrous coats, and soft or hard stools. According to the replication size formula and the formula for the chance of experimental animals dying by 10%, the minimum size for each group was 7 Rattus novergicus strain Wistar. So, for a study with five groups, a total of 35 Rattus novergicus Wistar strains are required.

While receiving X-ray radiation, mice were fastened using a cone-shaped wire designed specifically for rats. Paper pads were used to fixate the rats so they would not move during the X-ray radiation procedure, and tape was used to fixate the wire cones. The periapical dental X-ray cone is covered with plastic and bound with rubber to prevent direct contact with the test animals. where the cone is located in relation to the study item. The experimental animal is 20 cm away from the radiation beam4. Giving group 1, group 2, group 3, and group 4 each 0.002 mSv of periapical radiation exposure nine times each. So that the total radiation dose received by the experimental animals was 0.018 mSv (equivalent to the accumulated effective dose once exposed to panoramic radiography of 0.016 mSv and lateral cephalometric radiography of 0.0022 mSv).

The study examines radiation combination with panoramic radiography and lateral cephalometrics, focusing on the serum MDA level of Rattus novergicus strain. Control variables include upkeep of experimental animals, research room atmosphere, experimental animal fixation, and radiation exposure. The study aims to understand the effects of these treatments on the strain. The radiation beam was 20 cm away from the experimental animal. Periapical radiation exposure with a dose of 0.002 mSv was given nine times in groups 1 (K1), 2 (K2), 3 (K3), and 4 (K4), resulting in a total radiation dose of 0.018 mSv. This equals the accumulated effective dose of 0.016 mSv and 0.0022 mSv when exposed to panoramic and lateral cephalometric radiography. Group 1 (K1), group 2 (K2), group 3 (K3), group 4 (K4).

The procedure for measuring serum MDA begins with the experimental animals anaesthetized with ketamine and xylazine, taking blood through the heart. Blood samples were taken from the heart with the help of a 2 ml syringe in a tube without anticoagulant and centrifuged at 3000 rpm for 10 minutes, then stored at -20 °C for analysis (Poormoosavi et al., 2018). Determination of serum MDA levels using the Thiobarbituric Acid Reactive Substance (TBARS) method. The assay was started by adding 100 L of serum with 1 ml of 0.9% NaCl, followed by centrifugation at 8,000 rpm for 20 minutes. Then 550L of distilled water and 100L of TBA were added. After homogenization with vortex, 250 L of 1N HCl was added and vortexed again. Then 100 L Na-Thio was added and homogenized by centrifugation at 500 rpm for 15 minutes. The supernatant formed was transferred to a new microtube. Then heated in a water bath at 100 degrees C for 30 minutes. Absorbance was measured with a UV-1601 spectrophotometer at a wavelength of 535 nm (Vera et al., 2018).

FINDINGS AND DISCUSSION

The research results were analyzed descriptively to gain an overview of distribution and improvement. A 95% significance level (p=0.05) hypothesis test was conducted using analytical statistics to clarify the presentation. The study found an increase in MDA levels in group 1 (K1) against the control group (K) after radiation exposure, while MDA levels in groups 2 (K2), 3 (K3), and 4 (K4) decreased closer to the control group (K-). Data were analyzed using SPSS software, with normality tests using Shapiro Wilk and homogeneity tests using the Levenne Test. The results showed a p-value <0.05, indicating normal distribution and non-homogeneity. Non-parametric tests, such as Kruskal-Wallis and hypothesis testing using Mann-Whitney, were conducted to determine the data's homogeneity. Followed by the Mann-Whitney test to determine the difference between the two groups. Obtained a sig value <0.05, meaning that there is a significant difference between the 6 groups (K-, K+, K1, K2, K3, K4) followed by the Mann-Whitney test to determine the difference between each of the two groups.

This study investigated the difference in malondialdehyde levels in Wistar blood serum after exposure to panoramic radiation and lateral cephalometrics. Male white Wistar rats (Rattus Norvegicus strain Wistar) were used due to their physiological and anatomical similarities to humans. There was a significant rise in MDA levels (p0.05) in the mice euthanized on days 1, 3, 5, and 7 following radiation exposure to a combination of panoramic radiography and lateral cephalometric radiography. A combination of cephalometric and panoramic radiography was performed on the control group on the first day following radiation exposure. The rise in MDA levels on day one following radiation exposure in conjunction with panoramic radiography and lateral cephalometric radiography is consistent with earlier research by Zhang et al. (2023), which found that the proportion of cells undergoing apoptosis rose after radiation exposure. The study aims to investigate malondialdehyde levels in Wistar rats' blood serum after exposure to ionizing radiation from panoramic and lateral cephalometric radiography on days 1, 3, 5, and 7. There was a significant increase in MDA levels in K_1 (45.2704 + 2.08684). Significant decrease of MDA levels occurred in K_2 (34.7747 + 7.90103), K_3 (18.1266 + 5.33797), and K_4 (12.0494 + 1.91399).

Antioxidants play a part in preventing the body from creating oxidation processes by free radicals that might result in oxidative stress, which is the mechanism for reducing MDA levels. Enzymatic antioxidants are those that the body spontaneously produces. Salivary peroxidase (Px), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) are a few of the enzymatic antioxidants generated. Superoxide (O2•-), hydrogen peroxide (H2O2), hydroxyl radicals (•OH), and peroxide levels are often reduced by these enzymatic antioxidants24,25,31. Low quantities of enzymatic antioxidants found in cells can dramatically slow down the oxidation of oxidized substrates19. Antioxidants that are enzyme-based function by halting a series of processes. Free radicals will interact with enzymatic antioxidants and be transformed into more stable molecules. Enzymatic antioxidants will react with free radicals and convert them into more stable products so that the formation of reactive oxygen compounds cannot occur. The interaction between antioxidants and free radicals can change reactive compounds to become less reactive. No formation of reactive oxygen compounds will inhibit the process of lipid peroxidase. So that the production of MDA, which acts as the end product of the lipid peroxidase process, will decrease (Khonsary, 2017).

Ionizing radiation is an ionization process that interacts with matter, releasing electrons from the atomic nucleus and forming negative ions. Free radical damage is generated by ions and free radicals, which can damage biological macromolecules. In aerobic life, aerobic organisms produce free radicals due to biochemical reactions. Oxidative stress can cause necrosis, disrupting biological functions such as ion homeostasis, enzyme activity, membrane integration, and cell function. Oxidative stress can also lead to mitochondrial loss, cell energy deficiency, and accumulation of damaged cytotoxic mediators. Malondialdehyde (MDA) is a biomarker of oxidative stress, increasing with increasing stress and being stable in isolated bodies. It is not affected by dietary fat and is a specific product of lipid peroxidation.

CONCLUSIONS

There was an increase in MDA levels of blood serum of Wistar rats after radiation exposure to a combination of panoramic radiography and lateral cephalometric radiography on day one compared to the control group. There was a decrease in MDA levels of the parotid glands of Wistar rats after radiation exposure to a combination of panoramic radiography and lateral cephalometric radiography on day 3, day 5, and day 7 compared to day 1. However, the MDA levels have not returned to normal MDA levels, still higher than the control group.

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