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Original Research Paper

Histopathological Comparison of Nasopharyngeal and Lung Tissue on Wistar Rats Induced with Formaldehyde

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Abstract: Formaldehyde is a toxic substance commonly found in everyday life. Formaldehyde is also a carcinogenic exposure that often occurs in industrial areas. Formaldehyde itself is genotoxic and cytotoxic to tissues that can cause dysplasia to carcinoma. Inhalation of formaldehyde will expose the respiratory tract, which includes the nasopharynx and lung tissue to formaldehyde. Therefore, it is important to know the differences in tissue susceptibility to formaldehyde exposure related to the ability to cause dysplasia and carcinoma. Six Wistar rats were induced with formaldehyde through the inhalation method at a dose of 40 ppm for 16 weeks, and then terminated and subjected to histopathological examination. The degree of dysplasia will be assessed in both tissues, and then compared and tested statistically. The Mann-Whitney U statistical test showed a P value of 0.818, meaning that there was no significant difference between the degree of dysplasia in nasopharyngeal tissue and lung tissue. However, there was a difference in the rate of dysplasia progression between the two tissues, with nasopharyngeal tissue having a faster rate of progression. This difference in the rate of progression is related to the higher amount of formaldehyde deposition in the upper airway.

Keywords: Dysplasia, formaldehyde, lung, nasopharynx, wistar rats.

Introduction

Formaldehyde is a toxic chemical substance that can universally be found in the environment. This substance can also be found in man-made products such as synthetic fibers, furniture, carpet, and other household products (Bernardini et al., 2020). Formaldehyde is also used in tanatopraction treatments, where it is a modern technique used for the preservation of human cadavers from decomposition or damage caused by pathogenic microorganisms (Varlet et al., 2019). Inhalation of formaldehyde has a definitive effect on cardiovascular organs, it also increases lipid product peroxidation, and decreases liver antioxidant enzyme activities (Zhang et al., 2018). The progression from dysplasia to carcinoma in situ and subsequently from carcinoma in situ to invasive carcinoma can result from sustained exposure to carcinogenic factors, causing genetic and epigenetic alterations. The prevailing approach to evaluating lesions with a potential for malignancy in the head and neck involves conducting histopathologic examinations of dysplasia and carcinoma (Ranganath et al., 2022).

Chromosome and DNA damage may occur due to the genotoxic and cytotoxic properties of formaldehyde. Cancer risk may increase due to increased genomic instability from this genotoxic chemical (Kang et al., 2021). Inhalation of high levels of formaldehyde, around 15 ppm, has been associated with the appearance of nasal cancer

in rats and mice (Njoya et al., 2009). An increased risk of tissue-invasive cancer can be characterized by the presence of tissue dysplasia. Loss of uniformity between individual cells in their architectural orientation is one of the common signs of tissue dysplasia (Kumar, Abbas and Aster, 2018).

The end result of this abnormal development is carcinoma. Compared to other types of cancer, nasopharyngeal carcinoma is generally rare. However, there is a unique geographical distribution, with 70% of reported cases originating from East and Southeast Asia (Guo et al., 2019). This means that the probability of nasopharyngeal cancer cases in Indonesia, which is part of Southeast Asia, is also higher. Formaldehyde itself has been classified as a definitive carcinogenic substance in humans for nasopharyngeal cancer cases since 2004. Formaldehyde-related cancers have been included in occupational disease registries in countries and regions such as France, Denmark, Taiwan and Malaysia. A causal relationship between formaldehyde exposure and nasopharyngeal cancer has also been identified (Kwon et al., 2018). However, concentration and exposure duration contribute to the severity of its effect (Yahyaei et al., 2020).

Formaldehyde exposure also happens on anatomical pathology laboratories. Excessive death rates, with higher rates of brain tumor, malignancies, are analyzed to be caused by prolonged exposure to formaldehyde (Dugheri et al., 2021). Occupational exposures to carcinogenic substances are also accounted for 5-10% of all lung cancer causes (Schabath and Cote, 2020). For nasopharyngeal cancer, individual exposed to occupational carcinogens in workplace has increased risk compared to unexposed individuals. This risk increases linearly with duration (Chen et al., 2021). With all of these links between carcinogenic substance inhalation and malignancy, there is a large number of factory workers who are unaware of matters related to respiratory health leads to a high possibility of inhalation of carcinogenic substances (Wedayani, et al., 2023).

This raises the importance of studying mechanism of malignancy on respiratory tissues, that would help in the detection and management efforts of these cancers. It is important to detect nasopharyngeal cancer early, including the possibility of progression from dysplasia, because in addition to the morbidity of the disease, nasopharyngeal cancer therapy also brings side effects such as an increased incidence of anemia, increased blood urea nitrogen (BUN) (Kadriyan et al., 2022). Susceptibility to oncogenic events that lead to tumor formation is highly tissue-specific. There are different mechanisms of tumorigenesis that are tissue-specific and cell-specific (Schneider et al., 2018). Therefore, a study that compares histopathological characteristics between different tissues and cells is needed. Currently, there has been no study that compares the histopathological differences between nasopharyngeal and lung tissue after a prolonged exposure to formaldehyde by inhalation.

This study is conducted to investigate and to compare the degree of dysplasia on nasopharyngeal and lung tissue of Wistar rats after being induced with 40 ppm formaldehyde. It has been studied that formaldehyde affects both the upper and lower respiratory system (Kang et al., 2022). It was also shown that formaldehyde exposure had respiratory effects on mice (Li et al., 2017). The inducement of Wistar rats has also been proven to cause dysplasia (Susilawati et al., 2022). Continuous exposure to carcinogenic factors can cause genetic and epigenetic alterations that lead to progression from dysplasia to carcinoma in situ, and then from carcinoma in situ to invasive carcinoma. This study will help provide comparative data that can then be developed for more effective cancer treatment and provide data on the differences of dysplasia progression on different airway tissues in a prolonged formaldehyde inhalation setting.

Methods

This study is a post-test only experimental study. Six formaldehyde-induced Wistar rats were included in this study. Each rat was induced with 40 ppm formaldehyde with 10% solution concentration, and being fed and given distilled water for 16 weeks. The rat was kept in a cage that is specially designed to induce dysplasia

effectively and economically (Wedayani et al., 2023), with ventilation on two sides of the cage. This method is designed based on previous research, where similar design study with 16 weeks of induction period has successfully produced dysplasia in Wistar rats (Susilawati et al., 2022). On week 4, 8, 12, one rat is sacrificed to get the nasopharyngeal and lung tissue extracted. On week 16, 3 rats will be sacrificed. The ethics of this study has been reviewed, and clearance was obtained from Committee of Ethics, Faculty of Medicine, University of Mataram (066/UN17.F7/ETIK/2023).

Histological examination was done in the Pathological Anatomy Laboratory, University of Mataram Hospital. The sample were put in a tube with formalin solution for preservation. The sample then were fixated, dehydrated, cut, rehydrated, and colored with Hematoxylin-Eosin staining. The tissue slides then were examined to determine the degree of dysplasia. Obtained histopathological data then analyzed statistically with Statistical Package for the Social Sciences (SPSS) program, run in Microsoft Windows 11. As all of the data that will be compared are ordinal in nature, the data analysis method used to determine the difference of dysplasia degree between nasopharyngeal and lung tissue was the Mann-Whitney U test.

Results

Histopathological Result of the Nasopharyngeal and Lung Tissue

Tissue samples were stained with Hematoxylin-Eosin staining and viewed under the microscope. These are the samples of nasopharyngeal tissues.

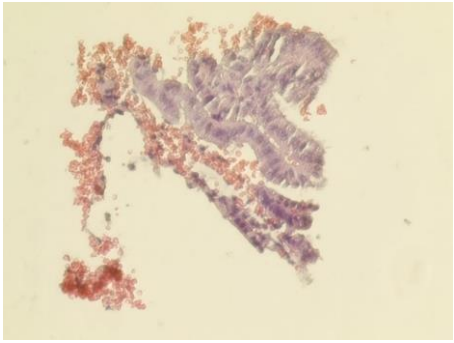

Image 1. Nasopharyngeal Tissue: Glands on Nasopharynx with Mild Dysplasia after 40 ppm Formaldehyde Induction on Week 4 (HE 40x)

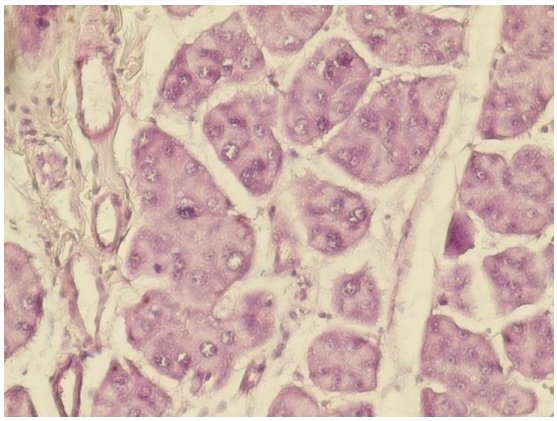

Image 2. Nasopharyngeal Tissue: Glands on Nasopharynx with Severe Dysplasia after 40 ppm Formaldehyde Induction on Week-16 (HE 100x)

Table 1. Nasopharyngeal Tissue Degree of Dysplasia

Group	Termination Time	Degree
Nasopharynx	Week 4	Mild
Nasopharynx	Week 8	Moderate
Nasopharynx	Week 12	Severe
Nasopharynx	Week 16	Severe
Nasopharynx	Week 16	Severe
Nasopharynx	Week 16	Mild

The histopathological analysis of the lung tissues showed that at week 4, 40 ppm formaldehyde-induced Wistar rats had normal histopathological examination results. At week 8, 40 ppm formaldehyde-induced rats were observed to have moderate dysplasia. At weeks 12 and 16, rats induced with 40 ppm formaldehyde were observed to have severe dysplasia.

Table 2. Lung Tissue Degree of Dysplasia

Group	Termination Time	Degree
Lung	Week 4	Normal
Lung	Week 8	Moderate
Lung	Week 12	Severe
Lung	Week 16	Severe
Lung	Week 16	Severe
Lung	Week 16	Mild

Analysis of the Histopathological Difference

The data in this study are ordinal, categorized as mild, moderate, and severe, so there is no need to test parametricity and can be directly tested non-parametrically with the

Mann-Whitney-U statistical test.

Table 3. Mann-Whitney U Statistical Test

Tissue	N	Mean Rank	Sum of Ranks
Nasopharynx	6	6.83	41.00
Lung	6	6.17	37.00
Total	12		
Exact Significance			0.818

The results of statistical analysis with the Mann-Whitney U Test showed that the comparison of the degree of dysplasia between nasopharyngeal tissue and lung tissue was not significant with a p value for two-tailed hypothesis of 0.937, where a p value is declared significant when it is smaller than 0.05. From the results of this significance test, it can be interpreted that in Wistar rats induced with 40 ppm for 16 weeks, there is no difference in the severity of dysplasia in lung tissue and nasopharyngeal tissue

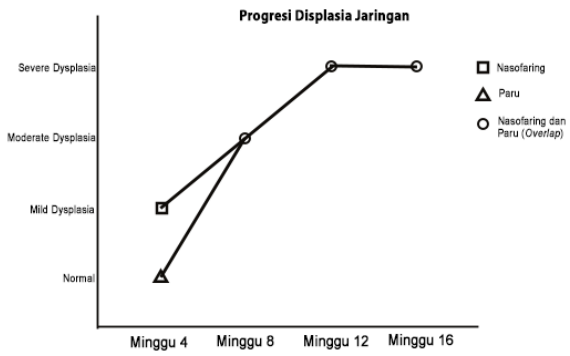

Image 4. Dysplasia Progression Graph

When viewed progression-wise, it can be seen that there are differences in the onset time of dysplasia in each tissue. Nasopharyngeal tissue has a faster onset of dysplasia, with findings of mild dysplasia in nasopharyngeal tissue from Wistar rats terminated in the fourth week. As for lung tissue from Wistar rats terminated in the fourth week, it was found that there was no dysplasia in the tissue yet. Both of tissue starts developing moderate dysplasia on week 8, and severe dysplasia on week 12.

Discussion

Histopathological Result of The Nasopharyngeal and Lung Tissues

Formaldehyde deposition on cells will cause metabolic and genomic interactions between formaldehyde and cells. This will induce degeneration and necrosis of respiratory epithelial cells, which has been confirmed histopathologically in rats, mice and monkeys exposed to formaldehyde through inhalation. After changes such as cell proliferation and squamous metaplasia, hyperplasia and dysplasia occur at the site of squamous metaplasia. Hyperplasia and dysplasia occurred at the site of squamous metaplasia, suggesting that these changes occur during progression from squamous cell metaplasia to squamous cell carcinoma (Nishikawa et al., 2021).

Formaldehyde inhalation for 16 weeks accumulated the deposition of formaldehyde in the nasopharyngeal and lung tissues of the Wistar rats. Combined with the aforementioned mechanism, this causes the cells to undergo cellular adaptations, combined with genomic and cytotoxic effects of formaldehyde, that resulted in dysplasia. This manifested in architectural changes such as loss of tissue polarity and elevated density. Cellular changes were also observed in the tissue samples of this study, that showed signs such as polymorphism, anisonucleosis, and hyperchromatic nucleus.

Analysis of the Histopathological Difference

The results of statistical testing as previously described showed that there is no significant difference between the degree of dysplasia in nasopharyngeal tissue and lung tissue in Wistar rats induced with formaldehyde by inhalation for 16 weeks. This means both tissue has the same susceptibility of developing dysplasia. This study yield different result when compared to theory based on the study done by Overton et al., (2001) which found that the respiratory tract as a whole would retain more than 95% of inhaled formaldehyde and the mass flow rate across a unit area of the respiratory tract (flux) in some tracheobronchial models would be more than 1,000 times higher than in the lungs, with no flux to the alveolar region (Overton, Kimbell and Miller, 2001). This study has substantial differences, where this study used a dose of formaldehyde of 40 ppm for 16 weeks which was directly tested in vivo on Wistar rats. (2001) used a dosimetric modeling method based on four levels of human activity.

When the results of this data are viewed progressively as illustrated in Image 4, it can be seen

that in the fourth week, there are still differences in the effects on the two tissues, where the nasopharyngeal tissue has been observed to have mild degree of dysplasia, while the lung tissue which is the lower airway, is still observed to be normal. This result is in line with the theory discussed in the final report of the carcinogen background document for formaldehyde, which states that the absorption of formaldehyde by the upper respiratory tract is close to 100% regardless of its concentration (National Toxicology Program, 2010). This means that by the fourth week, with the lowest accumulative dose, formaldehyde can already be fully absorbed in the upper airway mucosa, resulting in dysplasia, while in the lower airway, no tissue dysplasia has been found. Dysplasia, secondary to the uptake of formaldehyde in the tissues, could only be detected at week eight in the lung tissue, which means that the uptake of formaldehyde and the induction of dysplasia in the lung tissue is more dose-dependent (in this case, accumulative dose) than in the nasopharynx which is the upper airway.

The more effective absorption of formaldehyde in the upper airway, in this case nasopharyngeal tissue, is due to the highly water-soluble nature of formaldehyde, so it can be assumed that inhaled formaldehyde is easily dissolved in the mucus covering the mucosal epithelial surface (Nishikawa et al., 2021). In the most distal bronchioles, epithelial cells are cuboidal and do not produce mucin, and bronchiolar patency is stabilized by surfactant from adjacent alveoli (Fahy and Dickey, 2010). This leads to the conclusion that there would be more formaldehyde deposited in the nasopharynx than in the lung.

As formaldehyde deposition causes degeneration that leads to abnormal cell proliferation, the more substances that are deposited, the more interactions with cells that cause necrosis, and lead to metaplasia to dysplasia. The doses used in this study reflect high exposure scenarios, such as chemical accidents and chronic occupational exposure to formaldehyde. In contrast to exposure in daily life, occupational exposure occurs over a wider range of concentrations, as much as 20.94 mg/m3 in the anatomy laboratory and 60.77 ppm in the industrial plant in the extreme case (Kang et al., 2022). Other factors such as differences in the length of time of progression from normal to mild, and mild to severe may cause both tissues to still be in the mild phase at week eight, even though dysplasia has only occurred in the lung tissue for a longer time, as well as in other degrees. This reasoning is based on the theory that the likelihood or risk of progression of dysplasia increases as the degree of dysplasia increases (Datta et al., 2021).

The strength of this study comes from the design of this study, which is done with an experimental design, compared to older studies done on this topic. Long period between terminations caused the recorded progression to be less detailed. The subject is only induced with one dose of formaldehyde, which is 40 ppm, in which higher doses may yield a more significant result and can be applied in situations of greater exposure to formaldehyde. These weaknesses of this study should be taken note of and be improved on future studies regarding this topic.

Conclusion

This study shows that there is no significant difference between the degree of dysplasia in nasopharyngeal tissue and lung tissue. There is a difference in progression time in both tissues, where lung tissue takes longer to develop dysplasia than nasopharyngeal tissue. Both tissues have different progression times, due to differences in the susceptibility of formaldehyde deposition which is higher in the upper airway than the lower airway. In the case of prolonged exposure to high doses, both tissues have the same susceptibility to develop severe dysplasia.

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