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EGF Levels in *Rattus norvegicus* Induced with 40 ppm Formaldehyde for 1 Month in Relation to Nasopharyngeal Cancer

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Abstract: Nasopharyngeal carcinoma (NPC) is one of the most common malignancies in the ENT department, with a high incidence in Southeast Asia. One of the major risk factors for NPC is formaldehyde exposure, which induces oxidative stress, chronic inflammation, and disruption of growth factors such as Epidermal Growth Factor (EGF). This study aims to analyze changes in EGF levels in *Rattus norvegicus* exposed to 40 ppm formaldehyde for one month to better understand the molecular mechanisms contributing to nasopharyngeal carcinogenesis. This experimental study measured EGF levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Data were analyzed using the Kolmogorov-Smirnov normality test and a *Paired T-Test* to assess changes in EGF levels before and after treatment. The analysis showed a tendency for increased EGF levels after formaldehyde exposure; however, the difference was not statistically significant ($p = 0.355$). This suggests that one-month exposure to formaldehyde does not directly affect EGF expression in the tested tissue. This study found that 40 ppm formaldehyde exposure for one month did not significantly alter EGF levels. However, the observed trend in certain groups suggests that the effect of formaldehyde on EGF levels may be dose- and duration-dependent. Further studies with longer exposure durations and higher concentrations are needed to explore the link between formaldehyde exposure and nasopharyngeal carcinogenesis.

Keywords: Nasopharyngeal carcinoma; formaldehyde; Epidermal Growth Factor (EGF); *Rattus norvegicus*; toxicity

Introduction

Nasopharyngeal cancer (NPC) is one of the most common malignant tumors in the ENT department. The incidence rate of NPC is less than 1 per 100,000 people per year in most regions of the world, but the number is higher than 20 per 100,000 people per year in Southeast Asia and Southern China in recent decades. In Indonesia, the average prevalence is 6.2/100,000, with 13,000 new NPC cases annually, but apart from that, little has been documented about NPC in Indonesia (Adham *et al.*, 2012; Setiasari *et al.*, 2020; Kadriyan *et al.*, 2022). NPC is generally diagnosed at an advanced stage and has a poor prognosis due to its hidden anatomical location and nonspecific early-stage symptoms. The prognosis for about 30% of NPC patients is poor, mostly as a result of distant metastases. Patients with synchronous metastases rarely have an overall survival (OS) longer than 30 months. The overall survival (OS) for individuals with metachronous metastases is around 20 months from the date of metastatic illness diagnosis (Toumi et al., 2022). In China, the net 5-year survival rate is estimated to be 47% (Zhang et al., 2023).

Nasopharyngeal cancer (NPC), previously known as lymphoepithelioma, is a carcinoma that develops in the nasopharyngeal epithelium. One of the main risk factors for NPC is exposure to carcinogenic chemicals such as formaldehyde. Long-term exposure to formaldehyde is known to cause oxidative stress, chronic inflammation, and changes in the expression of various growth factors, including EGF. Several studies have shown that dysregulation of epidermal growth factor (EGF) and the EGFR pathway plays a role

in the development and progression of NPC, making it important to understand changes in EGF levels due to formaldehyde exposure in order to comprehend the molecular mechanisms of nasopharyngeal carcinogenesis. One of the molecular factors involved in NPC pathogenesis is the EGF pathway and its receptor, epidermal growth factor receptor (EGFR) (Möhner and Wendt, 2016; Li *et al.*, 2021; Peng *et al.*, 2021).

EGF is a protein that functions as a crucial growth factor for various cell types, including epithelial and endothelial cells. EGF binds to its receptor, EGFR, which then activates intracellular signaling pathways that stimulate cell proliferation and inhibit apoptosis. The activation of this pathway contributes to tumor growth, angiogenesis, and metastasis. In NPC, excessive EGFR expression can lead to uncontrolled cell proliferation and inhibit normal regulatory mechanisms, thereby increasing tumor aggressiveness (Chen *et al.*, 2021; Pascarelli *et al.*, 2021; Qiu *et al.*, 2022).

This study aims to analyze EGF levels in *Rattus norvegicus* exposed to 40 PPM formaldehyde for one month and assess its potential role in the development of nasopharyngeal cancer. By understanding changes in EGF levels due to formaldehyde exposure, this study is expected to provide further insights into the toxicity mechanisms of formaldehyde in nasopharyngeal carcinogenesis. Additionally, this study examines the potential impact of formaldehyde on the regulation of growth factors. The findings of this study may also contribute to the development of prevention strategies and interventions against the harmful effects of formaldehyde exposure in occupational and everyday environments.

Method

This study employs an experimental design using animal models to evaluate the effects of 40 ppm formaldehyde exposure on Epidermal Growth Factor (EGF) levels. Data analysis is conducted using the Kolmogorov-Smirnov test to assess data distribution and the relationship between the examined variables. Furthermore, EGF levels obtained through the Enzyme-Linked Immunosorbent Assay (ELISA) method are compared between the formaldehyde-exposed group and the control group that was not exposed. This approach allows researchers to objectively measure the impact of formaldehyde exposure on EGF expression, providing further insight into the toxic effects of this compound.

Result and Discussion

Descriptive Test

Based on the initial analysis, the mean ELISA EGF value before treatment (pre) was lower compared to the value after treatment (post). This data can be seen in Table 1, which shows the mean pre ELISA EGF at 11.591 ± 0.700 and post ELISA EGF at 12.274 ± 3.667.

Table 1. Deskripsi Data Pre dan Post ELISA EGF

		Statistic	Std. Deviation
Nilai pre ELISA EGF	Mean	11.59100	.700214
Nilai post ELISA EGF	Mean	12.27429	3.667306

Normality Test

The normality test using the Kolmogorov-Smirnov test showed that the pre ELISA EGF data were normally distributed ($p = 0.736 > 0.05$). Similarly, the post ELISA EGF data were also normally distributed ($p = 0.077 > 0.05$). Therefore, the assumption of normality was met.

Table 2. Uji Normalitas Data Pre dan Post ELISA EGF

	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Nilai pre ELISA EGF	.685	30	0.736
Nilai post ELISA EGF	1.277	30	0.077

Difference Test

The results of the difference test using the Paired T-Test showed that there was no significant difference between the pre and post ELISA EGF values overall (p = 0.355 > 0.05). This indicates that, in general, there was no significant change in EGF levels after treatment.This increase indicates a change in EGF levels after formaldehyde exposure.

Table 3. Hasil Uji Paired T-Test

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Nilai post ELISA EGF - Nilai post ELISA EGF	-.683287	3.985563	.727661	-2.171521	.804947	-.939	29	.355

Discussion

This study aims to evaluate changes in EGF levels following formaldehyde administration for one month in *Rattus norvegicus*. EGF plays a crucial role in modulating cell proliferation, differentiation, migration, survival, and adhesion. Its overexpression or variance may contribute to tumor development (Qiu et al., 2022). Excessive EGFR expression is observed in approximately 80% of oral squamous cell carcinoma cases, promoting keratinocyte proliferation and differentiation. One of the known etiological factors for human cancer is excessive activation of the EGFR pathway, which contributes to treatment resistance, metastasis, and cancer growth. In oral cancer, excessive EGFR expression is associated with a malignant phenotype, suppression of apoptosis, and a higher risk of metastasis (Boccellino et al., 2023).

Effect of Formaldehyde Induction on EGF Levels

The analysis results indicate that EGF levels before and after treatment did not show a statistically significant change (p = 0.355). This suggests that formaldehyde administration for one month may not directly affect EGF expression in the tested tissue. However, there was a tendency for changes in certain treatment groups compared to the positive control, indicating that the effect of formaldehyde on EGF levels may depend on the dose and duration of exposure.

Formaldehyde FA is a volatile, colorless, flammable, and highly reactive aldehyde widely used in industrial production and consumer products. Formaldehyde is found in both indoor and outdoor environments, originating from building materials, furniture, paints, textiles, and combustion emissions from vehicles, factories, and incinerators. Additionally, Formaldehyde is present in cigarette smoke and is a natural byproduct in biological systems. Inhalation exposure to FA has been linked to eye and respiratory tract irritation, asthma, and impaired lung function. The International Agency for Research on Cancer (IARC) classifies formaldehyde as a human carcinogen (Group 1) due to its association with nasopharyngeal cancer, leukemia, and sinonasal cancer. Genotoxicity plays a key role in its carcinogenic effects, with studies showing that formaldehyde

can cause mutations, micronucleus formation, as well as DNA and protein adducts and cross-linking. These changes have the potential to serve as biomarkers of exposure and indicators of biological effects associated with cancer development (Leso et al., 2020; Protano et al., 2022). Formaldehyde exposure at varying concentrations had a significant impact on the extent of nasopharyngeal dysplasia. The higher the formaldehyde exposure concentration, the greater the degree of dysplasia (Agung Ayu Niti Wedayani et al., 2024).

EGF and its receptor, EGFR, play a crucial role in the pathogenesis of NPC. EGFR is a tyrosine kinase receptor that mediates various cellular processes such as proliferation, differentiation, angiogenesis, migration, and metastasis. In NPC, excessive EGFR expression has been associated with increased tumor aggressiveness, poorer prognosis, and resistance to conventional therapies such as chemotherapy and radiotherapy. EGFR activation in NPC occurs through ligand binding, such as EGF, which induces receptor autophosphorylation and activates various intracellular signaling pathways, including PI3K/AKT, MAPK/ERK, and JAK/STAT. These pathways support cancer cell survival by inhibiting apoptosis, enhancing angiogenesis, and promoting tumor migration and invasion. High EGFR expression in NPC tissues is associated with increased expression of pro-angiogenic factors such as VEGF, contributing to faster tumor growth and spread. Additionally, the link between EGFR and NPC is further reinforced by findings that EGFR mutations or amplification can lead to constitutive activation of signaling pathways, making cancer cells more resistant to radiation- and chemotherapy-based therapies. Consequently, therapies targeting EGFR, such as tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (e.g., cetuximab), are being developed as potential therapeutic strategies for NPC treatment. High EGF expression in the tumor microenvironment also contributes to NPC progression by enhancing angiogenesis and cancer cell motility. Increased EGF and EGFR expression in NPC is often associated with environmental and genetic factors, including exposure to carcinogens such as formaldehyde, Epstein-Barr Virus (EBV) infection, and genetic polymorphisms in EGFR that may influence an individual's response to therapy (Liang and Zhu, 2021; Peng *et al*., 2021).

Conclusion

This study found no statistically significant change in EGF levels following one month of 40 ppm formaldehyde exposure in *Rattus norvegicus*, suggesting that short-term formaldehyde exposure may not directly affect EGF expression. However, the tendency for changes in certain treatment groups indicates that the impact of formaldehyde on EGF levels might be dose- and duration-dependent. Given the crucial role of EGF and EGFR in NPC pathogenesis, further research with extended exposure duration and higher concentrations is necessary to elucidate the precise molecular mechanisms linking formaldehyde exposure to nasopharyngeal carcinogenesis. These findings contribute to the understanding of formaldehyde's toxic effects and highlight the need for preventive strategies to minimize exposure, particularly in occupational and environmental settings.

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