Short Communications

Editorial Process: Submission:04/22/2025 Acceptance:08/29/2025 Published:09/13/2025

The Proliferation of Osteosarcoma: The Impact of Phenol and Ethanol on *Ki-67* Expressions

Rhyan Darma Saputra[1], Mahardika Frityatama[2]*, Udi Heru Nefihancoro[1], Novan Adi Setyawan[3]

Abstract

Background: Osteosarcoma is an aggressive primary bone malignancy characterized by a high rate of cellular proliferation, necessitating effective local control strategies. Ethanol and phenol have been utilized as adjuvant agents to inhibit tumor proliferation. However, comparative studies evaluating their efficacy in reducing osteosarcoma proliferation remain limited. This study aims to compare the effects of ethanol and phenol on reducing the proliferation of osteosarcoma, particularly in delineating tumor resection margins microscopically. **Methods:** This experimental study was conducted at Dr. Moewardi General Hospital and the Anatomical Pathology Laboratory of the Faculty of Medicine, Sebelas Maret University, Surakarta from August to December 2024. Samples were obtained using purposive sampling. Osteosarcoma specimens were analyzed using immunohistochemistry to measure *Ki-67* expression and divided into three treatment groups: ethanol, phenol, and NaCl (control). Statistical analyses included the Kruskal-Wallis test and Dunn's post hoc test to evaluate differences in *Ki-67* expression among groups. **Results:** A total of 22 osteosarcoma specimens were analyzed. The results demonstrated that both ethanol and phenol were effective as local control agents in reducing osteosarcoma proliferation, as measured by *Ki-67* expression. Ethanol exhibited significantly greater efficacy compared to phenol and NaCl ($p<0.05$). The median *Ki-67* expression levels were 5 (1–20) in the ethanol group, 10 (2–30) in the phenol group, and 20 (10–60) in the NaCl group. **Conclusion:** Both ethanol and phenol are effective as local control agents. However, ethanol demonstrates superior efficacy in reducing *Ki-67* expression, establishing its potential as a more effective adjuvant in the management of osteosarcoma.

Keywords: Ethanol- Phenol- *Ki-67*- Osteosarcoma

Asian Pac J Cancer Prev, **26 (9)**, 3133-3136

Introduction

Osteosarcoma is the most prevalent primary bone malignancy, distinguished by the aberrant production of osteoid matrix by malignant osteoblasts [1]. Globally, the incidence of osteosarcoma is approximately 4–5 cases per million annually [2]. In Indonesia, it accounted for majority of bone cancers, comprising 70.59% of all cases, with 219 instances documented over 13 years at Cipto Mangunkusumo Hospital [3]. Local control remains a cornerstone of its multimodal management, with chemical adjuvants such as ethanol and phenol frequently employed to enhance tumor eradication. Ethanol, a hydrocarbon compound widely recognized for its ablative properties, and phenol, an aromatic sclerosing agent, both hold promise as effective modalities for minimizing residual disease and recurrence [4, 5].

A key element in evaluating therapeutic efficacy is *Ki-67*, a nuclear protein and well-established marker of cellular proliferation. As a reliable predictor of tumor aggressiveness, *Ki-67* offers critical insights into the biological behavior of malignancies and serves as an essential tool for assessing the impact of treatment strategies [6]. Despite their clinical utility, the comparative efficacy of ethanol and phenol as adjuvants in achieving local control, particularly through their influence on *Ki-67* expression, remains insufficiently explored.

This study aims to assess and compare the effects of ethanol and phenol on reducing the aggressiveness and proliferation of osteosarcoma, particularly in delineating tumor resection margins microscopically.

Materials and Methods

Ethical Statement

This study was reviewed and approved by the Dr.

*[1]Orthopedics & Traumatology Department, Faculty of Medicine, Sebelas Maret University, Moewardi General Hospital, Surakarta, Indonesia. [2]Resident of Orthopedics & Traumatology Specialist Programe, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia. [3]Anatomical Pathology Department, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia. *For Correspondence: mahardikaf@gmail.com*

Moewardi General Hospital Research Ethics Committee (Approval No. 896/VIII/HREC/2024). Although the study did not involve direct experimentation on human subjects, it used human tissue specimens with prior informed consent and institutional oversight. Therefore, ethical principles regarding the use of human biological material were upheld in accordance with the Declaration of Helsinki, particularly its 2013 revision, concerning the use of identifiable human tissue and research ethics involving minimal risk.

Study Design and Setting

This experimental study investigated the comparative effectiveness of ethanol and phenol as local control adjuvants in managing osteosarcoma. The research was conducted at Dr. Moewardi General Hospital, Surakarta, in collaboration with the Pathology Anatomy Laboratory at Universitas Sebelas Maret, from August to December 2024.

Study Population and Sampling

The study population consisted of osteosarcoma specimens obtained from patients treated at RSUD Dr. Moewardi. Specimens were selected using purposive sampling based on predefined criteria.

Inclusion criteria required patients to have a confirmed diagnosis of high-grade osteosarcoma, completion of four cycles of standardized neoadjuvant chemotherapy (Cisplatin 33 mg/m² and Adriamycin 25 mg/m² in Weeks I and IV; Ifosfamide 1800 mg/m² with Mesna and Adriamycin 25 mg/m²/24 hours in Weeks VII and X), and informed consent agreement.

Exclusion criteria encompassed patients with prior exposure to alternative chemotherapy regimens, concurrent malignancies or autoimmune diseases, or known hypersensitivity to ethanol or phenol.

Samples were divided into three treatment groups: ethanol (96%) treatment group, Phenol (6%) treatment group, and NaCl group (control).

Sample Size Calculation

The sample size was determined using the formula for comparing two means, incorporating a standard deviation of 10, a significance level of 0.05 ($Z\alpha/2=1.96$), a power of 80% ($Z\beta=0.84$, and an anticipated effect size of 5. The minimum required sample size per group was 63. Given the limited population of eligible cases (N = 30), the final sample size was adjusted to 21 per group using a finite population correction factored using the formula for comparing two means.

Variables and Measurements

The independent variables were the two chemical agents under investigation, ethanol (96%) and phenol (6%), both applied as adjuvant therapies. The dependent variables included tumor necrosis rates, assessed histologically through hematoxylin-eosin staining under 40× magnification, and *Ki-67* expression levels, quantified via manual immunohistochemistry as a marker of tumor cell proliferation. Controlled variables included the immersion duration and dosages of the agents. Data were meticulously collected under standardized laboratory protocols to ensure accuracy and reproducibility.

Statistical Analysis

All statistical analyses were conducted using SPSS version 22.0. Homogeneity of variance was evaluated using Levene's test, while normality of data distribution was assessed via the Shapiro-Wilk test. For data exhibiting normal distribution and homogeneity of variance, one-way ANOVA was employed, with results expressed as mean ± standard deviation. For non-normally distributed or heterogeneous data, the Kruskal-Wallis test was applied, and findings were presented as median (minimum–maximum). Statistical significance was set at $p<0.05$.

Results

22 osteosarcoma specimens were utilized to evaluate the effects of Ethanol, Phenol, and NaCl on *Ki-67* expression. Table 1 presents the mean, standard deviation, and median *Ki-67* expression for each group.

Statistical Analysis

- The Shapiro-Wilk testing showed non-normal distribution of data across all groups ($p < 0.05$).
- The Levene's test indicated variance heterogeneity ($p < 0.05$), necessitating non-parametric analysis.
- The Kruskal-Wallis test confirmed significant differences among groups ($p < 0.0001$).
- The Post hoc Dunn's test as shown in Table 2

Table 1. *Ki-67* Expression Across Treatment Group

Treatment Group	Mean Ki-67 (%) ± SD	Median (Range)
Ethanol	7.27 ± 5.78	5 (1–20)
Phenol	14.86 ± 9.92	10 (2–30)
NaCl (Control)	26.59 ± 15.38	20 (10–60)

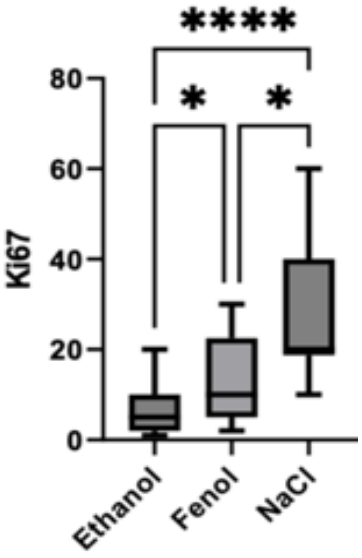
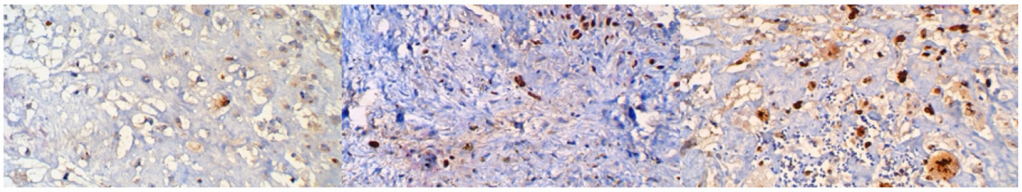


Figure1. Boxplot Diagram Comparing *Ki-67* Expression Among Treatment Specimen Groups.



(2a) (2b) (2c)

Figure 2. Histological Comparison of *Ki-67* Expression in Ethanol, Phenol, and NaCl Groups Visualization of Tumor Necrosis. 2a is Ethanol group, 2b is Phenol Group, 2c is NaCl group, observed under a magnification of 20x. Immunohistochemical slides illustrate reduced staining intensity in the ethanol group compared to phenol. Necrotic areas are more extensive in the ethanol-treated group compared to phenol.

Table 2. Post Hoc Comparisons of *Ki-67* Expression Between Treatment Groups Using Dunn's Test

Comparison	p-value
Ethanol vs Phenol	0.034
Ethanol vs NaCl	<0.0001
Phenol vs NaCl	0.031

revealed phenol is significantly reduced *Ki-67* expression compared to NaCl ($p = 0.031$); but the result showed ethanol's superiority over phenol ($p = 0.034$) and NaCl ($p < 0.0001$).

A boxplot (Figure 1) illustrated the lower and narrower *Ki-67* expression range for Ethanol compared to other treatments, emphasizing its effectiveness.

Discussion

As a reliable predictor of tumor aggressiveness, *Ki-67* expressed across all active cell cycle phases, emerged as a critical prognostic tool in osteosarcoma. Elevated *Ki-67* levels correlate with higher malignancy and poor clinical outcomes [7].

As shown in Figure 2., this study revealed that NaCl, serving as a negative control, exhibited the highest *Ki-67* expression levels (mean ± SD: 26.59 ± 15.38; median: 20). As an inert solution, NaCl lacked ablative or antimitotic properties, consistent with its role as a neutral rinsing agent without direct effects on tumor cell structure or proliferation [9]. Ethanol, however, demonstrated the lowest *Ki-67* expression (mean ± SD: 7.27 ± 5.78; median: 5), highlighting its superior efficacy in suppressing tumor cell proliferation. This effectiveness is attributed to Ethanol's sclerosant properties, such as cellular dehydration, protein denaturation, and vascular endothelial damage, which collectively inhibit tumor progression [7, 8].

Phenol demonstrated intermediate efficacy, with a median *Ki-67* expression of 10 (range: 2–30), outperforming NaCl but falling short of Ethanol. Phenol induces necrosis and intracellular protein denaturation but is limited by its shallow tissue penetration (~200 µm), restricting its impact to superficial tumor layers [5, 9]. Post hoc analysis confirmed Ethanol's superiority over Phenol ($p = 0.034$) and NaCl ($p < 0.0001$), while Phenol also showed significant effectiveness compared to NaCl ($p = 0.031$). These findings underscore Ethanol's potential as a more potent and versatile agent for local control in osteosarcoma treatment.

This study aligns with prior research demonstrating Ethanol's antitumor efficacy across various malignancies. Ban et al. reported Ethanol's ability to reduce *Ki-67* expression, cell viability, migration, and invasiveness, reinforcing its capacity to mitigate tumor aggressiveness. While Phenol's corrosive nature and systemic toxicity limit its clinical application, Ethanol's safety profile and deeper tissue penetration make it a more suitable option for clinical use [4, 5, 10].

Despite significant advances in surgical interventions, osteosarcoma's 5-year survival rate remains at 60–70%, plummeting to 10–20% for cases with pulmonary metastases. The demonstrated efficacy of Ethanol in reducing *Ki-67* expression presents a promising strategy for enhancing local tumor control and potentially improving long-term survival. Ethanol's accessibility, effectiveness, and safety further support its integration into osteosarcoma treatment protocols [4, 11].

This study's limitations include a small sample size and reliance solely on *Ki-67* as a biomarker. This study also did not control for tumor size or depth of sampling, which may introduce bias due to heterogeneity in tumor architecture and chemical penetration depth. However, this study is an ex vivo and uses histologic/immunohistochemical analysis rather than whole-tumor functional metrics, also the specimens were immersed in chemicals equally, regardless of tumor size, the direct exposure to agents could still be comparable. Variability in tumor characteristics across sample groups may also impact generalizability. Our study lacks clinical follow-up data such as recurrence rates or post-operative imaging. As this was an ex vivo experimental study focused on cellular proliferation, we were not able to assess long-term oncologic outcomes. Lastly, while ethanol and phenol were selected for their relevance in local practice, no comparison was made with other adjuvant agents such as liquid nitrogen, argon beam coagulation, or hydrogen peroxide, which may also influence residual tumor viability. Future studies should investigate additional biomarkers, larger cohorts, other adjuvant agents to validate these findings, enabling the broader clinical application of Ethanol in osteosarcoma

management.

In conclusion, ethanol has proven to be an effective agent for local control in osteosarcoma management by significantly reducing tumor proliferation, as measured by *Ki-67* expression. Its sclerosant properties, including cellular dehydration and protein denaturation, make it superior in efficacy compared to Phenol and NaCl, as evidenced by its lower median *Ki-67* expression values.

While Phenol also demonstrated effectiveness in reducing *Ki-67* levels, its impact was less pronounced than Ethanol due to its limited tissue penetration. These findings underscore Ethanol's potential as the preferred local control agent in osteosarcoma treatment, offering a more potent and clinically advantageous alternative.

Author Contribution Statement

Rhyan Darma Saputra: Lead conceptualization, equal data curation, equal formal analysis, equal investigation, lead methodology, equal project administration, lead supervision, equal writing, equal review and editing. Mahardika Frityatama: Equal conceptualization, equal data curation, equal formal analysis, equal investigation, equal methodology, equal project administration, equal supervision, Lead software, Lead writing, equal review and editing. Udi Heru Nefihancoro: Equal conceptualization, equal data curation, equal formal analysis, Lead investigation, leead methodology, lead project administration, equal supervision, equal writing, equal review and editing. Novan Adi Setyawan : Equal conceptualization, Lead data curation, equal formal analysis, equal investigation, lead methodology, equal project administration, eual supervision, equal writing, equal review and editing

Acknowledgements

None.

If any scientific Body approved it/ if it is part of an approved student thesis

This research was part of an academic thesis . It was conducted under the supervision and approval of the local institutional research committee.

Any conflict of interest

The authors declare no conflict of interest.

How the ethical issue was handled (name the ethical committee that approved the research)

The study was reviewed and approved by the Dr. Moewardi general hospital research ethics committee No.896/VIII/HREC/2024, and all procedures were conducted in accordance with the ethical standards of the institutional and national research committees, as well as with the Helsinki Declaration and its later amendments

Availability of data (if applicable to your research)

The data that support the findings of this study are available from the corresponding author upon reasonable request

Was the study registered in any registration dataset (for clinical trials, guidelines, meta-analysis)

This study was not registered in any clinical trial registry or systematic review database.

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