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Interleukin-17 expression is associated with survival in pediatric osteosarcoma

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ABSTRACT

Background: Osteosarcoma is a cancerous tumor that develops from bone-forming mesenchymal cells. The cause of osteosarcoma appears to be multifaceted, and cytokine production is a potential causative factor. T helper 17 cells are responsible for producing the cytokine interleukin 17 (IL-17), and its association with cancer progression remains a subject of ongoing debate. This study aimed to analyze IL-17 expression before and after neoadjuvant chemotherapy and the relationships between prognostic factors and survival.

Methods: We investigated the expression of IL-17 in osteosarcoma tissues from 22 pediatric patients who underwent neoadjuvant chemotherapy. Histopathological and immunohistochemical methods were used to assess IL-17 expression prior to the patients undergoing neoadjuvant chemotherapy. IL-17 expression before chemotherapy was classified as low or high based on the median data. Survival was calculated using the Kaplan–Meier survival analysis with the log-rank test.

Result: The following characteristics were found to be associated with longer survival: male, age > 10 years old, distal location of tumor, low-grade tumor, and tumor with no metastasis; however, there were no statistically significant differences ($p > 0.05$). Binary logistic analysis showed that high expression of IL-17 based on the median data before neoadjuvant chemotherapy (>1.62%) were significantly associated with reduced survival ($p = 0.010$). The mean estimated overall duration of survival was 19.4 months (95% confidence interval (CI): 14.5-24.3) for low IL-17 expression and 14.0 months (95%CI: 8.3–19.7) for high IL-17 expression based on the median data. There was no statistically significant difference in the overall survival ($p > 0.05$).

Conclusion: High expression of IL-17 before neoadjuvant chemotherapy after neoadjuvant chemotherapy were associated with poor survival among pediatric osteosarcoma patients. These findings suggest that IL-17 is a potential prognostic biomarker for pediatric osteosarcoma.

Keywords: IL-17, Pediatric, Osteosarcoma, Survival.
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INTRODUCTION

Osteosarcoma is the most common type of bone tumor in children and adolescents. In the United States, data from the Surveillance, Epidemiology, and End Results (SEER) Program for the year 2023 revealed that new osteosarcoma cases constituted 0.2% of all cancer cases, with a significant portion (24.4%) occurring in individuals under the age of 20.[1] At the international level, the Cancer Incidence in Five Continents (CI5) statistics on the osteosarcoma age-standardized incidence rates (ASRs) per million, number of cases by time period and age category, and average annual percent change (AAPC) in incidence (1988–2012) show that there are 1.3 cases per million and 9.2 cases per million for the 0–9 years and 10–19 years age groups, respectively.[2] According to Fukushima et al., among the 3,457 patients with primary malignant bone tumors between 2006 and 2013 in Japan, 1,497 had osteosarcoma (43.3%), and the majority of cases were recorded in patients < 14 years old (77.7%).[3]

Osteosarcoma is classified as a rare type of cancer in children; however, it has a low average survival rate. When a combination of surgery and chemotherapy is applied, the survival rate for osteosarcoma after five years is 60%.[4] Osteosarcoma has several prognostic variables. The presence of lung metastases at the time of initial diagnosis and a histological response of more than 10% following neoadjuvant chemotherapy are risk factors for a poor prognosis in osteosarcoma cases. In addition, 10–15% of osteosarcoma patients who respond well to chemotherapy may experience relapse in the form of lung metastases.[4]

Activated CD4+ T cells can give rise to T helper 17 (Th17) cells, which produce the cytokine interleukin 17 (IL-17). IL-17 plays crucial roles in human autoimmune disorders, inflammation, and pathogen defense responses.[5] Despite IL-17 being

associated with a poor prognosis in many types of human cancer, its contribution to cancer growth is still debated, and its potential role in the development of cancer has not been fully elucidated.[5]

It has been demonstrated that IL-17 causes angiogenesis-stimulating proteins such as vascular endothelial growth factor (VEGF) to be produced in stromal cells and that IL-17 receptor expression is linked to the generation of VEGF in osteosarcoma-derived cell lines.[5] It has also been demonstrated that the interaction between IL-17A and its receptor (IL-17RA) promotes metastasis in nude mice (non-T-cell athymic) that have been injected with an osteosarcoma-derived cell line exhibiting high levels of IL-17RA and then transfected with the IL-17 gene.[6] Additionally, another study showed that people with osteosarcoma have greater serum IL-17 levels than healthy individuals and that these levels are substantially higher in those with metastases.

In a systematic review conducted by Mikola et al., it was reported that IL-17 has both pro- and antitumorigenic effects, depending on the type of cancer and the molecular structure and location of IL-17.[7] The review examined studies on the following types of cancer: lymphoma, leukemia, and oral, skin, lung, breast, liver, colorectal, ovarian, prostate, bladder, and pancreatic cancer. Colorectal cancer was the most studied type of cancer, accounting for eight of the included studies.[7] Notably, the roles that IL-17 plays in cancer have been examined in several types of cancer but rarely in osteosarcoma.

Several studies have shown that IL-17F expression is reduced in patients with colorectal cancer. For example, using immunohistochemistry, Al-Samadi et al. demonstrated that colorectal cancer patients had lower levels of IL-17F than healthy controls.[8] This finding was consistent with those of Liu et al., who found that colorectal cancer samples contained less IL-17F than ulcerative colitis or polyp samples.[9] Additionally, Tong et al. showed that IL-17F mRNA expression was decreased in colon cancer.[10] However, Chen et al. recently revealed that colorectal tumor mucosa samples had higher levels of IL-17F than corresponding non-tumor mucosa samples.[11] Similarly, studies that have examined IL-17 expression in lung cancer have also reported some disparate outcomes. According to Li et al., IL-17F expression negatively correlated with lymph node metastasis and TNM staging and favorably correlated with tumor differentiation.[12] In addition, Yang et al. reported that individuals with more advanced illness had lower serum levels of IL-17F.[13]

To date, no studies have investigated how IL-17 expression affects survival in patients with osteosarcoma. Hence, in this study, we aimed to determine whether IL-17 expression is a potential predictive indicator of survival following neoadjuvant treatment in children with osteosarcoma.

METHODS

Data were accessed from the patients' medical records stored in the Pediatric Cancer Database of the Department of Pediatrics at Dr. Moewardi General Hospital on 28 June 2023. This study was approved by the Ethical Committee of Dr. Moewardi General Hospital. The Ethical Committee waived the need for informed consent because the study was completed anonymously (ethical code: 1.754/VI/HREC/2023).

Patients

Pediatric patients who had a confirmed diagnosis of osteosarcoma between January 2018 and December 2022 at Dr. Moewardi General Hospital were included in this study. Data on the following variables: patient sex, patient age, exact anatomic site of the tumor, tumor grade, metastasis and outcome after neoadjuvant chemotherapy were recorded. The patients were divided into subgroups to determine the effect of variables on survival rate (i.e., whether any variables are potential prognostic factors). The categories and classifications were as follows: sex (male and female), age (< 10 years old and ≥ 10 years old), exact anatomic site of the tumor (proximal and distal), tumor grade (low, intermediate high and high), metastasis (yes and no) and outcome (died and survived).

We also obtained data on IL-17 expression at various stages and grades of osteosarcoma to examine the effect of this potential carcinogenesis mediator. We evaluated IL-17 expression before and after neoadjuvant chemotherapy, analyzed the relationships between the potential prognostic factors (i.e., patient sex and age; tumor grade, metastasis and location) and outcome or survival, and determined whether IL-17 is a potential predictive indicator of survival following neoadjuvant treatment in children with osteosarcoma.

Osteosarcoma was diagnosed through history taking, physical examination, computerized tomography scanning or magnetic resonance imaging, and histopathological examination. Surgical excisions and biopsies were performed in the Department of Orthopedics, Traumatology, and Anatomical Pathology. For some problematic cases, multidisciplinary team discussions were held that involved the orthopedic surgeon, pathologist, and pediatric oncologist to determine the tumor stage, management plan, and prognosis.

Osteosarcoma patients who had a localized tumor underwent a first-line chemotherapy regimen of doxorubicin (25 mg/m^2) on Day 1 and cisplatin (100 mg/m^2) on Days 1 to 3 for three to four cycles over 7–10 weeks. A second line of chemotherapy was administered that included doxorubicin and cisplatin (at the same doses as used in the first-line treatment). Ifosfamide was administered during cycles 2 and 4 in patients with metastatic disease. To lessen the negative effects of chemotherapy, mesna (1,600 mg/m^2) was concurrently infused intravenously. After completing the neoadjuvant chemotherapy, each patient underwent limb salvage surgery or limb ablation and amputation.

Collection of osteosarcoma specimens

The specimens used in this study were blocks of osteosarcoma tumor tissue samples from pediatric patients diagnosed with osteosarcoma between 1st January 2018 and 31st December 2022. The samples were embedded in paraffin and fixed with formalin. The tissue blocks were obtained from the archives of the Anatomical Pathology Section at Dr. Moewardi General Hospital and the Faculty of

Medicine at Sebelas Maret University. Sections were prepared from the entire tissue blocks and stained to determine IL-17 expression.

Reagents

Recombinant IL-17A was obtained from Abclonal (Woburn, USA). The source of the Il-17A reagents was from rabbit, with immunoglobulin (Ig)G isotype and purified with affinity purification. It was stored at -20 °C without freeze or thaw cycles and buffered with phosphate-buffered saline (PBS) with 0.05% proclin300 and 50% glycerol at pH 7.3. All tools and materials were from the Anatomical Pathology Laboratory of the Faculty of Medicine at Sebelas Maret University.

Immunohistochemistry

The staining technique used in the immunohistochemical examination is an indirect immuno-peroxidase consisting of three phases with Avidin Biotin Complex (ABC). Paraffin was removed from the tissue by deparaffinization with acetone, xylol, 100% alcohol, 90% alcohol, 80% alcohol, 70% alcohol, and water. Phosphate Buffer Saline (PBS) with a pH of 7.4 was used to wash the tissue, then incubated with Trypsin 0.125% at 37°C for 5-10 minutes to open the masking antigen, 30 minutes with 0.5% H2O2 in methanol to remove endogenous staining at room temperature. After that, the tissue was washed with running water for 1 minute and then with distilled water. The tissue was marked, washed with PBS pH 7.4 for five minutes, incubated with 3% sera in 1% BSA for twenty minutes, and then washed twice for three minutes with PBS pH 7.4. Tissue incubation was performed in murine monoclonal antibodies against the expression of IL-17. Tris-PBS 1:200 was used to dissolve monoclonal antibodies. 1µL of monoclonal antibody is required for 1 cm2 of tissue. The tissue was then incubated in a humid room for 30 minutes. The tissues were then rinsed twice for three minutes with PBS pH 7.4. After being washed, the tissues were treated with biotinylated anti-murine antibodies (Dako Kit) for 30 minutes. The incubated tissue was then washed again with PBS pH 7.4 for 3 minutes twice. After that, incubate them with streptavidin-biotin (Dako Kit) for 30 minutes, then wash the tissue with PBS pH 7.4 for 3 minutes twice. The substrate (Dako Kit) was then used to incubate the tissue once more until it turned brown. The tissues were then rinsed twice for three minutes each in PBS pH 7.4. Following a PBS pH 7.4 wash, tissues were then rinsed under running water. The washed tissue is then covered with a deck glass and ready to be viewed under a light microscope. A positive result will give a brownish color.

The immunohistochemical and histopathological examinations were performed by two anatomical pathologists separately, and the results were analyzed using the Kappa test. IL-17 expression was evaluated by observing the entire field of view of each osteosarcoma tissue preparation using an Olympus Cx 21 microscope with a 400 × magnification (10 × ocular lens and 40 × objective lens). IL-17 expression was quantitated and recorded as a percentage using Image J software. The high and low cutoff points for IL-17 expression were determined by median data.

Statistical Analysis

Based on the obtained median data, the IL-17 expression levels before undergoing neoadjuvant chemotherapy were categorized as either low or high. The prognostic value of IL-17 levels was tested with the $\chi 2$ test and Fisher's test, including known prognostic factors: sex, age, exact anatomic site of the tumor, tumor grade (low, intermediate high, and high), and metastasis (yes and no). To investigate the relationship between IL-17 expression and patient outcomes, statistical tests including the $\chi 2$ test and Fisher's test were employed. Overall survival was defined as the time elapsed from the date of diagnosis to either the date of death or the date of the last follow-up. Survival analysis was conducted using the Kaplan–Meier method in combination with the log-rank test, and statistical significance was considered when p-values <0.05. All statistical analyses were carried out using the SPSS version 25 software.

RESULTS

Patient Characteristics

Out of the 22 individuals enrolled in this research, the predominant proportion were males (54.5%), and the majority were older than 10 years (77.3%). Most of the patients had tumors that were proximally located (54.5%), high-grade osteosarcoma (59.1%), and has no metastasis (63.6%).

Table 1. Patient Characteristics

Variabel	N	%
Sex		
Male	12	54.5
Female	10	45.5
Age (years old)		
≤10	5	22.7
> 10	17	77.3
Exact anatomic site of tumor		
Proximal	12	54.5
Distal	10	45.5
Grade		
1 (low grade)	5	22.7
2-3 (intermediate-high grade)	4	18,.2
3 (high grade)	13	59.1
IL-17 Expression		
High	11	50.0
Low	11	50.0
Outcome		
Died	10	45.5
Survived	12	54.5
Metastasis		
Yes	8	36.4
No	14	63.6

Over the observation period, 10 patients died, while 12 patients survived. The characteristics of the study participants can be found in Table 1.

Survival of Osteosarcoma Patients Based on Patient and Tumor Characteristics

Figure 1 and Table 2 shows the survival probabilities calculated for each of the evaluated prognostic factors in pediatric osteosarcoma. The following attributes were found to extended survival: male (17.5 months with 30.6% survival rate), age > 10 years old (16.5 months with 27.7% survival rate), distal location of tumor (19.3 months with 44.4% survival rate), low-grade tumor (100% survival rate), and tumor with no metastasis (19.2 months and 49% survival rate). However, no statistically significant difference was found for any of the characteristics in terms of overall survival (P > 0.05).

IL-17 Expression in Pediatric Osteosarcoma Patients Before and After Neoadjuvant Chemotherapy

The levels of IL-17 identified in the samples obtained before and after chemotherapy underwent analysis through a normality test (Shapiro–Wilk test), and the results are shown in Table 3. The Wilcoxon test was used to determine whether there were differences because the IL-17 expression data was not normally distributed. As illustrated in Table 3, it is evident that the average IL-17 level was significantly higher prior to the initiation of neoadjuvant chemotherapy in comparison to after its completion (3.52% vs. 0.87%, p = 0.007). This indicates that neoadjuvant chemotherapy was effective in notably decreasing IL-17 expression. The results of the differential test of the mean percentage of IL-17 expression before and after neoadjuvant chemotherapy are shown in Table 4. Additionally, the average levels of IL-17 before and after neoadjuvant chemotherapy are shown in Figure 2, and microscopy images showing IL-17 staining, which indicates IL-17 expression, are presented in Figure 3.

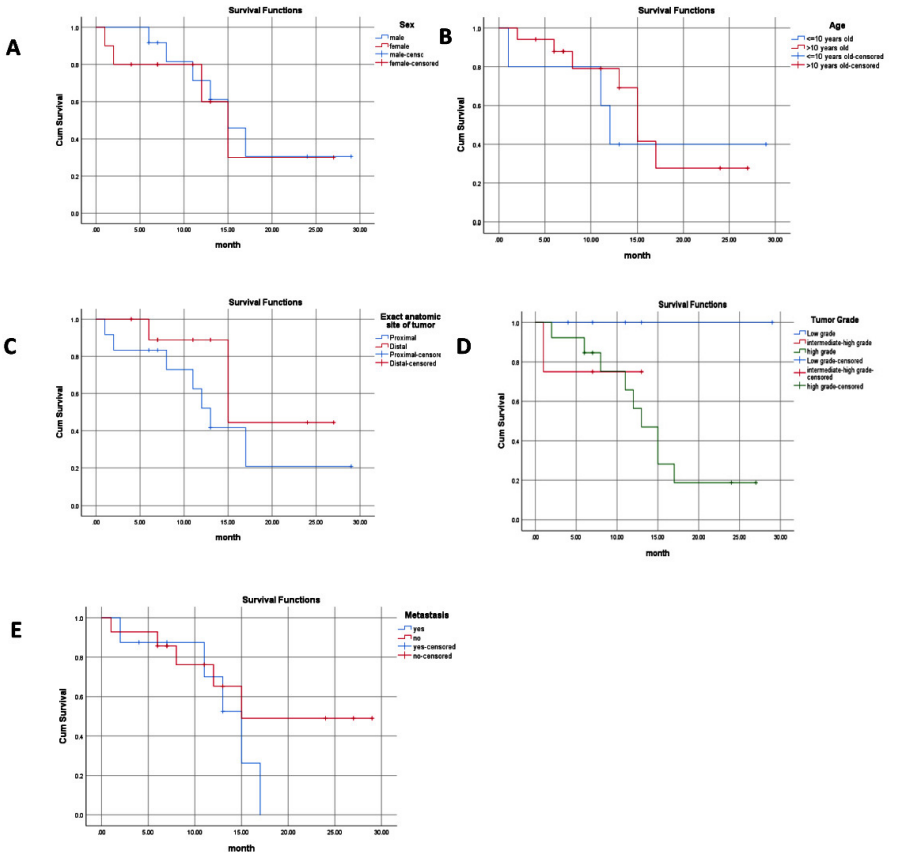

Figure 1. Kaplan–Meier survival curves of prognostic factors (A: Sex, B: Age, C: Exact anatomic site of tumor, D: Histological subtype, E: Metastasis) for survival.

Table 2. Survival probability of pediatric osteosarcoma based on prognostic factors.

Variables	Survival Cumulative Probability	Mean time survival (months)	Log-rank test
Sex			
Male	30.6 %	17.5 (11.9-23.1)	0.773
Female	30 %	15.3 (8.1-22.5)	
Age			
≤ 10 years	40 %	16.4 (6.7-26)	0.578
>10 years	27.7 %	16.5 (11.8-21.1)	
Exact anatomic site			
Distal	44.4%	19.3 (13.3-25.4)	0.174
Proximal	20.8 %	14.4 (8.5-20.4)	
Histological grade			
Low grade	100 %	*	0.224
Intermediate-high grade	75%	*	
High grade	18.8%	*	
Metastasis			0.302
Yes	0%	12.9 (9.1-16.6)	
No	49%	19.2 (12.8-25.6)	

Table 3. Normality test of IL-17 expression before and after neoadjuvant chemotherapy

Variable	Kolmogorov-Smirnov[a] P	Shapiro-Wilk P
IL-17 pre	0.000*	0.000
IL-17 post	0.000*	0.000

Table 4. Differential test of mean percentage of IL-17 expression before and after neoadjuvant chemotherapy

Group	N	Mean IL-17 Expression (%)	Median	SD (%)	p-value
Beforechemotherapy	22	3.52	1.62	5.73	0.007
After Chemotherapy	22	0.87	0.85	2.30	

Notes: *significance p<0.05

Table 5. The relation of IL-17 expression to the outcome

Variabel IL-17 Based on the Median	The Outcome		OR (95%CI)	p-value
	Died	Survived		
≤1.62	2 (9,1%)	9 (40.9%)	0.083 (0.011-0.633)	0.010
>1.62	8 (36.4%)	3 (13.6%)		

Notes: Chi Square/Fisher exact test; *significance at p < 0.05.

Table 6. Survival probability of pediatric osteosarcoma prognostic factors based on IL-17 expression

Variables	Survival Cumulative Probability	Mean time survival (months)	Log-rank test
IL-17 Expression Based on Median			
Low expression	60.0 %	19.4 (14.5-24.3)	0.199
High Expression	21.8 %	14.0 (8.3-19.7)	

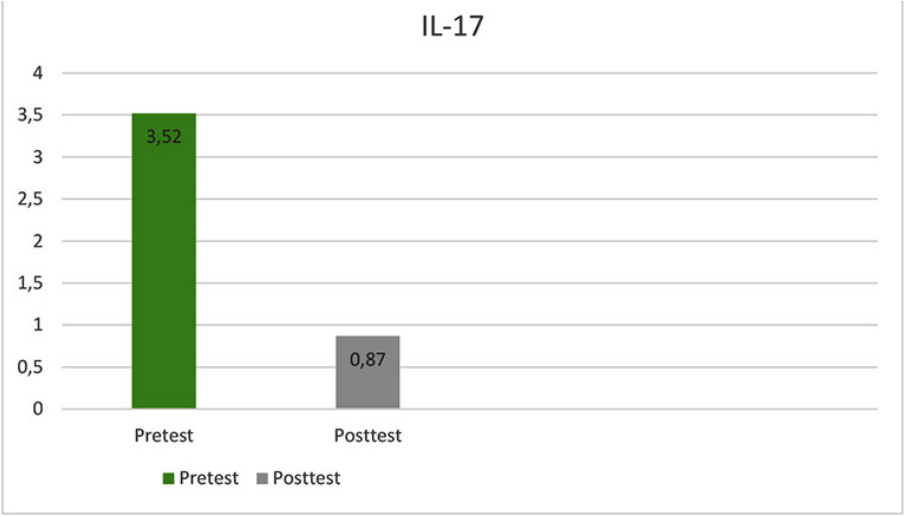

Figure 2. The average expression of IL-17 in osteosarcoma patients before and after neoadjuvant chemotherapy.

Relationship Between IL-17 Expression and Outcome Based on the Median Data

Table 5 shows the relationship between IL-17 expression and outcome based on the median data. It can be seen that the IL-17 variable was found to have an odds ratio (OR) value of 0.083 (0.011–0.633). This means that when IL-17 expression is <1.62% (low expression; determined via immunohistochemical staining) it acts as a protective factor and the patient is likely to survive. There was a significant correlation found between IL-17 expression and outcome, as indicated by the *p* value of 0.046.

Survival of Osteosarcoma Patients Based on IL-17 Expression

Table 6 shows the survival probability associated with the assessed patient and tumor characteristics of pediatric osteosarcoma. Fig 5 shows that the mean estimated overall duration of survival was 19.4 months (95% confidence interval (CI): 14.5-24.3) for low IL-17 expression and 14.0 months (95%CI: 8.3–19.7) for high IL-17 expression based on the Median Data. The variable (IL-17 expression) showed no statistically significant difference in overall survival (p = 0.199).

DISCUSSION

In this study, IL-17 expression in samples from osteosarcoma patients decreased significantly after chemotherapy. This finding indicates that the neoadjuvant chemotherapy regimens, which consisted of doxorubicin, cisplatin, and cyclophosphamide, that the pediatric patients involved in this study underwent inhibited the expression of IL-17.

Interleukin-17 contributes to the growth of many types of cancer, including ovarian and stomach cancer. According to a study by Xiang et al., chemotherapy with temozolomide (TMZ) and three-dimensional conformal radiation (CRT) were used to treat gliomas, and the levels of IL-17 were found to be much lower after treatment than before.[14] This result is in accordance with those of our study. Moreover, Gaba et al. showed that an increase in plasma IL-17A levels was a sign of failure of cervical cancer treatment that consisted of three cycles of cisplatin

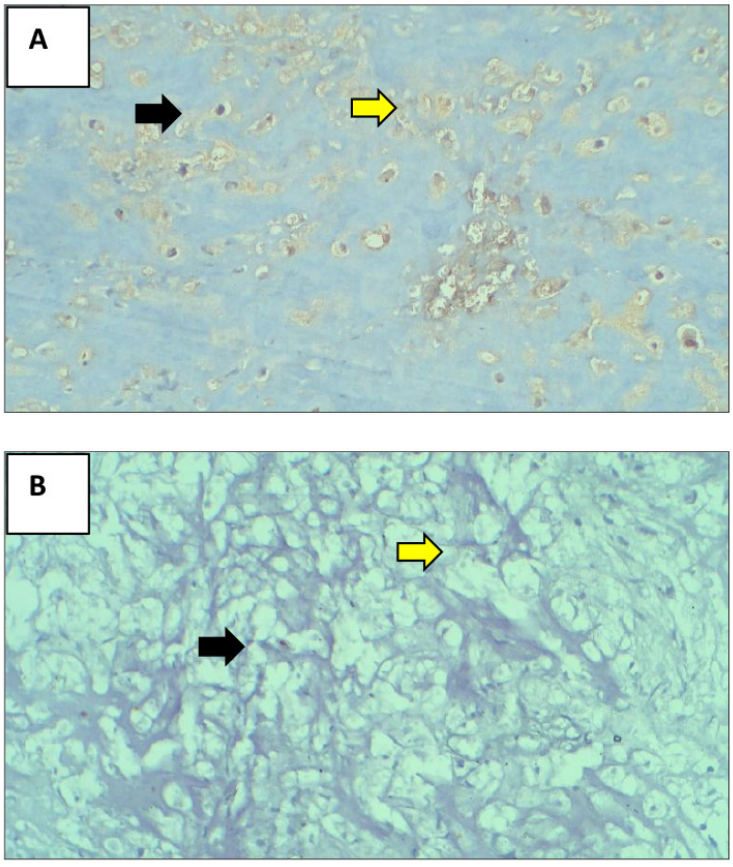

Figure 3. **Microscopic view IL-17 expression.** Figure A is an image of tissue IL-17 expression before chemotherapy. Figure B is a picture of tissue IL-17 expression after chemotherapy. In Figure A, the black arrows show strong expression in the cytoplasm with a dark brown color. The yellow arrows show strong intercellular expression with dark brown color. Figure B black arrows show expression of IL-17 in the cytoplasm, and yellow arrows show expression of IL-17 in intercellular after neoadjuvant chemotherapy with light brown color. The brown color is a reaction between staining with IL-17 antibody and IHC. Examination using an Olympus type Cx 21 microscope with a magnification of 400 x.

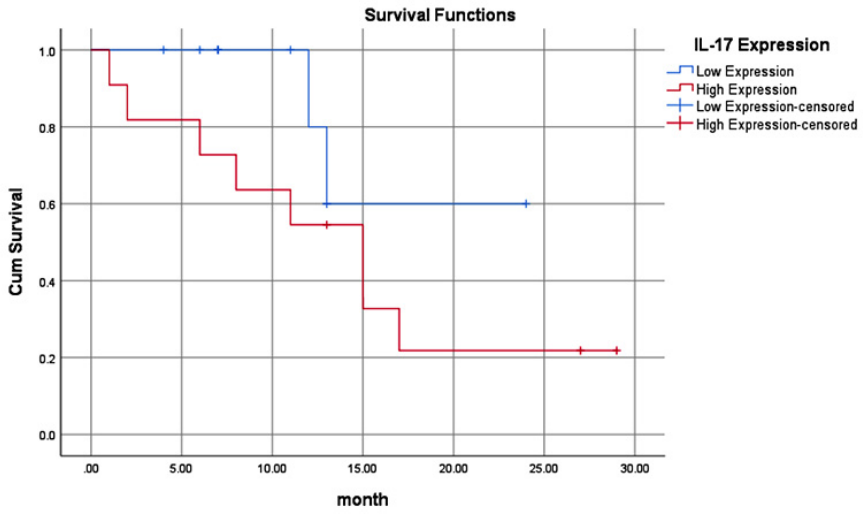

Figure 4. Kaplan–Meier survival curves of IL-17 expression as a prognostic factor for survival based on the median data.

and 5-fluorouracil (5FU).[15] Hence, there is strong evidence connecting IL-17 and chemotherapeutic failure, and the tumor response to chemotherapy is inversely correlated with IL-17 expression following chemotherapy. However, tumor growth is reduced when chemotherapy is combined with reducing IL-17R levels.[5,16]

During chemotherapy, both normal cells and cancer cells are damaged. Given that IL-17 is known to play roles in signaling and cellular mechanisms involved in tissue repair and tumorigenesis, it is likely that IL-17 contributes to tumor healing after chemotherapy.[17] IL-17 can also prevent cancer cells from dying during cisplatin treatment and encourage their growth; however, cisplatin inhibits IL-17 signaling, and IL-17 signaling is further reduced as more cells undergo apoptosis during cisplatin treatment.[18]

Our results showed that there was a significant correlation between IL-17 expression and survival in the studied patients. High IL-17 expression (> 1.62%; detected using immunohistochemistry) before neoadjuvant chemotherapy tended to correlate with poor survival. Improved survival was observed in the pediatric osteosarcoma patients who had low IL-17 expression (19.4 months; 95%CI: 14.5–24.3). These results were in accordance with those of other studies. A study conducted in Taiwan in 2018 showed that a poor prognosis for head and neck cancer could be predicted by elevated IL-17 in peripheral blood: patients with a higher percentage of IL-17-expressing cells had a significantly lower five-year overall survival rate compared to patients with lower percentages of such cells.[19] The findings of a systematic review conducted in China suggested that increased IL-17 expression confers poor clinical outcomes in lung carcinoma.[20] Furthermore, it has been shown that high expression of IL-17 inhibits apoptosis and promotes the proliferation of cisplatin-treated cancer cell lines and that cisplatin synergistically increases tumor cell death by inducing apoptosis, which in turn inhibits IL-17 signaling.[18] Therefore, based on our statistically significant findings, low IL-17 expression can be considered a predictor of survival in children with osteosarcoma after neoadjuvant chemotherapy.

However, several studies have shown increased IL-17 expression after chemotherapy. A 2019 study by Ariyana et al. showed that the concentration of IL-17 increased by 46.36 pg/mL after chemotherapy for breast carcinoma.[21] An increase in the severity of the cancer can lead to an increase in the IL-17 level. In patients suffered from cancer, T cells are induced to differentiate into Th17 cells, and this process is triggered by tumor growth factor (TGF) and other cytokines (IL-21, IL-6, and IL-23) and involves transcription factors (e.g., related orphan nuclear receptor (ROR)-gamma). Thus, the level of IL-17 continues to increase as long as a person has cancer. It remains unclear why the level of IL-17 increases after chemotherapy. Ariyana et al. concluded that IL-17 levels may have increased due to the severity of breast cancer and other concurrent conditions.[21]

Furthermore, our findings were in line with those of a 2013 study by Wang et al. that demonstrated that interaction between IL-17A and IL-17RA increased in osteosarcoma metastasis in naked mice.[22] While upregulating IL-17RA in MG63 cells was found to increase their response to IL-17A and exert greater metastasis, downregulating IL-17RA in U-2 cells was found to reverse the heightened metastasis produced by IL-17A. A possible explanation for the increased metastasis of the osteosarcoma cells is that the IL-17A/IL-17RA interaction stimulated the production of vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP9), and CXC chemokine reseptor-4 (CXCR4) in the osteosarcoma cells. Additionally, signal transducer and activator of transcription 3 (Stat3) activity was essential for the IL-17A/IL-17RA interaction that promoted osteosarcoma metastasis and resulted in poor outcome.[22]

In contrast, in their study on patients with late TNM-stage non-small cell lung carcinoma (NSCLC), Song et al. showed that an increased percentage of peripheral Th17 cells positively correlated with better five-year overall survival (P = .002). As mentioned earlier, Th17 cells are a newly discovered subtype of helper T cells that differ from T helper type 1 (Th1) and T helper type 2 (Th2) cells and that secrete IL-17, a proinflammatory cytokine.[23]

Another systematic review, conducted in 2015, showed that high IL-17 expression in tissue was correlated with poor survival.[24] In 18 of the 27 reviewed studies, there was a correlation between a high IL-17C cell count and a poor prognosis. Hepatocellular carcinoma (HCC; six studies) and colorectal carcinoma (three studies) were the most studied cancer types that correlated with poor survival.[24] Five studies on gastric adenocarcinoma, cervical carcinoma, esophageal squamous cell carcinoma (ESCC), recurrent ovarian carcinoma, and pancreatic ductal adenocarcinoma found a connection between a high IL-17C cell count and better survival. It is significant to note that in two of these five studies, IL-17C cells were measured in regions with the greatest lymphocytic infiltrate. There was a substantial association between a high IL-17 level and a poor prognosis in 18 studies overall, and the association was 3.5 times stronger than that between IL-17 level and a better prognosis.[24]

A 2014 meta-analysis conducted in China found that in cases of HCC, NSCLC, and ESCC, IL-17 has potential as a unique prognostic marker for monitoring patients' prognoses and evaluating the effectiveness of clinical treatments.[25] High expression of IL-17 predicted poor survival in both NSCLC and HCC (HR = 2.02; 95%CI: 1.44–2.83; p=0.001; I2 = 0%), but was linked to favorable survival in ESCC (HR = 0.63; 95%CI: 0.51–0.79; p=0.001; I2 = 0%).[25]

Together, our and the previous findings suggest that the IL-17-mediated immune response in patients with cancer may be context-dependent. With only a limited number of studies conducted on each cancer type, especially on osteosarcoma, it is not possible at this stage to identify whether IL-17 has a significant effect on survival. However, further research on the role that IL-17 plays in the development of osteosarcoma may reveal critical insights and result in the development of IL-17 as a unique disease marker for osteosarcoma and more effective therapeutic approaches that specifically target IL-17.

This study also subjected into several limitations. The cytokine interleukin-17 is influenced by various factors that can either enhance or impede its expression. However, due to constraints in time and cost, this study did not investigate these factors. Consequently, the analysis of pro-inflammatory cytokines in this study was confined to immunohistochemical examination, with more comprehensive assessments, such as examinations at the mRNA level, remaining unexplored. Employing the PCR technique could yield more precise and detailed data. Also, the success of a cohort often hinges on having a considerable number of competent subjects. In this particular study, the research involved 22 subjects. To enhance the statistical robustness and significance, there is an aspiration to augment the number of research subjects. Notably, samples in this study were sourced from various hospitals, introducing variations in pre-analytic stages and resulting in non-uniform research samples. To address this, the research was conducted prospectively, aiming to achieve greater uniformity in the pre-analytical stage.

CONCLUSION

The expression of IL-17 in children with osteosarcoma was shown to decrease in a statistically significant manner after neoadjuvant chemotherapy. High expression of IL-17 before neoadjuvant chemotherapy has been statistically proven to be a prognostic factor for poor survival in children with osteosarcoma and associated with a shorter survival duration than low expression. These findings suggest that IL-17 is a potential prognostic biomarker in pediatric osteosarcoma.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ETHICAL STATEMENT

This study has been approved by Institutional Review Board of Dr. Moewardi General Hospital (No:142/II/HREC/2023).

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AUTHOR CONTRIBUTION

MR responsible for concept and design of the study, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation and editing, manuscript review, and guarantor. HS, BW, VW, BP, PU, S, and FAH responsible for manuscript review and act as guarantor. FZ responsible for data analysis, statistical analysis, manuscript preparation and editing. JW responsible for manuscript preparation and manuscript editing.

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