Association between the expression of GSTP1 and mutant p53, poor chemotherapy response and metastasis in patients with osteosarcoma

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Abstract. Osteosarcoma, a primary malignant tumor of the bone, is known for its aggressive behavior and tendency to metastasize to the lungs. The standard treatment for osteosarcoma includes surgery combined with chemotherapy. However, genetic changes and chromosomal contributions influence the tumor's aggressive behavior and influence the effectiveness of chemotherapy, often resulting in drug resistance and metastasis. The present study aimed to assess the association between increased expression of mutant p53 and glutathione S-transferase P1 (GSTP1), poor chemotherapy response and the occurrence of metastasis in patients with osteosarcoma. The study is a cross-sectional study using paraffin blocks retrieved from the Department of Anatomical Pathology at Cipto Mangunkusumo Hospital (Jakarta, Indonesia) containing tissues gathered from patients diagnosed with osteosarcoma who received the first-line drug of neoadjuvant chemotherapy for three cycles from January 2019 to December 2021. Immunohistochemical examinations of GSTP1 and mutant p53 were conducted, using the Fedchenko and Reifenrath immunoreactive scoring system. The results obtained after the immunohistochemical examination and evaluation of GSTP1 expression and mutant p53 expression were subjected to bivariate association analysis with chemotherapy response (Huvos Score) and the occurrence of metastasis. This study involved a total of 36 patients. Statistical analysis using the Fisher's exact test revealed a significant association between increased GSTP1 expression and a poor chemotherapy response, as well as between increased mutant P53 expression and a poor chemotherapy response. By contrast, no significant association was found between GSTP1 or mutant P53 expression and the occurrence of metastasis. In conclusion, a significant association was found between the increasing expression levels of GSTP1 and mutant p53 and a poor chemotherapy response in patients with osteosarcoma, while no significant association was found between the increasing expression of GSTP1 and mutant p53 and the occurrence of metastasis in these patients.

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Abbreviations: GSTP1, glutathione S-transferase P1; FFPE, formalin-fixed paraffin-embedded; IRS, immunoreactive scoring system; CC, contingency coefficient; GOF, gain-of-function

Key words: osteosarcoma, GSTP1 expression, mutant p53 expression, poor response to chemotherapy, metastasis

Introduction

Osteosarcoma is the most common primary malignant bone tumor, predominantly affecting children and adolescents, characterized by its highly aggressive nature and a propensity for early metastasis, particularly to the lungs (1). Despite advancements in chemotherapy regimens, the prognosis for patients, especially those with metastatic disease or a poor response to chemotherapy, remains dismal. Previous studies have elucidated the role of genetic factors in modulating these clinical outcomes (2,3). Among these, mutations in the tumor

suppressor gene TP53 and polymorphisms in the glutathione S-transferase P1 (GSTP1) gene have been implicated.

p53, known as the 'guardian of the genome,' is a tumor suppressor protein that plays a crucial role in preventing cancer formation. Under normal conditions, p53 regulates the cell cycle and promotes apoptosis or cell repair in response to DNA damage (4,5). Mutations in the TP53 gene, which encodes the p53 protein, are among the most common mutations found in human cancers. These mutations often result in a loss of normal p53 function and can confer oncogenic properties to the protein, contributing to tumor progression and resistance to chemotherapy (6,7). The missense hot-spot mutation R175H was specifically selected for detailed analysis in the present study as it is a canonical 'hot-spot' allele with well-documented gain-of-function effects that promote chemoresistance in sarcoma models, and since institutional molecular prescreening performed between 2017 and 2022 identified R175H in 22% of sequenced osteosarcoma samples, the highest single-allele frequency observed locally (7), making it highly relevant to the patient population of the present study (8-11).

GSTP1 is part of the GST family, which plays a significant role in detoxification by conjugating glutathione to a wide range of substrates, including chemotherapy agents. This process can lead to the inactivation of drugs intended to kill cancer cells, contributing to chemotherapy resistance (12). High levels of GSTP1 expression have been associated with poor responses to chemotherapy. Furthermore, GSTP1 expression is often upregulated in response to oxidative stress and chemotherapy, which can further enhance resistance to treatment (13,14). Whereas earlier pharmacogenetic studies centred on the germline Ile105Val (rs1695) polymorphism, protein-level assessment, such as that undertaken in the present study, captures genotype-independent mechanisms, including somatic copy-number gain, promoter methylation, nuclear factor erythroid 2-related factor 2 (NRF2)-driven transcription and post-translational stabilisation (15,16). This integrative read-out may better reflect the functional detoxification capacity of the tumor.

Despite extensive investigation into the roles of mutant p53 or GSTP1 expression in osteosarcoma, the findings have been inconsistent and have often failed to account for the complex interplay between a specific TP53 hot-spot and GSTP1 expression (17,18). The present study, therefore, investigates whether the R175H-mutant p53 and upregulation of GSTP1 are jointly associated with histological chemoresponse and metastatic behaviour in osteosarcoma within a uniformly treated cohort.

Materials and methods

Patients and samples. This cross-sectional study included tissue samples from patients with osteosarcoma who had received neoadjuvant chemotherapy as their first-line treatment at Cipto Mangunkusumo General Hospital (Jakarta, Indonesia) between January 2019 and December 2021. Ethical approval was obtained from the Institutional Review Board (approval no. KET-420/UN2.FI/ETIK/PPM/00.02/2023). Written informed consent was obtained from all participants, with parental or guardian consent obtained for those <18 years old. Participants included 36 patients with osteosarcoma [20 males (55.6%) and 16 females (44.4%)] who received first-line chemotherapy consisting of 120 mg/m^2 cisplatin administered on day 1 and 75 mg/m^2 doxorubicin given as 25 mg/m^2/day on days 1-3, repeated every 21 days for a total of three cycles. The mean age was 18.14±8.37 years (age range, 8-41 years). Exclusion criteria included patients with other diagnosed malignancies that cause p53 mutant type and upregulated GSTP1 expression. Each patient tissue sample was associated with data about Enneking staging (Table I) (19) and Huvos score (Table II) (20). In addition, osteosarcoma cases were classified according to the 2020 World Health Organization criteria and staged using the AJCC 8th edition of the Tumor-Node-Metastasis system, following Japanese Clinical Oncology Group recommendations to ensure standardized reporting across cohorts (21,22). Samples consisted of osteosarcoma tissues obtained during surgical resection after completion of neoadjuvant chemotherapy. Tissue samples were retrieved from the Department of Anatomical Pathology, and formalin-fixed, paraffin-embedded (FFPE) resection specimens were used for immunohistochemical (IHC) examination. All tissues were fixed in 10% neutral buffered formalin at room temperature for 24 h before routine paraffin embedding.

IHC analysis

Immunostaining of GSTP1 and mutant P53. FFPE biopsy samples were cut into 5-μm sections and mounted onto glass slides before deparaffinization in xylene baths and rehydration in descending ethanol concentrations. Antigen retrieval was performed by heat-induced epitope retrieval using EnVision FLEX Target Retrieval Solution (cat. no. DM828; Dako; Agilent Technologies, Inc.) at 99˚C for 40 min. The slides were then allowed to cool to room temperature. Next, slides were placed at room temperature to allow them to cool gradually. Endogenous peroxidases were blocked with EnVision™ FLEX Peroxidase-Blocking Reagent (cat. no. SM801; Dako; Agilent Technologies, Inc.) for 5 min at room temperature. The IHC slides were incubated with GSTP1 primary antibody (anti-GST3/GST pi antibody; rabbit monoclonal; cat. no. ab138491; Abcam) or mutant p53 (R175H) primary antibody (rabbit monoclonal; clone HL1129; cat. no. GTX636395; GeneTex, Inc.) for 1 h (diluted 1:100). This antibody was validated for use in immunohistochemistry-paraffin [IHC-P] by: i) In-house optimisation (U2OS-R175H vs. SaOS-2 FFPE pellets); ii) orthogonal confirmation with Sanger sequencing; and iii) parallel staining with a pan-p53 DO-7 clone (data not shown). Additionally, the same recombinant clone is listed as validated for IHC-P by Abcam (cat. no. ab308342). Detection was performed using the Mouse/Rabbit PolyVue Plus HRP/DAB Detection System (cat. no. PVP100D; Diagnostic BioSystems, Inc.) according to the manufacturer's instructions. Briefly, sections were incubated sequentially with Mouse/Rabbit PolyVue Plus™ HRP/DAB Detection System (catalog no. PVP250D; Diagnostic BioSystems, Inc.) for 10 min each at room temperature, followed by DAB/Plus chromogen for 5 min at room temperature. Slides were counterstained with Mayer's hematoxylin for 2 min at room temperature, rinsed in running tap water, dehydrated through graded ethanol, cleared in xylene and mounted.

Evaluation of IHC slides was performed by a musculoskeletal consultant or a histopathologist using light microscopy. Semi-quantitative assessment of p53 mutant-type and GSTP1

Table I. Enneking staging for malignant bone tumors (11).

Stage	Grade	Site	Metastasis
IA	Low grade (G1)	Intracompartmental	No
IB	Low grade (G1)	Extracompartmental	No
IIA	High grade (G2)	Intracompartmental	No
IIB	High grade (G2)	Extracompartmental	No
III	Any grade	Any extent	Yes (regional or distant)

Table II. Huvos grading system (score to evaluate histopathological response to chemotherapy) (12).

Grade	Percent necrosis	Response
I	Little or no evidence of necrosis (<50% tumor necrosis)	Poor
II	Partial response with 50-90% necrosis	Poor
III	Good response with 90-99% necrosis	Good
IV	Complete response with 100% necrosis (no viable tumor cells)	Good

Table III. Immunoreactive scoring system (13).

Measurements	Score
SI	
No staining	0
Weak	1
Moderate	2
Intense	3
PP	
No stained cells	0
<10%	1
10-50%	2
51-80%	3
>80%	4
IRS[a]	
Negative expression	0-1
Positive, weak expression	2-3
Positive, moderate expression	4-8
Positive, strong expression	9-12

[a]IRS ranges from 0-12 as a product of the multiplication of SI and PP values. SI, staining intensity; PP, percentage of positive cells.

expression was performed using the Fedchenko and Reifenrath immunoreactive scoring system (IRS), evaluating two parameters: The intensity of immunostaining and the percentage of positive tumor cells stained (Table III) (23). Fig. 1 shows a representative histopathology image from this study with an IRS score of 9 (strong positive) and Fig. 2 shows a representative image with an IRS score of 6 (moderate positive).

Statistical analysis. Data were analyzed using SPSS software version 26.0 (IBM Corp.). Categorical variables, including patients with GSTP1 and mutant p53 expression, chemotherapy response (based on Huvos grade) and metastatic status, are presented as frequencies and percentages, and were analyzed using Fisher's exact test. This test was chosen instead of the χ^2 test since >20% of the cells in several contingency tables had expected counts of ≤5, violating the χ^2 assumption. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics. The demographic and clinical characteristics of the 36 patients are summarized in Table IV. The most common location for osteosarcoma was the distal femur (52.8%). The majority of patients underwent surgical interventions, with 23 patients (63.9%) receiving amputations and 13 patients (36.1%) undergoing limb salvage surgery. Notably, 16 patients (44.4%) presented with metastatic osteosarcoma, while 20 patients (55.6%) had non-metastatic disease.

Analysis of p53 mutant-type expression and GSTP1 expression in association with chemotherapy response. p53 mutant-type and GSTP1 expression was characterized by histopathology using brown staining and was found in both the cell nucleus and cytoplasm. p53 mutant-type and GSTP1 immunoreactivity were evaluated using the IRS, and the analysis results are displayed in Table V.

In patients with a good chemotherapy response (n=6), a lack of GSTP1 expression was found in 2 patients (33.3%), while positive expression was found in 4 patients (66.7%). In those with a poor chemotherapy response (n=30), all 30 patients exhibited positive GSTP1 expression (100.0%). Using Fisher's exact test, the association between GSTP1 expression and chemotherapy response was determined to be significant (P=0.024).

A lack of mutant p53 expression was found in 2 patients (33.3%) with a good chemotherapy response, while positive expression was exhibited in 4 patients (66.7%). In those with a

Table IV. Demographic characteristics.

Characteristics	Value
Sex, n (%)	
Male	20 (55.6)
Female	16 (44.4)
Mean age ± SD, years	18.14±8.37
Location, n (%)	
Distal femur	19 (52.8)
Proximal humerus	4 (11.1)
Proximal tibia	7 (19.4)
Distal tibia	1 (2.8)
Pelvis	1 (2.8)
Calcaneus	1 (2.8)
Talus	1 (2.8)
Proximal femur	1 (2.8)
Distal radius	1 (2.8)
Subtype of osteosarcoma, n (%)	
Osteoblastic	21 (58.3)
Chondroblastic	2 (5.6)
Fibroblastic	3 (8.3)
Osteoblastic and chondroblastic	7 (19.4)
Fibroblastic and chondroblastic	2 (5.6)
Osteoblastic and fibroblastic	1 (2.8)
Lung metastasis, n (%)	
Metastasis	16 (44.4)
Without metastasis	20 (55.6)
Type of surgery, n (%)	
Limb salvage surgery	13 (36.1)
Amputation	23 (63.9)

Table V. Fisher's exact test analysis of p53 mutant-type expression and GSTP1 expression with regard to chemotherapy response.

	Chemotherapy response, n (%)		
Variable	Good	Poor	P-value
GSTP1 expression			0.024
Negative	2 (33.3)	0 (0.0)	
Positive	4 (66.7)	30 (100.0)	
Mutant p53 expression			0.024
Negative	2 (33.3)	0 (0.0)	
Positive	4 (66.7)	30 (100.0)	

GSTP1, glutathione S-transferase P1.

poor chemotherapy response, all 30 patients exhibited positive mutant p53 expression (100.0%). Using Fisher's exact test, the association between mutant p53 expression and chemotherapy response was determined to be significant (P=0.024).

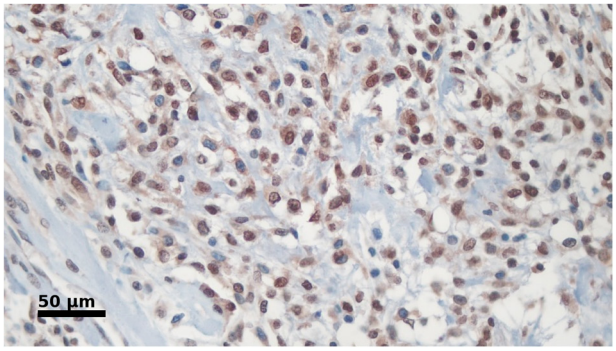

Figure 1. Nuclear and cytoplasmic positive expression of glutathione S-transferase P1 in tumor cells (brown color) with an immunoreactive scoring system score of 9 (positive, strong).

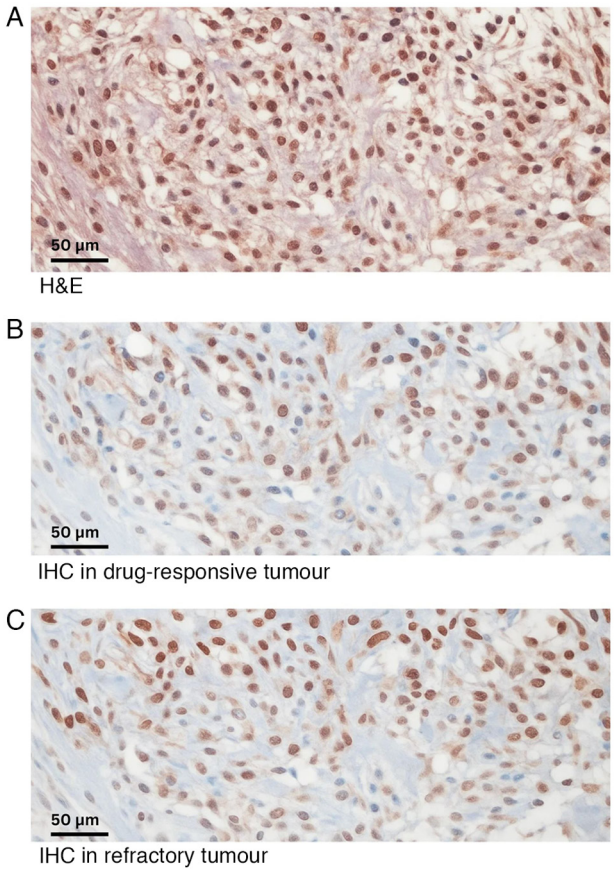

Figure 2. Nuclear and cytoplasmic positive expression of mutant p53 in tumor cells is shown in (A) H&E-stained osteosarcoma tissue, (B) a drug-responsive tumor with lower nuclear positivity and (C) a refractory tumor with stronger and more diffuse nuclear positivity (scale bar, 50 μm).

Analysis of p53 mutant-type expression and GSTP1 expression in association with metastasis. According to Table VI, only 1 patient (6.3%) with non-metastatic disease lacked the expression of GSTP1, while positive expression was observed in 15 patients (93.8%) with non-metastatic disease. Meanwhile, in patients with metastasis, a lack of GSTP1 expression was also observed in 1 patient (5.0%), while positive expression was exhibited by 19 patients (95.0%). Fisher's exact test revealed that there was no significant association between GSTP1 expression and the occurrence of metastasis (P>0.999).

Table VI. Fisher's exact test analysis of p53 mutant-type expression and GSTP1 expression with regard to metastasis.

	Metastasis, n (%)		
Variable	No	Yes	P-value
GSTP1 expression			>0.999
Negative	1 (6.3)	1 (5.0)	
Positive	15 (93.8)	19 (95.0)	
Mutant p53 expression			>0.999
Negative	1 (6.3)	1 (5.0)	
Positive	15 (93.8)	19 (95.0)	

GSTP1, glutathione S-transferase P1.

There was a lack of p53 mutant-type expression in 1 non-metastatic patient (6.3%), while positive expression was observed in 15 patients (93.8%). Meanwhile, in those with metastasis, 1 patient (5.0%) was negative for mutant p53 expression, while positive expression was exhibited by 19 patients (95.0%). Fisher's exact test revealed that there was no significant association between the expression of mutant p53 and the occurrence of metastasis (P>0.999).

Discussion

The present study findings reveal a significant and robust correlation between GSTP1 expression and mutant p53 expression in chemotherapy response. The tumor suppressor protein p53, encoded by the TP53 gene, plays a crucial role in regulating cellular responses to stress, particularly DNA damage. p53 is often referred to as the 'guardian of the genome' due to its role in preventing genomic instability by inducing cell cycle arrest, DNA repair, apoptosis or senescence in response to cellular stressors (24-26). The correlation between p53 status (wild-type vs. mutant-type) and response to chemotherapy in cancer treatment is complex and multifaceted; its status in cancer cells significantly affects the effectiveness of chemotherapy and radiation therapy, two main pillars of cancer treatment (27,28).

The present study cohort consisted predominantly of male adolescent cohort patients (mean age, 18 years), with tumors chiefly in the distal femur. High amputation rates (63.9%) reflected site-specific surgical constraints, yet the histopathological markers, mutant p53 and GSTP1, proved far more informative for treatment response than for metastatic behaviour. Both proteins were upregulated in every case with a poor chemotherapy response (IRS≥6), showing strong, significant associations with chemoresistance. This dichotomy underscores that chemo-protective mechanisms (e.g., defective apoptosis via mutant p53 or drug detoxification via GSTP1) operate independently of the biological pathways driving metastatic spread, highlighting the value of these markers for predicting chemoresponsiveness across clinical subgroups while signaling the need for additional molecular profiling to clarify determinants of metastasis in osteosarcoma.

The observation that mutant p53 and GSTP1 expression specifically predicts chemoresponse but not metastasis is consistent with the distinct prognostic roles of other established histopathological parameters. Beyond the Huvos score, which remains the standard measure of tumor necrosis, parameters such as post-treatment mitotic index and microscopic vascular invasion (MVI) are also prognostically significant. For example, a high mitotic index (≥10 mitoses/10 high-power field) in the resection specimen is an independent predictor of poor survival, reflecting a failure to achieve cell cycle arrest post-treatment (29). This aligns with the present molecular findings, as both indicate a chemoresistant phenotype. The prognostic utility of mitotic rate appears specific to post-treatment evaluation, as its value in pre-treatment biopsies has been shown to be limited (30).

Conversely, and in contrast to the present p53 and GSTP1 results, MVI is a powerful predictor of metastatic progression; its presence is strongly associated with higher rates of metastasis, local recurrence and mortality. This risk is markedly amplified in patients who are also poor chemo-responders, whose 5-year survival has been reported to be as low as 24% (31). This comparison reinforces the conclusion that the molecular mechanisms of chemoresistance, indicated in the present cohort by mutant p53 and GSTP1 expression, appear to be distinct from the biological pathways, such as MVI, that primarily govern metastatic dissemination.

This lack of association contrasts sharply with a recent study that estimated that the association of GSTP1 upregulation and mutant TP53 led to to a roughly two-fold increase in metastatic risk; therefore, the null finding of the present study most plausibly reflects the limited cohort size and relatively short follow-up, which together reduce power to capture late-emerging metastases (32). Future multicentre studies with larger samples and extended surveillance will be required to resolve this discrepancy.

Mutations in the TP53 gene often result in the loss of wild-type p53 function, which disrupts the ability of a cell to undergo apoptosis in response to DNA damage induced by chemotherapy (33). This loss of function can occur through various mechanisms, including disruptions in DNA binding, which prevents p53 from activating its target genes involved in cell cycle arrest and apoptosis (34,35). Mutant p53 can modulate the tumor microenvironment to create conditions that support tumor survival and growth, as well as resistance to chemotherapy (36). This includes promoting angiogenesis, immune evasion and the secretion of factors, such as IL-6, IL-8, HGF or IGF-1, that support tumor cell survival (37,38).

p53 mutations have been linked to increased expression of multidrug resistance proteins, such as P-glycoprotein, which can actively efflux chemotherapy agents from cancer cells, reducing their effectiveness (39). Additionally, p53 mutations can modulate the expression of genes involved in DNA repair, allowing cancer cells to survive and proliferate despite the genotoxic effects of chemotherapy (25).

Mutant p53 proteins often acquire gain-of-function (GOF) properties that contribute to tumor progression and chemotherapy resistance. These GOF properties include enhanced drug efflux, metabolism, promotion of survival, inhibition of apoptosis, increased DNA repair, suppression of autophagy, enhanced microenvironmental resistance and induction of a

stem-like phenotype. For example, specific p53 mutations, such as R175H and R273H, have been shown to increase resistance to chemotherapeutic agents, such as cisplatin and etoposide, by disrupting the normal p53-mediated apoptosis pathway (40,41).

These mutations can also affect the expression of various genes involved in metabolism and drug effects, further contributing to resistance. Specifically, in osteosarcoma, the R270C p53 mutant does not suppress the transcriptional activation function of wild-type p53, leading to resistance to doxorubicin, a common chemotherapy agent (42). Additionally, the expression of mutant p53 is associated with increased expression of ONZIN (Plac8), which contributes to osteosarcoma metastasis through the CXCL5-MAPK signaling pathway (43).

GSTP1 is a critical enzyme involved in the detoxification of xenobiotics and carcinogens by catalyzing the conjugation of glutathione to reactive intermediates. This enzyme plays a crucial role in cellular defense mechanisms against toxic and carcinogenic compounds. However, its expression in cancer cells has been associated with resistance to chemotherapy, affecting the efficacy of treatment various types of malignancies, including breast, ovarian, lung, colorectal, prostate, gastric and hepatic cancers, as well as leukemias, where elevated GSTP1 levels have been consistently linked to reduced sensitivity to commonly used chemotherapeutic agents (44,45).

GSTP1 is a phase II metabolic enzyme that catalyzes the conjugation of reduced glutathione to various substrates, including chemotherapy agents, leading to their detoxification and excretion. Upregulation of GSTP1 in osteosarcoma cells has been associated with an increased capacity to deactivate chemotherapy drugs, thereby reducing their cytotoxicity and effectiveness (46). A study indicated that osteosarcoma cells with increased GSTP1 expression exhibit resistance to various chemotherapeutic drugs, including doxorubicin, cisplatin and methotrexate, which are commonly used in the treatment of osteosarcoma (47).

The GSTP1 mechanism contributes to chemotherapy drug resistance by its ability to conjugate glutathione to chemotherapy drugs, thereby neutralizing their cytotoxic effects. This conjugation increases the solubility of the drugs, leading to their rapid elimination from cells and reducing the effects of their cytotoxic chemotherapy. For example, GSTP1 has been shown to detoxify platinum-based compounds, such as cisplatin and oxaliplatin, which are commonly used in the treatment of various types of cancer (13,48). Additionally, GSTP1 can modulate signaling pathways associated with cell survival and apoptosis. For instance, GSTP1 has been shown to inhibit the pathway of Jun N-terminal kinase, a protein involved in critical mediation of apoptosis or pro-apoptotic signaling, thereby promoting the survival of cancer cells from chemotherapy (49).

By contrast, pharmacogenetic investigations have centred on the germline Ile105Val (rs1695) polymorphism of GSTP1, yet its association with chemoresponse and survival has been inconsistent (50). Measuring GSTP1 at the protein level provides a more integrative read-out, as expression reflects not only such coding variants but also somatic copy-number changes, promoter methylation, NRF2-driven transcription and post-translational stabilisation during oxidative stress; this broader regulatory capture may explain why expression status, but not Ile105Val genotype alone, was robustly associated with chemoresistance.

In addition, in the present study, contrary results were found between GSTP1 expression and mutant p53 expression regarding metastasis events in patients with osteosarcoma at Cipto Mangunkusumo General Hospital, showing no significant associations. Several studies have explored the genetic and molecular underpinnings of GSTP1 expression in osteosarcoma, particularly its potential role in metastasis. A comprehensive study conducted by Li *et al* (50) investigated the role of genes involved in metabolic and transport pathways, including GSTP1, in osteosarcoma survival after chemotherapy. The findings revealed no significant association between GSTP1 expression and metastatic disease in patients with osteosarcoma. Similarly, a pharmacogenetic analysis of primary and metastatic osteosarcoma failed to identify GSTP1 among the genes whose expression is associated with metastasis (51).

The mechanistic role of GSTP1 in cancer metastasis remains unclear. Although its detoxification function could theoretically influence metastatic potential by altering the tumor microenvironment or modulating responses to chemotherapy, current studies have not established a direct mechanistic link between GSTP1 expression and metastatic behavior in osteosarcoma cells. Some investigations have examined GSTP1 in relation to chemoresistance and apoptosis, but they have not directly addressed its impact on metastasis. Although GSTP1 participates in detoxification and has been implicated in chemotherapy resistance, its direct contribution to osteosarcoma metastasis remains uncertain as available evidence has not demonstrated a clear association between GSTP1 expression and metastatic progression. The complexity of metastasis, which involves multiple genetic and environmental factors, likely diminishes the influence that any single gene, including GSTP1, may exert on metastatic outcomes (46,52).

The complexity of cancer metastasis, which involves many genetic and environmental factors, likely diminishes the potential impact of a single gene, such as GSTP1, on metastasis outcomes. Other studies suggest various mechanisms and pathways that could be more directly involved in the osteosarcoma metastasis process. For example, research has highlighted the importance of signaling pathways such as PI3K/AKT/mTOR and their roles in tumor growth and metastasis in osteosarcoma. These pathways may offer a more direct link to metastatic behavior than GSTP1 (53,54).

Several studies have investigated the correlation between p53 mutations and metastasis in osteosarcoma, with varying conclusions. For example, a study by Wunder *et al* (55) found no evidence that p53 mutations predict the development of metastases in patients with high-grade osteosarcoma. The p53 mutation status did not differentiate between patients who presented with localized or metastatic disease. Similarly, research by Overholtzer *et al* (56) reported that the p53 mutation status was concordant between primary tumors and matched metastases in patients with osteosarcoma, suggesting that p53 mutations are early events in tumorigenesis rather than drivers of metastasis.

Mutant p53 plays a critical role in osteosarcoma metastasis. The R270C mutant p53, equivalent to human R273C, has been shown to bind to chromatin near the transcription start sites of various genes, altering their expression. This mutant form

exhibits a different binding profile from wild-type p53 and does not suppress the transcriptional activation function of the wild-type protein in osteosarcoma cells. Although the deletion of this mutant p53 reduces tumor growth, it does not affect invasion or prevent metastasis *in vivo*. This suggests that while mutant p53 contributes to tumor growth, it may not directly affect the metastatic ability of osteosarcoma cells (42).

The clinical implication based on the results of the present study is that the expression of mutant p53 and GSTP1 can be used as for diagnostic purposes in patients with osteosarcoma, thereby serving as a predictor of chemotherapy response, prognosis and decision-making in the management of affected patients.

The present study focused on a specific population of patients with osteosarcoma, providing valuable insights within a defined geographic and demographic context, thus yielding relevant data for that population. Additionally, this study was more feasible to implement due to its cross-sectional design, which eliminates the need for long-term follow-up. Conducted at a single point in time, this study offers a faster and more cost-effective approach compared with longitudinal studies.

A key limitation of this study is its cross-sectional design, which involves a single data collection point, thereby providing only a snapshot of the population at a particular moment. This limitation is particularly pertinent in research involving dynamic biological processes, such as chemotherapy response or metastasis development, where longitudinal changes and trends may be crucial to understanding the underlying mechanisms. Moreover, the relatively small, single-centre cohort and the retrospective design may reduce statistical power, introduce selection bias and limit the generalisability of the study findings.

In conclusion, the present study found a significant association between mutant p53 expression and GSTP1 expression and poor chemotherapy response in patients with osteosarcoma. Meanwhile, the association between mutant p53 expression and GSTP1 expression and the occurrence of metastasis was not significant in patients with osteosarcoma. Further research should focus on longitudinal studies that track the expression levels of GSTP1 and mutant p53 from initial diagnosis to the development of metastasis and the treatments provided. Further research requires comprehensive genomic profiling studies to explore the interactions of GSTP1 and mutant P53 with other genes, as well as the signaling pathways involved in metastasis and chemotherapy response in osteosarcoma, to provide a more comprehensive understanding of the biology of this disease.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BP, AFK, EK, YP, AK, NCS, MNSBI, RM, IGEW and III were responsible for study design. BP, AFK and EK were responsible for data analysis. BP, AFK and EK wrote the manuscript. BP, AFK and EK confirm the authenticity of all the raw data. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of the Cipto Mangunkusumo General Hospital, Faculty of Medicine, University of Indonesia (Jakarta, Indonesia; approval no. KET-420/UN2.FI/ETIK/PPM/00.02/2023). Written informed consent was obtained from all participants, with parental or guardian consent obtained for those <18 years old.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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