Prognostic value of Serum miR-217 Level in Osteosarcoma Patients

Jia Ye
Wuhan University Renmin Hospital
Zhi-Hui Jin
Wuhan University Renmin Hospital
Ren Chen
Wuhan University Renmin Hospital
Sen Chen
Wuhan University Renmin Hospital
Yi-Jun Ren
Wuhan University Renmin Hospital
Wei-Chun Guo
Wuhan University Renmin Hospital
Hui He (hehui880@126.com)
First People's Hospital of Jiangxia District

Research article

Keywords: Serum miR-217, prognosis, osteosarcoma

Posted Date: March 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-16386/v1

Abstract

Background MicroRNA (miR)-217 is a tumor suppressor significantly associated with osteosarcoma. We try to evaluate serum levels miR-217 in osteosarcoma patients and evaluate its prognostic significance. Methods A total of 163 consecutive osteosarcoma patients and 96 healthy participates were enrolled. Serum miR-217 levels were evaluated by using real-time quantitative reverse transcription polymerase chain reactions(RT-PCR). The association between serum miR-217 level and survival outcomes was evaluated by univariate and multivariate analysis. Results Serum miR-217 levels in osteosarcoma patients was significantly lower than healthy volunteers ($P<0.05$). Low serum miR-217 was significantly related to advanced cancer and metastasis (both $P<0.05$). Moreover, patients with a low serum miR-217 had a poorer overall survival than those with a high serum miR-217 levels ($P<0.05$). Serum miR-217 level also been showed as independent risk factor for osteosarcoma in multivariate analysis (HR, 0.42; 95%CI: 0.12–0.98; $p<0.01$). Conclusions Serum miR-217 levels was significantly downregulated in osteosarcoma patients and remarkably associated with poor prognosis, indicating that serum miR-217 might serve as a useful diagnostic and prognostic indictor for osteosarcoma.

Introduction

Osteosarcoma is one of the most commonly diagnostic and lethal primary sarcoma of the bone in adolescents(1, 2). Despite considerable advancements of therapeutic and diagnostic strategies over the previous decades, the long-term prognosis of osteosarcoma remains unsatisfactory(3, 4). Recurrent and metastasis were considered the main contributors to the low long-term survival in patients with osteosarcoma(5, 6). Moreover, the potential molecular mechanisms underlying the histological heterogeneity, response to treatments and recurrent are still unclear. Therefore, furtherly understanding of the complex and definite molecular mechanisms concerning the progression and aggressiveness of osteosarcoma is important for risk stratification and individual treatment.

MicroRNAs (miRNAs) is a subset of endogenous small non-coding RNAs, which can specifically regulate gene expression by inhibiting the translation and/or decreasing of the stability of specific protein-coding gene(7, 8). Previous studies showed that dysregulation of miRNAs was significantly associated with development of various malignancies(9–12). It has been revealed that miRNAs can maintain the stable state in serum samples with appropriate and measurable concentration(13–15). In details, serum miRNAs can be resistant to endogenous ribonuclease activity by binding to specific proteins or being packaged into apoptotic bodies or exosomes(16, 17). Thus, various serum miRNAs expression have been confirmed as valuable indictors for diagnosis or prognosis of cancer(18, 19).

MiRNA-217 is a tumor suppressor miRNA targeting several oncogenes in different cell type(20, 21). MiR-217 could serve as a tumor suppressor which can inhibit tumor growth (22). Moreover, miR-217 could also function as a oncogene (23). However, no previous study has been showed to evaluate the value of miR-217 in osteosarcoma, especially for serum miR-217. Therefore, in current study, we try to evaluate serum level of miRNA-217 in osteosarcoma patients and analyze its prognostic value.

Materials And Methods

Patients

This study protocol was planned by basing on the relevant guidelines or regulations and conformed to the Declaration of Helsinki. All subjects have provided signed informed consent prior to enrollment. This study was also approved by the institutional review boards of the First People's Hospital of Jiangxia District. The data of 163 patients with primary osteosarcoma admitted to Department of Orthopaedics between July 1, 2014 and July 1, 2018 were retrospectively collected. Exclusion was conducted based on following criteria: preoperative comorbidity, relapse and metastasis, incomplete clinical and histopathological data and life expectancy less than 4 months. All enrolled patients received the standard preoperative neoadjuvant chemotherapy, then resection and postoperative chemotherapy according to the 2018 European Sarcoma Network Working Group Clinical Practice Guidelines for osteosarcoma(24). The tumor specimens of all patients were pathologically diagnosed as osteosarcoma. A control group enrolled 96 age and sex-matched healthy participates.

All relevant clinical and pathological data of each patient were collected and confirmed. The clincal stages were evaluated basing on the Enneking staging system (ESS). All Patients were regularly followed up though clinical visiting or telephone. Follow-up was lasted from the enrollment to death or June 2019. the primary outcome of interest was survival status. Overall survival (OS) was defined as from the date of enrollment to the date of death or endpoint.

Rna Isolation

Peripheral venous blood (5 mL) was collected from each subjects. Serum was extracted and transferred to RNase/DNase-free tubes and immediately stored at − 80 °C for further process. Total RNA was extracted from each serum sample by using of a miRNA easy Serum/Plasma Kit (Qiagen, Valencia, CA, USA). The RNA concentration and integrity were evauated by using a NanoDrop ND-1000 spectrophotometer (Nanodrop technologie, USA).

Quantification Of Mirna By Qrt-pcr

Total RNA from each subjects was used to reversely transcribe miRNAs to a strand cDNA by using a miScript Reverse Transcription Kit (Qiagen, Valencia, CA, USA). Amplifications were conducted by using a miScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA). The RT-PCR was performed on Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Sequence of primer for miR-217 was GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACG AAA CCC A. The miR-217 expression levels in each sample were normalized against the miR-16 expression. And the threshold cycle (Ct) values ≥ 40 was confirmed as an undetectable level. The relative expression level of serum miR-217 was quantitatively evaluated by using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

All statistical analyses were conducted by using the SPSS 20.0 (IBM, USA). The statistical results were considered as significant while $P < 0.05$ (two sided). Continuous variables that expressed as mean ± SD were compared by using of analysis of variance (ANOVA), whereas comparisons of categorical variables were conducted by using chi-square or Fisher's exact test, which were presented as frequencies (%). Receiver operating characteristic curve (ROC) analysis was performed to evaluate the prognosis value of serum miRNA-217 levels in predicting survival. Kaplan-Meier survival curves for serum miRNA-217 levels predicting survival were analyzed by log-rank test. Univariate and multivariate Cox hazard regression model was employed to evaluate the prognostic factors for osteosarcoma.

Results

Serum miR-217 expression in patients with osteosarcoma

Serum miR-217 level was significantly downregulated in osteosarcoma patients compared with the healthy subjects ($P < 0.05$, see Fig. 1). Furthermore, ROC curve analysis revealed that serum miR-217 level could be used to distinguish osteosarcoma patients from healthy subjects, with a sensitivity of 68.6% and a specificity of 73.2%. The area under the curve (AUC) was 0.75 (95%CI:0.66–0.96, $P < 0.05$, Fig. 2).

Relationship Between Serum 217 And Clinical Characteristics

The median value of serum miR-217 level in all 163 osteosarcoma patients was considered as the cut-off point to divide patients into the high miR-217 group (n = 77) or low miR-217 group(n = 86). The association between serum miR-217 levels and clinical characteristics were evaluated by using Chi-squared test. A statistically significant difference was observed between a high serum miR-217 levels and distance metastasis and clinical stage (both $P < 0.01$, Table 1). However, other clinical variables, including age, sex, tumor site and size, and pathology were not closely associated with serum miR-217 expression (all $p > 0.05$). (Table 1).

Table 1
Correlation between serum miR-217 level and clinicpathologic characteristics of osteosarcoma patients

Characteristic	Serum miR-217		p
	High (n = 77)	Low (n = 86)	
Age(years)			0.63
≥25	36	37	
25	41	49	
Gender			0.26
Male	54	67	
Female	23	19	
Tumor site			0.48
Tibia/femur	46	56	
other	31	30	
Tumor size (cm)			0.90
≥8	33	36	
8	44	50	
Distant metastasis			< 0.01
Yes	18	54	
No	59	32	
Clinical stage			< 0.01
IIA	54	16	
IIB + III	23	70	
Pathology			0.10
Osteogenic or chondrocytic	46	62	
Fibrocytic or mixed	31	24	

Prognostic significance of serum miR-217 level in osteosarcoma patients

The results of Kaplan–Meier method and log-rank test showed that patients with a low expression of serum miR-217 had a significantly poorer OS than those with a high expression of miR-217 (p = 0.03,

Fig. 3.).

The univariate and multivariate analysis enrolled age and gender of patients, tumor size, distant metastasis, clinical stage, histological type and serum miR-217 level to determine independent prognostic indictor for osteosarcoma patients. After adjustment for potential confounders, a low serum miR-217 level (hazard ratio, 0.42; 95% confidence interval [CI]: 0.12–0.98, P < 0.01) was also an independent predictive factor for survival outcome of osteosarcoma patients (Table 2.)

Table 2
The prognostic factor of osteosarcoma patients

	Univariate			**Multivariate**		
	HR	95%CI	p	HR	95%CI	p
Age(years)	1.21	0.72–2.26	0.52			
Gender	1.23	0.66–2.28	0.91			
Tumor site	1.01	0.52–1.62	0.84			
Tumor size	1.62	0.89–3.55	0.18			
Distant metastasis	0.72	0.32–0.99	0.02	0.68	0.22–0.96	< 0.01
Clinical stage	1.68	1.01–4.28	0.01	1.26	0.92–2.13	< 0.01
Pathology	1.22	0.71–2.27	0.63			
Serum miR-101	0.49	0.22–0.99	0.03	0.42	0.12–0.98	< 0.01
CI, confidence interval; HR, hazard ratio; miR-217, micro RNA-217.						

Discussion

In this study, the results showed that the serum level of miR-217 was significantly decreased in osteosarcoma patients comparing with healthy participates. Moreover, serum miR-217 level can be used as a superior marker to discriminate osteosarcoma patients from healthy subjects. Furthermore, we evaluated the significance of serum miR-217 in predicting prognosis of osteosarcoma patients and found that a low serum miR-217 level was significantly associated with shorter survival in osteosarcoma patients. We also observed a significant association between serum miR-217 level and classical unfavorable clinical characteristics for osteosarcoma patients. Based on such results, we suggested that miR-217 can be used as a diagnostic biomarker for osteosarcoma patients, which also can serve as a promising prognostic indictor.

MiR-217, as a novel tumor biomarker, play critical roles in biological process of cancer development(25). The miR-217 has been confirmed as a potential tumor suppressor in many malignancies including osteosarcoma(26, 27). Shen et al. reported that miR-217 was decreased both in cancer cell lines and

tissues, which was significantly correlated with distant metastasis, and functioned as a tumor suppressive miRNA and inhibits the osteosarcoma tumorigenesis through targeting WASF3(28). Moreover, miR-217 may be involved in inhibiting of tumor cells proliferation and metastasis through targeting KRAS oncogene(29). In our study, miR-217 was remarkably downregulated in osteosarcoma patients and significantly correlated with poor prognosis, which are consistent with the previous studies mentioned above.

However, the underlying function and origin of serum miR-217 in malignancy have not yet been fully understood. Several potential mechanisms for circulating miRNAs releasing have been reported, including passive leakage from cells in setting of chronic inflammation or injury, active secretion, complex formation with lipoproteins or RNA binding proteins(30–32). Yan et al. reported that serum miR-217 expression was significantly decreased in acute myeloid leukemia (AML) patients compared to controls, which was identified as an independent marker for the diagnosis and prognosis of AML(33). It has been reported that low expressions of certain miRNAs were remarkably associated with advanced cancer stage(34). In this study, we found that low serum level of miR-217 was significantly associated with clinical stage of osteosarcoma patients, which is consistence with previous studies.

Our study firstly reported that the downregulation of serum miR-217 level in a considerable scale osteosarcoma patients group, which can serve as a serum diagnostic and prognostic biomarker, as well as a novel therapeutic target for osteosarcoma. However, there were several limitations in this study. One limitation was a single center, small sample size and retrospective design of study. A large-scale, prospective and multicenter study is required to furtherly reevaluate such results. Furthermore, the underlying roles and mechanisms of miR-217 in development of osteosarcoma have not yet been fully evaluated. Future experiments are needed to be performed to elucidate the mechanisms of serum miR-217 in carcinogenesis.

Conclusions

In this study, we found that serum miR-217 levels were downregulated in osteosarcoma patients. Moreover, low serum miR-217 level was significantly associated with poor survival of osteosarcoma patients, indicating that miR-217 acting as a tumor suppressor might not only serve as a diagnostic and prognostic indictor for osteosarcoma, but also a potential novel treatment target.

List Of Abbreviations

MiR, microRNA; RT-PCR, real-time quantitative reverse transcription polymerase chain reactions; ESS, Enneking staging system; OS, overall survival; ROC, receiver operating characteristic curve; AUC, area under the curve.

Declarations

Ethics approval and consent to participate:

This study was approved by the institutional review boards of the First People's Hospital of Jiangxia District.

Consent for publication

All subjects have provided signed informed consent prior to enrollment.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

There was no funding for this study.

Authors' contributions

J.Y., Z.H.J., R. C., S. C. analyzed and interpreted the patient data, study concepts and manuscript editing. Y.J.R. and W.C.G. for manuscript editing and statistical analysis. H. H. for manuscript review. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Zhou J, Xiao X, Wang W, Luo Y. Association between PTEN and clinical-pathological features of osteosarcoma. Biosci Rep. 2019;39(7).
2. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin. 2019.
3. Gao F, Xu F. Reduced expression of miR-564 is associated with worse prognosis in patients with osteosarcoma. Eur Rev Med Pharmacol Sci. 2018;22(18):5851-6.
4. Han X, Wang W, He J, Jiang L, Li X. Osteopontin as a biomarker for osteosarcoma therapy and prognosis. Oncology letters. 2019;17(3):2592-8.
5. Simpson S, Dunning MD, de Brot S, Grau-Roma L, Mongan NP, Rutland CS. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics.

Acta Vet Scand. 2017;59(1):71.

6. Smeland S, Bielack SS, Whelan J, Bernstein M, Hogendoorn P, Krailo MD, et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort. Eur J Cancer. 2019;109:36-50.
7. Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis. 2015;35(1):3-11.
8. Sheu-Gruttadauria J, Pawlica P, Klum SM, Wang S, Yario TA, Schirle Oakdale NT, et al. Structural Basis for Target-Directed MicroRNA Degradation. Mol Cell. 2019.
9. Jakob M, Mattes LM, Kuffer S, Unger K, Hess J, Bertlich M, et al. MicroRNA expression patterns in oral squamous cell carcinoma: hsa-mir-99b-3p and hsa-mir-100-5p as novel prognostic markers for oral cancer. Head Neck. 2019.
10. Kim DH, Khan H, Ullah H, Hassan STS, Smejkal K, Efferth T, et al. MicroRNA targeting by quercetin in cancer treatment and chemoprotection. Pharmacol Res. 2019;147:104346.
11. Liu JB, Yan YJ, Shi J, Wu YB, Li YF, Dai LF, et al. Upregulation of microRNA-191 can serve as an independent prognostic marker for poor survival in prostate cancer. Medicine (Baltimore). 2019;98(29):e16193.
12. Li Y, Sun H, Guan J, Ji T, Wang X. Serum microRNA-381: A Potential Marker for Early Diagnosis of Gastric Cancer. Yonsei Med J. 2019;60(8):720-6.
13. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6(11):857-66.
14. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513-8.
15. Ichikawa D, Komatsu S, Konishi H, Otsuji E. Circulating microRNA in digestive tract cancers. Gastroenterology. 2012;142(5):1074-8 e1.
16. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011;108(12):5003-8.
17. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011;13(4):423-33.
18. Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci. 2010;101(10):2087-92.
19. Zhang J, Wang T, Zhang Y, Wang H, Wu Y, Liu K, et al. Upregulation of serum miR-494 predicts poor prognosis in non-small cell lung cancer patients. Cancer Biomark. 2018;21(4):763-8.
20. He S, Wang Z, Tang H, Dong J, Qu Y, Lv J. MiR-217 Inhibits Proliferation, Migration, and Invasion by Targeting SIRT1 in Osteosarcoma. Cancer Biother Radiopharm. 2019;34(4):264-70.
21. Jiang B, Zhu SJ, Xiao SS, Xue M. MiR-217 Inhibits M2-Like Macrophage Polarization by Suppressing Secretion of Interleukin-6 in Ovarian Cancer. Inflammation. 2019.

22. Li J, Li D, Zhang W. Tumor suppressor role of miR-217 in human epithelial ovarian cancer by targeting IGF1R. Oncol Rep. 2016;35(3):1671-9.
23. Lin Y, Cheng K, Wang T, Xie Q, Chen M, Chen Q, et al. miR-217 inhibits proliferation, migration, and invasion via targeting AKT3 in thyroid cancer. Biomed Pharmacother. 2017;95:1718-24.
24. Casali PG, Bielack S, Abecassis N, Aro HT, Bauer S, Biagini R, et al. Bone sarcomas: ESMO-PaedCan-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29(Supplement_4):iv79-iv95.
25. Safaralizadeh R, Ajami N, Nemati M, Hosseinpourfeizi M, Azimzadeh Isfanjani A, Moaddab SY. Disregulation of miR-216a and miR-217 in Gastric Cancer and Their Clinical Significance. J Gastrointest Cancer. 2019;50(1):78-83.
26. Su J, Wang Q, Liu Y, Zhong M. miR-217 inhibits invasion of hepatocellular carcinoma cells through direct suppression of E2F3. Mol Cell Biochem. 2014;392(1-2):289-96.
27. Wang B, Qu XL, Liu J, Lu J, Zhou ZY. HOTAIR promotes osteosarcoma development by sponging miR-217 and targeting ZEB1. J Cell Physiol. 2019;234(5):6173-81.
28. Shen L, Wang P, Yang J, Li X. MicroRNA-217 regulates WASF3 expression and suppresses tumor growth and metastasis in osteosarcoma. PLoS One. 2014;9(10):e109138.
29. Zhao WG, Yu SN, Lu ZH, Ma YH, Gu YM, Chen J. The miR-217 microRNA functions as a potential tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS. Carcinogenesis. 2010;31(10):1726-33.
30. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18(10):997-1006.
31. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal. 2009;2(100):ra81.
32. Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res. 2010;38(20):7248-59.
33. Yan J, Wu G, Chen J, Xiong L, Chen G, Li P. Downregulated miR-217 expression predicts a poor outcome in acute myeloid leukemia. Cancer Biomark. 2018;22(1):73-8.
34. Cheng G. Circulating miRNAs: roles in cancer diagnosis, prognosis and therapy. Adv Drug Deliv Rev. 2015;81:75-93.

Figures

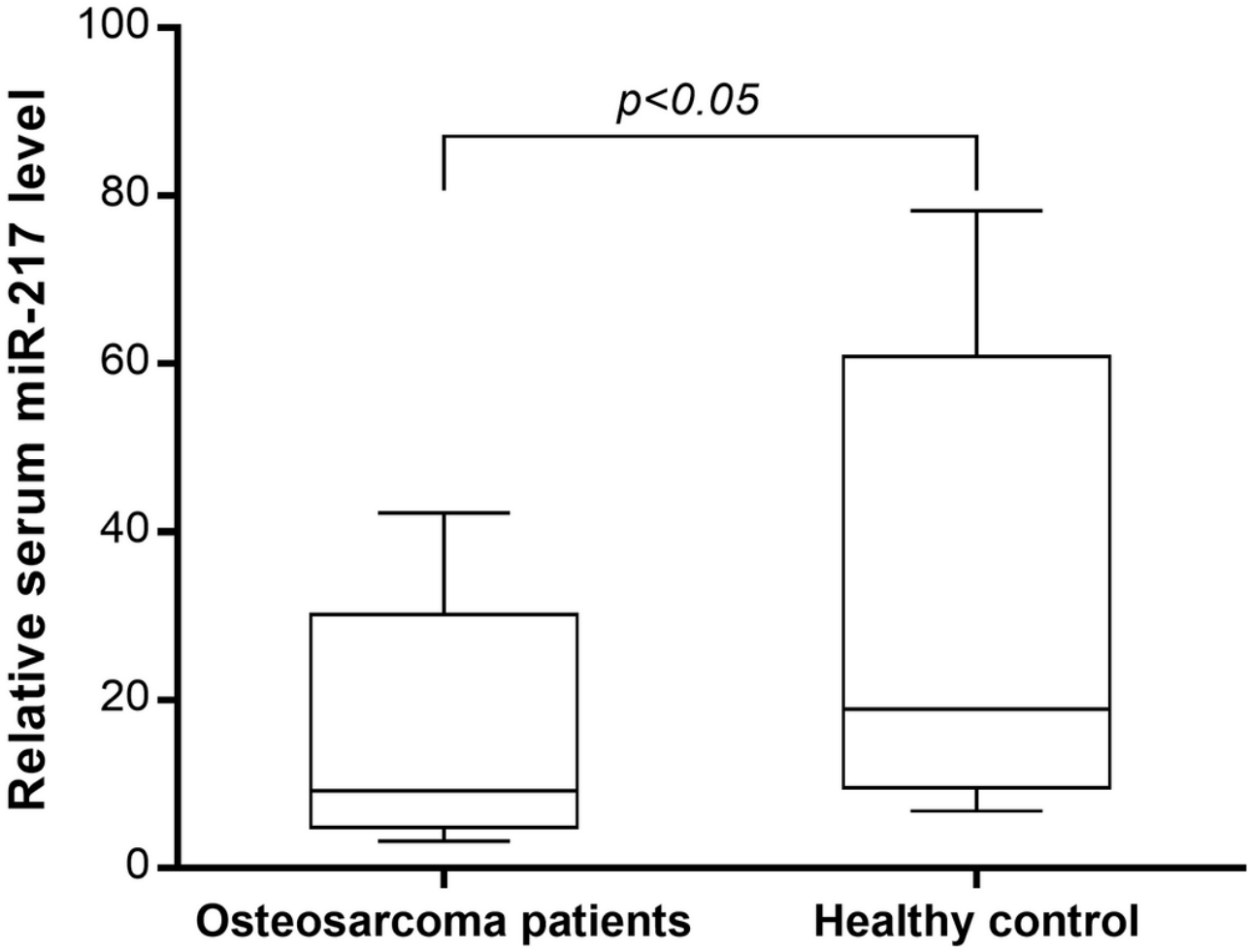

Figure 1

Serum miR-217 level in osteosarcoma patients and healthy controls. The mean serum miR-217 level of 163 osteosarcoma patients was significant lower than that of 96 age-matched healthy volunteers (P<0.05).

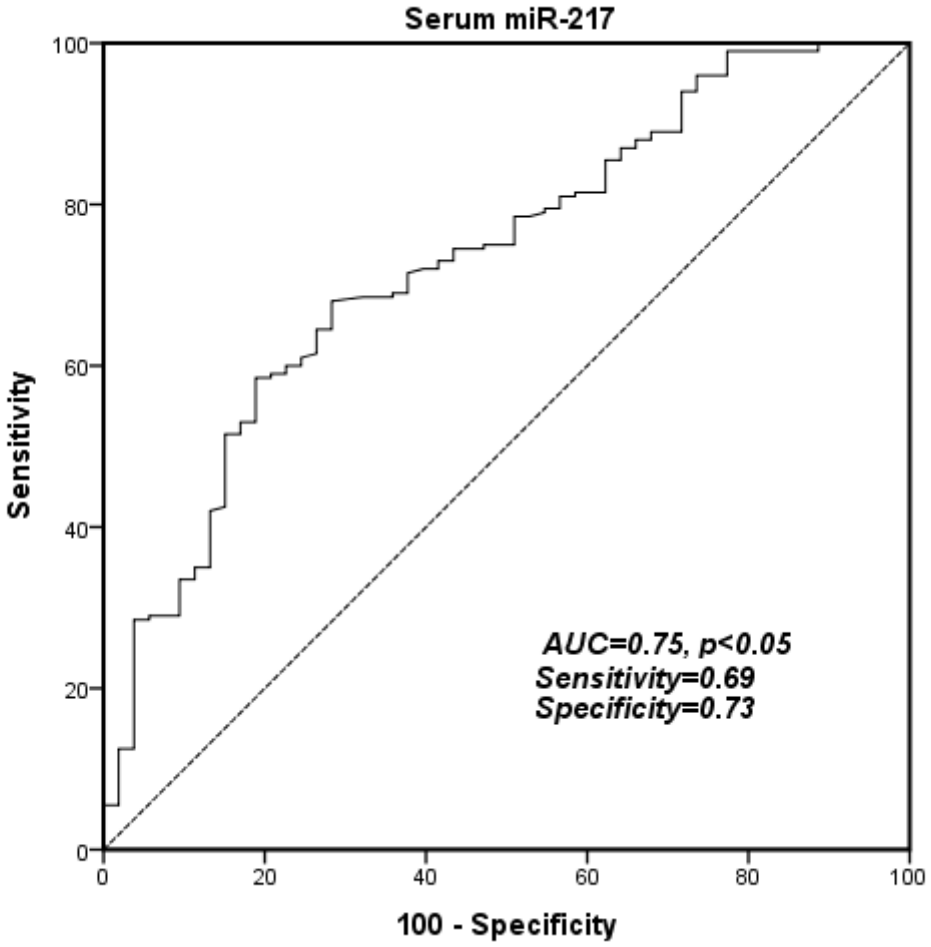

Figure 2

Receiver-operator characteristic curve for osteosarcoma detection. ROC analysis showed a AUC of 0.75 for miR-217 with a 95% confidence interval between 0.66 and 0.96, P<0.05.

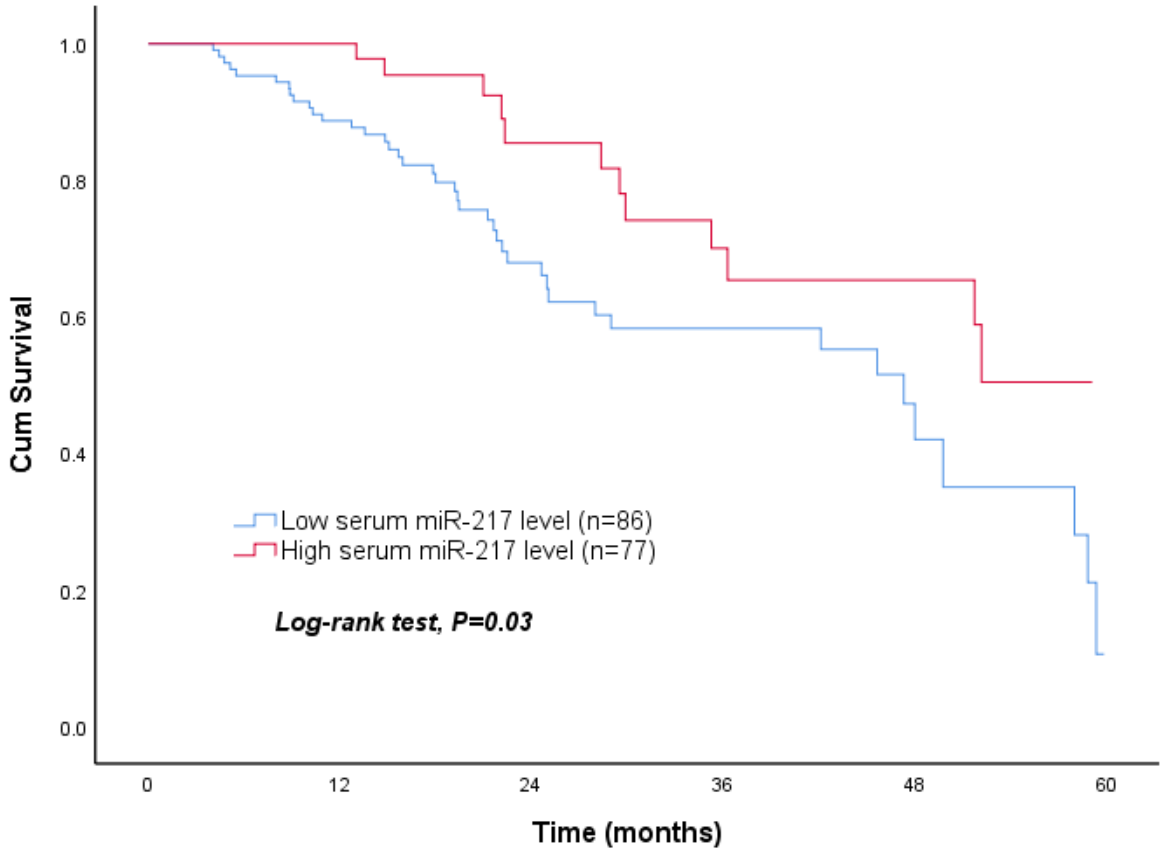

Figure 3

Lower serum miR-217 level was associated with poorer prognosis for osteosarcoma. The Log-rank analysis revealed that a low serum miR-217 level was significantly associated with a poor overall survival (P = 0.03).