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Exploring the impact of demographic and environmental factors on interferon–γ expression in bone and soft tissue tumor: A case-control study

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ABSTRACT

Bone and soft tissue tumor are rare but have a high morbidity and mortality rate, with a recent increase in incidence. Interferon–γ, a part of the immune cell microenvironment, plays a crucial role in tumor development. However, the expression level of IFN–γ in both benign and malignant tumor is unclear. The impact of age, sex, and demographic-environmental factors on IFN–γ expression is also unknown. Therefore, a case-control study was conducted using tissue blocks from patients with bone and soft tissue tumor. Tissue blocks were divided into malignant (cases) and benign (controls). Independent variables include distance to shoreline, population density, elevation, as well as distance to farmland, urban center, and river. Linear, Tobit, and robust regression analyses were also performed to assess the effect of various demographic and environmental factors on IFN–γ expression levels. The results showed that there was a significant association between malignancies and population density, with positive associations for malignancies and population density, and negative for distance to shoreline. Malignant bone and soft tissue sarcoma type had higher IFN–γ expression than the benign type. Furthermore, IFN–γ expression was positively associated with population density and negatively associated with distance to shoreline. In conclusion, this comprehensive approach allows for further investigation into how proximity to different surroundings and demographic features influences tumor biological behavior, potentially informing prognosis and personalized treatment methods.

Keywords: bone and soft tissue tumor; demographic-environmental determinants; interferon–γ; malignancies

1. INTRODUCTION

Bone and soft tissue tumor are rare but have a high morbidity and mortality rate, with a recent increase in incidence despite advances in treatment (Bessen et al., 2019; Bourcier et al., 2019; Lupo et al., 2021). The state of the immune cell microenvironment has a strategic role for the benefit of disease management, ranging from early detection, diagnosis, prevention, clinical predictors to therapy (Alves et al., 2019; Dancsok et al., 2019; Hohtari et al., 2019; Romadhon & Kurniati, 2024; Stahl et al., 2019). In this context, IFN–γ is one component of the immune cell microenvironment worth exploring. IFN–γ is a pleiotropic cytokine, known to play a role in antitumor mechanisms in cell-mediated immune responses (Liu et al., 2023; Lyu et al., 2023). High levels have been cited as a good predictor of response to combination therapy in gastric cancer (Li et al., 2023). Although IFN–γ is an antitumor immune factor, knowledge on the protumor also has a long history. The protumor effect of IFN–γ when under certain circumstances, is found in the disruption of the inhibitory feedback mechanism.

High levels induce PDL-1 expression, enabling tumor cells to escape immune system attack (Jorgovanovic et al., 2020; Yang et al., 2021). Chronic exposure also induces the selection of tumor clones with a more malignant phenotype. This gives rise to the "dark side" of IFN–γ into protumor factor (Jorgovanovic et al., 2020). Another mechanism is through suppression or loss of receptors and downstream signalling mediators (Ilyushin et al., 2023). Data regarding differences in IFN–γ levels across various types of cancer have inconclusive results. Studies on gastrointestinal stromal tumor (GISTs) found that serum levels of IFN–γ, along with ICAM-4 and vitamin D3, were significantly higher in malignant than benign types (Al-Shimmery et al., 2023). A comparative study of IFN–γ levels from bronchoalveolar lavage between malignant vs benign lung cancer found that there was no significant difference between the two (Malignant vs. Benign = 57.2 ± 26.8 vs 68.8 ± 32.9, p = 0.121) (Hogea et al., 2023). Studies on thyroid adenoma tumor found differences between poor vs good prognosis groups, where serum IFN–γ levels were significantly higher in good prognosis(Min et al., 2021). Furthermore, evidence on how IFN–γ affects primary bone tumor is limited. One study on existing primary bone tumor found that in malignant tumor types, high PDL-1 expression was found, followed by high serum IFN–γ levels (Eghtedari et al., 2023). However, this study has not evaluated how IFN–γ expression in various bone tumor tissues. The mechanism of IFN–γ levels in the primary tumor of bone tissue and soft tissue has not been explored.

Environmental pollution is a major cancer trigger and has a strong correlation with increased cancer mortality (Cazzolla Gatti et al., 2023; Kurniati & Nafiah, 2019; Romadhon et al., 2024). Epidemiologically, poor air quality has the strongest correlation with cancer mortality (Cazzolla Gatti et al., 2023; Turner et al., 2020). Evidence from epidemiologic studies suggests an understanding of the mechanisms linking toxic substances to disease progression. This suggests that exposure to environmental pollutants increases the risk of developing cancer. Water pollutants also play an important role in increasing the risk of cancer. On the other hand, more than 80% of waste from human activities (anthropogenic pollutants) is discharged into rivers and oceans without any treatment. This action results in environmental pollution, which epidemiologically causes more than 50 diseases, including cancer (Lin et al., 2022). The dominant water pollutants as cancer risk factors include heavy metals, insecticides, fertilizers, nanomaterials, and pharmacological substances (Chen et al., 2022a; De et al., 2023; Garg et al., 2018; Jiang et al., 2022; Luo et al., 2020; Mbedzi et al., 2020; Priyono et al., 2022; Ranjani et al., 2021). Moreover, the amount of waterborne pollutants accumulated in rivers, estuaries, and beaches near estuaries is directly proportional to the density of the surrounding population (De et al., 2023; Mbedzi et al., 2020; Priyono et al., 2022; Ranjani et al., 2021). The higher the amount of pollutants in the river water further downstream, the greater the pollution load. This phenomenon indicates that household, agricultural, and industrial pollutants discharged in rivers accumulate downstream, namely at the mouth of the river that goes to the coast (Chen et al., 2022b; Garg et al., 2018; Luo et al., 2021). Increasing industrial activities will potentially elevate the use of metals, leading to accumulation in coastal areas and oceans. Metals are considered highly toxic pollutants due to the non-degradable nature. In general, metal pollutants used by industry include mercury (Hg), hexavalent chromium (Cr(VI)), arsenic (As), cadmium (Cd), copper (Cu), Lead (Pb), Zinc (Zn), and Nickel. Most of these materials are toxic and carcinogenic (Damaianto & Masduqi, 2014). Various studies suggest that alarming conditions occur in Indonesia, especially on the island of Java (Hiwari et al., 2019). A study conducted on the coast of Tuban found that the area was mildly to moderately polluted (Damaianto & Masduqi, 2014). Similar conditions were also found in Kupang and Rote, East Nusa Tenggara Province (Hiwari et al., 2019). Investigative studies on the island of Bali regarding the influence of micrologams and microplastics also showed that marine biota were polluted with these pollutants (Siregar & Soegianto, 2024).

Based on available evidence, areas with a high probability of exposure to these pollutants were defined as proximity to the shoreline, river, residential population density, city center, agricultural areas, and elevation, with upstream water sources being higher, while downstream are lower. Adyasari et al. (2021) explained the contamination of downstream areas or river estuaries, and beaches in Indonesia. The locations overlap with the residences of certain patients evaluated in this study.

Studies have shown that microplastics can infiltrate cells, disrupt biological processes, and potentially trigger a carcinogenic environment. The process starts with DNA damage and oxidative stress, triggering an inflammatory response and disrupting cellular pathways. Moreover, microplastics have multifaceted capabilities towards cancer development (Kumar et al., 2024). These environmental contaminants modulate epigenetic control of gene expression, inflammatory mediator status, redox homeostasis, cell cycle proteins, and mimic endocrine mediators such as estrogen and androgen to trigger carcinogenesis (Goswami et al., 2024). A study conducted in the USA found that variants in the promoter sequence of the cytokine IL-6 (interleukin 6) modulate gene transcription and response to environmental carcinogens. According to in-vitro evidence, the rs1800797 variant found in the sample will modulate the expression of IL-6, which plays a role in the pathogenic mechanism associated with squamous cell lung cancer in uranium miners (Leng et al., 2016).

Based on the description above, this study aimed to assess the association between IFN–γ expression in bone and soft tissue tumors with several demographic and environmental factors. The difference in IFN–γ expression between malignant and benign bone and soft tissue tumor was examined, as well as associated factors including age, sex, and demographic-environmental factors, namely residential population density, proximity to the city center, farmland, shoreline, elevation, and distance from the river.

2. MATERIALS AND METHODS

2.1 Study framework

This study examined the association between IFN–γ expression in bone and soft tissue tumor as well as various demographic and environmental variables (Figure 1). The framework used geospatial and statistical methodologies to classify and geocode tumor instances to extract distances from coastlines, farmlands, metropolitan centers, and rivers, as well as population density and elevation information. Moreover, the Google Geocoding API (application programming interfaces) and MODIS (moderate resolution imaging spectroradiometer) satellite imagery data were used to calculate Euclidean distances. Regression methods, such as Tobit, and robust, were then applied to uncover significant associations. This approach allows comprehensive investigation of how proximity to different surroundings and demographic features influences tumor biological behavior, potentially informing prognosis and personalized treatment methods. Ethical considerations were strictly followed, preserving the confidentiality and privacy of patient data throughout the study process.

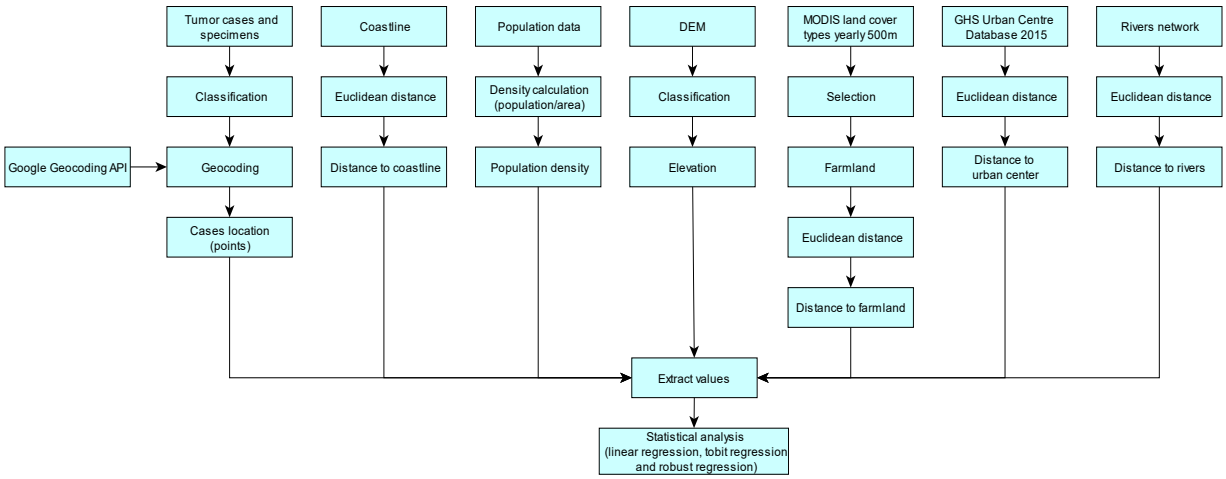

Figure 1. Environmental factor acquisition process

2.2 Tumor data

The tumor specimens were obtained from patients treated at the Department of Oncology, National Orthopedic Hospital at Surakarta in 2021. The study defined the diagnosis into four groups, namely Giant cell tumor of bone, osteosarcoma, benign soft tissue tumor, and soft tissue sarcoma. Interferon–γ expression.

Three stages were carried out in the immunohistochemistry examination of IFN–γ, namely sample preparation, slide processing, and result reading. The preparation stage started with the selection of specimens taken from histopathology, with two categories of diagnosis, namely bone and soft tissue tumor, according to the WHO classification. Identification was then carried out to confirm that the preparations were primary tumor, not a metastasis, infectious lesion, or tumor-like lesion. Afterwards, each category was differentiated between benign and malignant, in order of the most cases. The benign bone tumor category was represented by samples with a diagnosis of giant cell tumor, while malignant ones originated from preparations with a diagnosis of osteosarcoma. Soft tissue tumor preparation categories, both benign and malignant, were each selected in the order of the three most common cases. This first selection yielded 35 cases of giant cell tumor, 28 osteosarcoma, 18 cases of soft tissue sarcoma, and a total of 16 cases of benign soft tissue tumor.

The slides of all these categories were collected to check the quality (adequate) and availability of the tumor cells present. The selected preparation contained at least 200 viable tumor cells and less than 25% necrosis area. This is important because it will affect the results of immunohistochemistry staining. The microscopic quality of the preparations in the slides is shown in Figure 2.

The painting process was carried out in the Anatomical Pathology Laboratory of Universitas Sebelas Maret, while the reading stage was conducted in the Anatomical Pathology Laboratory of Universitas Muhammadiyah Surakarta by a competent pathologist. The reading was performed randomly and blindly (slide numbers were closed) from 60 existing immunohistochemistry slides. Interferon–γ immunohistochemistry examination.

IFN–γ expression was determined using the immunohistochemistry staining technique, which is an immune morphology analysis (Li & Yang, 2020). This examination used the rabbit polyclonal antibody IFN–γ (Catalog No. A12450SP142, ABclonal Systems, Inc.) an antibody which belongs to interferon class II, with gene ID 3458 derived from recombinant protein fusion containing amino acid sequences 24-166 of human IFN–γ. In the negative control, the primary antibody was omitted. Preparations from rat lung organs were used as positive controls, as mentioned in the manufacturer recommendations.

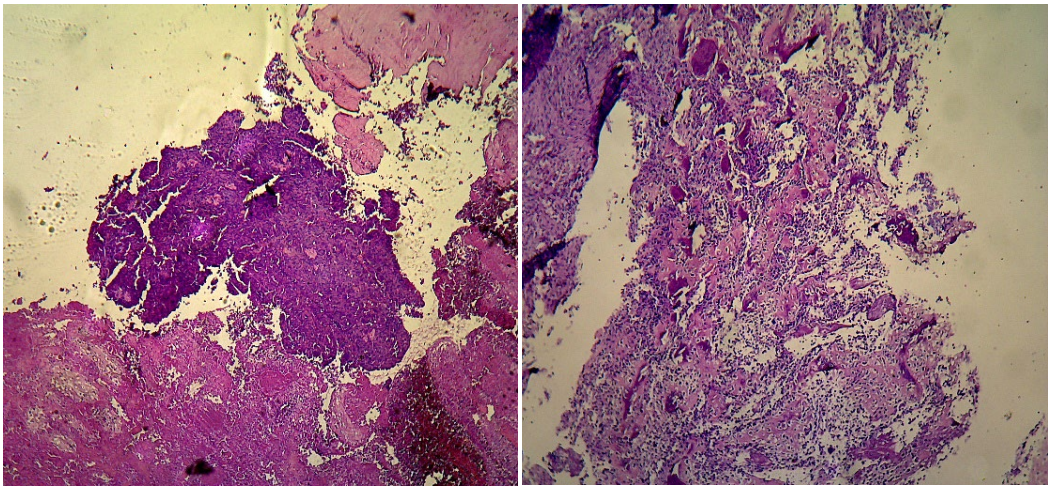

Figure 2. Microscopic of tumor, HE slide, 40x magnification
Note: There are areas of necrosis > 50% of a small field of view (left). It is not good for immunohistochemistry examination. There are more than 200 viable cells with a few necrotic areas (right).

Immunohistochemical examination of IFN–γ expression was performed directly on histopathological tissue blocks of bone tumor and soft tissues with IF concentration 1:100. The procedures started with cutting blocks using a thickness of 4–5 µm, placing on poly-L-lysine slides, followed by incubation at 37°C overnight, and deparaffinization. Antigen retrieval was carried out in a microwave oven with Tris EDTA (ethylene diamine tetraacetic acid) pH 9 at a temperature of 90°C for 3 min. The procedure continued at low temperature for ± 15 min, closed with mounting, and covered with deck glass. The primary data obtained pertains to the scoring of tumor cells positively stained for the IFN–γ antibody in the sample. A positive result was indicated by the cytoplasm of the cells being stained brown (Figure 3).

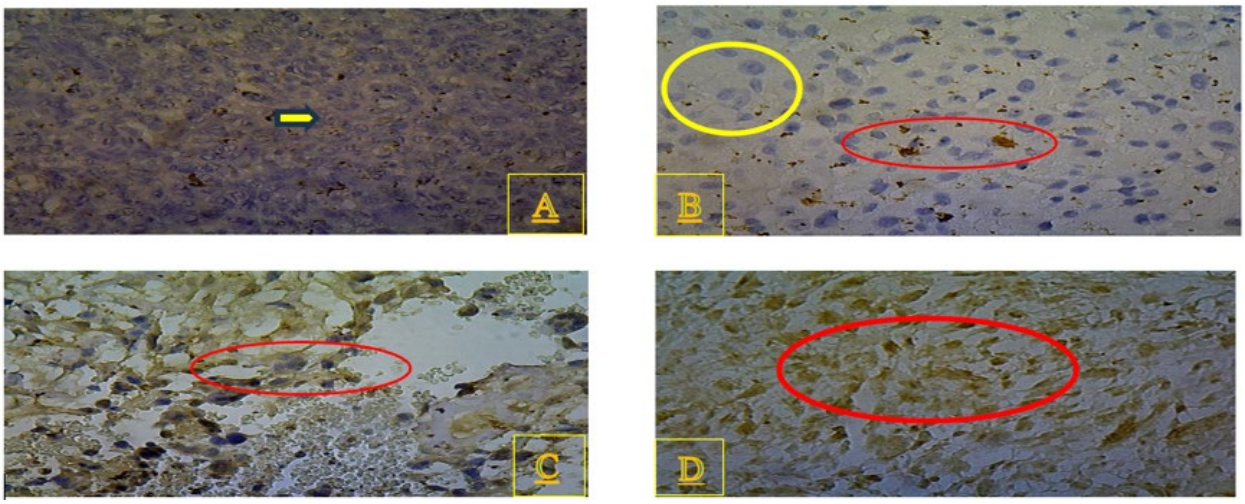

Figure 3. Immunohistochemistry Interferon-γ results with 40x magnification; (A) score 0, (B) score 1, (C) score 2, and (D) Score 3
Note: Yellow marker for negative expression cells and red marker for positive expression cells

IFN–γ staining results were assessed using a semi-quantitative method with the determination of an histo-score (H-score), as shown in Table 1. The H-score for IFN–γ is calculated by summing the products of the percentage of cells stained at each intensity level and the weighted intensity of staining. The evaluation was performed by randomly selecting and assessing 10 fields of view (at 400X magnification) for each slide. The mean values were calculated for each group, and a positive result was indicated when the mean expression of IFN–γ staining was greater than zero. Conversely, a negative result was determined when the mean expression of IFN–γ staining was equal to zero out of 10 fields of view (Kurniati et al., 2017; Price et al., 2023).

Table 1. Semi-quantitative method for determining the H-score of IFN–γ expression

IFN–γ expression score	Number of cells reflected / large field of view (400x magnification)
0	0–25%
1	26–50%
2	51–75%
3	76–100%

(Kurniati et al., 2017)

2.3 Covariates

Six demographic-environmental variables were used based on the previous studies (Romadhon et al., 2024) (Figure 4). These variables include (a) distance to shoreline (km), (b) population density (people/km^2), (c) elevation (meter above sea level), (d) distance to farmland (km), (e) distance to urban center, and (f) distance to river. Population density is defined as the concentration of people living in a certain area. It is calculated using the ratio of the number of people living in a predetermined area, such as sub-district or village (Leurent, 2022; Salvucci et al., 2023). In this study, the population density was generated based on the government report (Badan Pusat Statistik Indonesia, 2021). The distance between the residence and the shoreline was determined based on the Euclidean distance between the cancer incident and the shoreline, generated from the topographic map (Almeida et al., 2021; García-Rubio et al., 2015; Verrou et al., 2023). Furthermore, the elevation of the residence was determined based on digital elevation model of SRTM 30-m resolution from USGS (in meters) (Sahid, 2024). The distance of the residence to farmland was based on the Euclidean distance of the cancer incident location to the nearest farmland. The farmland was selected from MODIS land cover type yearly global 500m (class 12 and 14 of LC_Type1). Data were then extracted, used for Euclidean distance analysis in ArcGIS, and overlaid with the cancer incidence. Distance to the nearest urban center was based on GHS Urban Centre Database 2015 (Florczyk et al., 2019). Euclidean distance was used to calculate the distance from the urban center to the cases location. Finally, the distance to the river was acquired from HydroRIVERS (Lehner & Grill, 2013). Euclidean distance was also used to determine the distance from the provided river network.

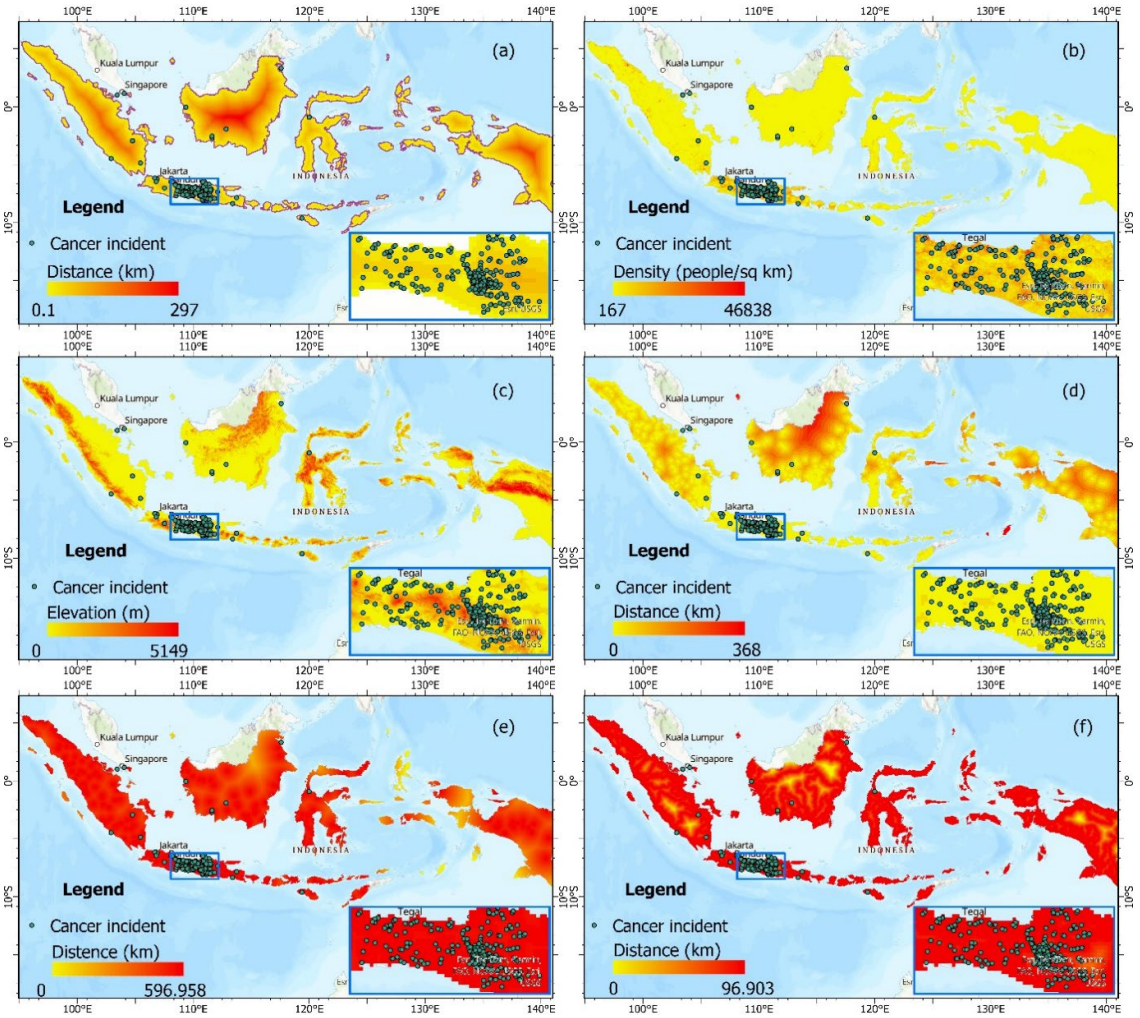

Figure 4. Demographic-environmental factors; (a) distance to coastline (km), (b) population density (people/km^2), (c) elevation (m), (d) distance to farmland (km), (e) distance to urban center (km), (f) distance to river (km)

2.4 Statistical data analysis

A case-control study used a semi-quantitative method to assess IFN–γ staining with the determination of an H-score. The study was conducted using tissue blocks from patients having bone and soft tissue tumor, with malignant (case) and benign types (control). Multiple linear regression with ordinary least square (OLS) estimator was used to obtain the best linear unbiased estimator (BLUE), where the regression model was $y = X'\beta + e$ (Gujarati & Porter, 2009). The assumptions that must be met are that the residuals are normally distributed, the residual variance is homogeneous (no heteroscedasticity), and there is no multicollinearity (Pallant, 2020). Moreover, data normality testing was performed with standardized normal probability plot, kernel density plot, and Shapiro-Wilk W test (Michels & Musshoff, 2022; Pallant, 2020). Heteroscedasticity was examined with Bresuch Pagan test while multicollinearity was determined with variance inflated factor (VIF) below 10 (Gujarati & Porter, 2009). Checking for outliers is also necessary to ensure unbiased parameter estimates using several measures, namely standardized residual <3 (Tabachnick et al., 2018). Data are called outliers when the cook distance value is >4/n (0.067), and DFIT is > $2\sqrt{k/n}$ (0.774) (Arimie et al., 2020). Another measure is the Mahalanobis distance, which detects multivariate outliers when the Mahalanobis distance value is > Chi square distribution table with p = 0.001 and k = the number of variables (27.87) (Tabachnick et al., 2018).

The minimum point of the dependent variable response (IFN–γ level) is 0, indicating that an IFN–γ value of zero suggests no malignancy. Conversely, when the value is more than 0, this indicates malignancy. The data pattern includes a sensor because there is a lower limit of 0 (Amore & Murtinu, 2021). The results show a total of 12 patients with IFN–γ values of zero. Aside from multiple linear regression analysis, Tobit regression analysis was also estimated for comparison. The Tobit regression model was $y^* = X'\beta + e$, where e follows a normal distribution. The value of $y = y^*$ when $y^*>0$ and $y = 0$ otherwise, with the dependent variable being the left censor (Amore & Murtinu, 2021). This analysis requires assumptions such as linear regression, namely normality of the residuals and homoscedasticity of the residual variance (Amore & Murtinu, 2021), as well as the maximum likelihood estimator (MLE) (Amore & Murtinu, 2021)Aside from Tobit regression, this study also used robust MM regression estimation as an alternative to obtain the best model. The regression is essential due to the presence of potential outliers (Chen, 2002).

3. RESULTS AND DISCUSSION

3.1 Association between IFN–γ expression and demographic-environmental factors

This study analyzed four major groups of bone and soft tissue tumor (Table 2). Benign type include giant cell tumor of bone and soft tissue tumor. On the other hand, the malignant types include osteosarcoma and soft tissue sarcoma. This data categorizes bone and soft tissue tumor, providing case counts for specific categories and groupings. The giant cell tumor category contains only 15 examples of bone, while 15 cases of benign soft tissue tumor contain neurofibroma, lipoma, and schwannoma. the osteosarcoma contains only 15 cases of osteosarcoma. The most diversified group is soft tissue sarcoma, which includes rhabdomyosarcoma, fibrosarcoma, malignant spindle cell tumor, undifferentiated pleomorphic sarcoma, and liposarcoma. This classification aids in analyzing the occurrence and distribution of tumor types throughout the study, offering a clear picture of the data structure.

The characteristics of the patients based on gender indicate a balanced representation of both males and females in the sample, suggesting a well-rounded representation of the population (Table 3). The analysis of mean ages across different tumor groups shows distinct variations. Osteosarcoma has the lowest mean age (SD) of 21.87 (10.11) years, indicating a tendency for this type of tumor to affect individuals at a relatively young age. In contrast, patients with soft tissue sarcoma have a considerably higher mean age of 50.13 (22.30) years, suggesting a prevalence among older individuals. Giant cell tumor patients fall between these two extremes with an mean age of 35.47 (11.29) years, while those with benign soft tissue tumor have a slightly higher mean age of 40.20 (13.41) years. These results underscore the significant age differences among the four tumor groups. Standard deviation values represent the degree of variability in age within each tumor group. A larger standard deviation indicates a wider age range among individuals. For instance, the relatively high standard deviation of 22.30 years for soft tissue tumor implies a greater diversity in ages among individuals affected. The characteristic of patient residence that showed statistical significance was proximity to the shoreline ($p<0.000$), with the closest mean distance to the shoreline found in osteosarcoma. Other geographical characteristics, such as proximity to farmlands, rivers, and city centers, did not show statistically significant differences (Table 4).

Table 2. Tumor classification and the number

Group of tumor	Detailed type of tumor	N	Sub-total
Giant cell tumor	Giant cell tumor of bone.	15	15
Benign soft tissue tumor	Schwannoma	4	15
	Neurofibroma	2	
	Lipoma	9	
Osteosarcoma	Osteosarcoma	15	15
Soft tissue sarcoma	Rhabdomyosarcoma	5	15
	Fibrosarcoma	3	
	Malignant spindle cell tumor	5	
	Undifferentiated pleomorphic sarcoma	1	
	Liposarcoma	1	

Table 3. Patients characteristics in the four group types of tumor

Variable/attribute	Type of tumor				*P*-value
	Benign		Malignant		
	Giant cell tumor of bone	Soft tissue tumor	Osteosarcoma	Soft tissue sarcoma	
	Mean (SD) /n (%)	Mean (SD) /n (%)	Mean (SD) /n (%)	Mean (SD) /n (%)	
IFN–γ	111.00 (89.29)	22.00 (36.00)	191.00 (37.95)	149.67 (64.21)	< 0.000
Age (years)	35.47 (11.29)	40.20 (13.41)	21.87 (10.11)	50.13 (22.30)	< 0.000
Sex					
Women	9 (60.0)	6 (40.0)	6 (40.0)	8 (53.3)	0.614
Men	6 (40.0)	9 (60.0)	9 (60.0)	7 (46.7)	
Population density (person/km^2)	1875.40 (2330.14)	1301.93 (582.15)	2253.60 (1912.19)	1806.47 (1474.86)	0.660
Elevation (Meters above sea levels)	195.00 (252.07)	154.87 (168.49)	136.80 (175.04)	286.87 (333.32)	0.405
Distance to shoreline (km)	32.61 (18.66)	50.87 (20.56)	15.85 (19.82)	49.55 (17.63)	< 0.000
Distance Urban Center (km)	10.69 (7.59)	10.10 (7.78)	7.91 (7.78)	8.26 (8.01)	0.541
Distance to farmland (km)	1.29 (1.27)	2.66 (2.84)	1.72 (1.61)	1.62 (2.38)	0.302
Distance to river (km)	1.89 (4.78)	0.54 (0.45)	0.34 (0.32)	0.30 (0.29)	0.266

Table 4. Descriptive statistics and association

	Mean	Standard deviation	I	II	III	IV	V	VI	VII	VIII	IX	X
I	118.4	86.51	1									
II	37.12	17.88	-0.050	1								
III	1.48	0.50	0.070	0.088	1							
IV	1.5	0.50	0.605***	-0.049	-0.033	1						
V	1993.36	2056.20	0.160	-0.036	0.187	0.199	1					
VI	184.76	232.09	0.005	0.217**	-0.005	0.043	-0.098	1				
VII	37.22	35.58	-0.392***	0.292*	0.149	-0.193	0.210	0.258**	1			
VIII	9.239	7.682	-0.043	-0.069	-0.097	-0.152	-0.429***	0.056	-0.334**	1		
IX	1.821	2.124	-0.030	-0.261*	-0.114	-0.071	-0.353***	-0.237*	-0.251*	0.326**	1	
X	0.483	0.594	-0.221*	-0.127	0.154	-0.278*	-0.046	-0.103	0.051	0.149	0.033	1

I: interferon-γ, II:age, III: sex, IV: type of tumor, V: population density, VI: elevation (meter above sea level), VII: distance to shoreline, VIII: distance to city center, IX: distance to farmland, X: distance to river. ***sig ≤ 0.001, **sig 0.01, *sig 0.05

The variable significantly associated with IFN–γ expression was the type of tumor (r = 0.605). This indicates that the type of tumor affects the increase in IFN–γ expression. Malignant tumor have higher expression than benign. A positive association was also found in the variable distance to shoreline (r = -0.392). The farther the distance between the beach and the house where the tumor patient lives, the lower the IFN–γ expression. An association was also found between IFN–γ expression and the distance from home to farmland. The farther the distance, the lower the expression.

The linear regression analysis assumption was conducted using residual normality, which can be checked with a normal probability plot (P-P plot) graph. The results showed a normally distributed data plot with a distribution of residual points around the diagonal line. Furthermore, this was strengthened by the kernel density estimate graph. The residual normality test results were reinforced by the Shapiro-Wilks test, which showed a *p*-value of 0.109>0.05, suggesting the residual pattern was normally distributed (Figure 5).

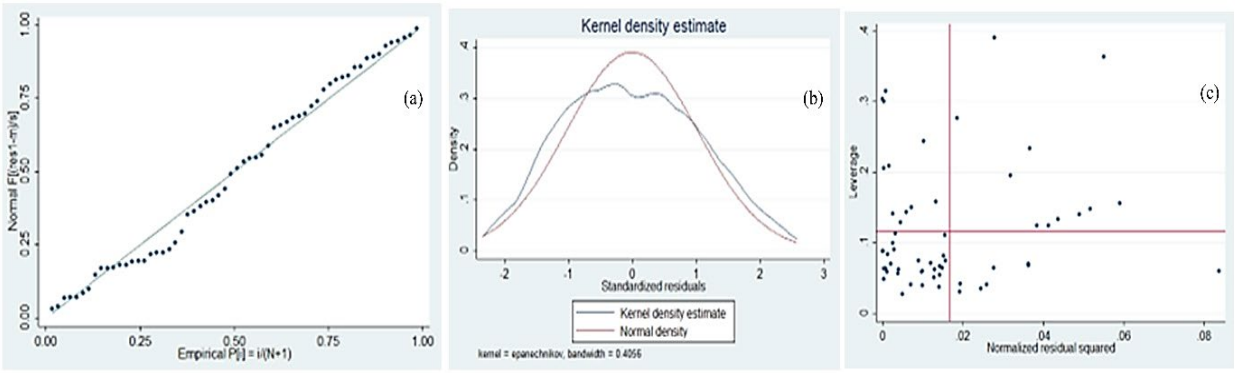

Figure 5. The result of normality test; (a) normal probability plot of residual, (b) normality kernel density of residual, (c) outlier detection

Heteroscedasticity testing with the Bresuch Pagan test shows satisfactory results with a *p*-value of 0.149 (>0.05), suggesting the residual variance is constant. However, assessment of outliers with the normalized residual squared and leverage diagram shows that there are two data points suspected as potential outliers. Furthermore, the standardized residual test shows that there are no outliers because the value is <3 (Tabachnick et al., 2018). However, the following three measures indicate the presence of potential outlier data. Based on the cook distance measure, some data points are potentially classified as outliers because the maximum cook distance value is 0.307>0.067. Meanwhile, the Mahalanobis distance value shows that there is no outlier data, as indicated by a maximum value of 23.961. Where α = 0.001 and k (the number of independent variables) = 9, the value of Chi square table (0.001;9) is 27.887. However, the maximum DFFIT check result is 50.10>0.774, which indicates potential outliers. (Table 5). These results necessitate the examination of other regression analyses besides linear regression with OLS, namely MM robust linear regression (Table 6).

Table 5. Regression assumption checking

Test	Statistics test	Result
Normality test	Shapiro Wilks	Z statistik (1.228), *p*-value (0.109)
Heterocedasticity test	Breusch-Pagan / Cook-Weisberg test for heteroskedasticity	Chi square (2.06), *p*-value (0.149)
Multicollinearity	Variance inflated factor	max (1.48)
Outlier	Standardized residual	min (-2.050), max (2.853)
	Cook distance	max (0.307)
	Mahalanonis distance	min (3.261), max (23.961)
	DFFIT	min (-76.9), max (50.10)

Table 6. Model estimation results

Variable	Linear regression		Tobit regression		Robust MM regression	
Dependent variable (IFN–γ)	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
Age		0.262		0.091		-0.285
Sex		21.398		21.192		11.036
Type of tumor		84.356***		95.407***		145.22***
Population density	0.010*	0.005	0.012*	0.006	0.009*	0.014***
Elevation	0.054	0.030	0.074	0.046	0.071	0.052
Distance to shoreline	-1.844***	-1.396***	-2.309***	-1.727***	-2.265***	-0.605***
Distance to urban centre	-1.075	-0.312	-1.019	-0.153	-0.866	0.247
Distance to farmland	-0.173	0.377	-0.637	-0.381	-3.988	4.595
Distance to river	-22.639	-8.693	-20.921	-5.781	-25.105	2.616
Constant	178.92	-6.562	178.719	-19.655	195.29	-140.39
Model quality						
F statistic	3.32	5.25				
P-value F statistic	0.007	0.000				
LR Chi square			21.4	41.70	44.73	318.52
P-value Chi square			0.001	0.000	0.000	0.000
R-square adj / pseudo R-square	0.191	0.394	0.034	0.065	0.293	0.602

***sig 0.01, **sig 0.05, *sig 0.10

The model estimation results are a comparison between multiple linear, Tobit, and robust mm regression models. The estimation results with multiple linear regression show that in model 1, the significant variables are population density (0.010) and residential distance to shoreline (-1.844). A rise in population density will increase the expression of IFN–γ. Conversely, the farther the distance of residence from the shoreline, the lower the expression. In model 2 or full model, the adjusted R-square value increased to 0.394 with the inclusion of demographic variables, including gender, age, and tumor type. The significant variables were tumor type and distance from the beach. Although population density was not significant in model 2, the regression coefficient was still in the direction of the hypothesis, which was positive. An alternative model with Tobit regression was used due to the suspicion that the left-censored data on IFN–γ expression was 0. This test also showed relatively similar results to linear regression. The population density variable was significant at 10% alpha with a regression coefficient of 0.012 in model 1. Residential distance to shoreline was also significant, with a regression coefficient of (-2.309). In model 2, tumor type and residential distance to shoreline remained significant. Both models were accepted with log likelihood ratio Chi square test p-value <0.05.

Robust MM regression showed relatively similar results in model 1, with linear regression analysis. The significant variables were population density and distance to shoreline. However, in model 2, the significant variables were tumor type, population density, and distance to shoreline. The pseudo R-squared value obtained was quite high at 0.602. Based on the three regression analyses, the variables with the potential to change the expression of IFN–γ include population density, distance to shoreline, and tumor type.

3.2 Bone and soft tissue tumor IFN–γ expression association

This study showed that the malignant bone and soft tissue tumor are significantly associated with high IFN–γ expression. The high expression is contrary to most literature that examined the antitumor effect of IFN–γ (Ligon et al., 2021; Luo et al., 2020; Smrke et al., 2021). IFN–γ is not a single biomarker for evaluating the effectiveness of therapy or disease progression. It requires joint evaluation of other biomarkers such as PD-1 or PDL-1 (Lam et al., 2021; Wang et al., 2023). Therefore, a comprehensive evaluation is needed to understand the mechanism by which the protumor phenotype occurs. The protumor properties can be obtained under conditions of suppression and loss of IFN–γ receptors and downstream signaling mediators, as well as the amplification of molecules that inhibit the signaling pathway. This mechanism is found in tumor cells that can escape the immune system. Other microenvironmental contributors include insensitivity of IFN–γ signaling, downregulation of major histocompatibility complex (MHC) molecules, upregulation of indoleamine 2,3-dioxygenase, and checkpoint inhibitors such as programmed cell death ligand 1 (PDL-1) (Ilyushin et al., 2023; Jorgovanovic et al., 2020).

3.3 Environmental factors affecting the level of malignancies bone and soft tissue tumor

The majority of the participants in this study lived in coastal/estuarine areas, rice fields, and dense housing with reports of high pollutants, including heavy metals, organics, plastic debris, and microplastics. The probability of malignant bone and soft tissue tumor is higher in these areas (Romadhon et al., 2024). Proximity to urban areas, industrial plants, and metal processing plants in a Spanish case-control study increased the risk of bone tumor in children (García-Pérez et al., 2019, 2020). Meanwhile, a study conducted in China found the opposite, with rural areas having a higher incidence and mortality rate of bone malignancies (Xi et al., 2023). The hypothesis in this study was that tumor tissue IFN–γ expression associates with the malignancy rate of bone and soft tissue tumor. The impact of demographic (age and sex) and residence characteristics was further explored on overall bone and soft tissue tumor IFN–γ expression levels. The residential characteristics explored included (a) distance to shoreline (km), (b) population density (people/km^2), (c) elevation (meter above sea level), (d) distance to farmland (km), (e) distance to urban center, and (f) distance to river.

Based on the results, population density in the residence was positively associated with IFN–γ expression. Increasing population in an area tends to heighten pollutant debris, whether liquid, solid or gaseous emissions discharged into the environment (Crippa et al., 2021; Schuyler et al., 2021; Zheng et al., 2021). A study in South Korea evaluated the effect of air pollution on breast cancer incidence with the inclusion of population density as one of the potential confounders (Hwang et al., 2020). Another study in Córdoba, Argentina, evaluating risk factors for colorectal cancer incidence through spatiotemporal analysis, found that the risk of cancer incidence increased in areas with high versus low urbanization (incidence rate ratios (IRR): 1.66) (Canale et al., 2023). Although not directly related to cancer, a population study in China showed an associative relationship between high population density and high incidence of inflammatory bowel diseases, namely Crohn's disease and ulcerative colitis (Ng et al., 2019). However, in the context of COVID-19-related metabolic diseases, the effect of population density shows inconclusive results in the worsening of clinical conditions related to systemic inflammation (Jumadi et al., 2022a, 2022b). The worsening of these cases is more related to individual postural conditions, such as obesity and the presence of comorbid metabolic diseases (Krams et al., 2021). The association between population density and IFN–γ expression is more related to the accumulation of anthropogenic pollutants, attributed to human behavior in the production of untreated waste. The residence characteristics of the study population have strong ties to high pollution areas, as reported by Adyasari et al. (2021).

The distance of residence to shoreline was negatively associated with IFN–γ expression. This result is in accordance with the initial hypothesis stating that the characteristics of the shoreline area, in the context of study population, include the accumulation of waste in any form discharged into the river. As the principle mentioned earlier, downstream areas have a significantly higher pollution load than upstream areas (Chen et al., 2022b; Garg et al., 2018; Luo et al., 2021). Indicators of pollution are reflected in the content of contaminants such as heavy metals, microplastics, and various types of pesticide residues in aquatic organisms, both animal species and aquatic plants consumed by humans (Kadim & Risjani, 2022; Özgenç et al., 2023). Another pathway for pollutants

to enter the human body is through air transmission (Chen et al., 2022b). Pollutants that successfully enter the human body in the long term elicit various hallmark mechanisms of cancer, until malignancy is clinically established. This IFN–γ-related hallmark of cancer pathways includes genomic instability, reactive oxygen species, and chronic inflammatory processes in the tumor microenvironment (Baines et al., 2021; Barguilla et al., 2022; Ozturk et al., 2022; Zhang et al., 2024). Almost half of the patients in this study resided on the north coast of Java, which is the main transportation route for large vehicles such as freight trucks, and the burden of air pollution is very large compared to other areas (Malisan et al., 2023; Nainggolan et al., 2023; Nugraha et al., 2023). The population living in the coastal area in this study has a double burden of pollutants from the main transportation route, and is the downstream area of the river on the island of Java.

One limitation of this study is the reliance on a small sample size, which necessitates the need for validation through larger-scale population-based studies. In addition, bone and soft tissue tumor are a rare group. By expanding the participant pool, future studies can provide more robust and reliable results, allowing for a better understanding of the association between geographical factors and cancer prevalence. The use of larger populations will enhance the generalizability and statistical power of the results, ultimately strengthening the validity and impact of the conclusions.

4. CONCLUSION

In conclusion, factors that significantly influence the level of IFN–γ expression in tissues include the level of tumor malignancy, population density, and distance of residence to the shoreline. IFN–γ expression was found to be significantly higher in malignant than in benign bone and soft tissue tumor. Furthermore, the expression in giant cell tumor of bone was found to be of the biphasic type with a low predominance characteristic of benign tumor, and a small proportion of expression equivalent to the malignant type. The distance between residence and coastline was significantly and negatively associated, suggesting that the closer the residence is to the coastline, the higher the IFN–γ levels and the degree of malignancy. This comprehensive approach provides insight into how proximity to different surroundings and demographic features influences tumor biological behavior, potentially informing prognosis and personalized treatment methods.

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