Correlation vitamin D [25(Oh)D3] with neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP) in cervical cancer patient

Nurlailah Mi'raj[1], Muji Rahayu[2], Dwi Retnoningrum[3], Banundari Rachmawati[4], Edward Kurnia Setiawan Limijadi[5]*

[1]Clinical Pathology Specialist Programme, Faculty Medicine, Universitas Diponegoro, Semarang, Indonesia; nurlailah03@gmail.com (N.M.).
[2,3,4,5]Department of Clinical Pathology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia; mujirahayu70@yahoo.co.id (M.R.) dwiretnodr@gmail.com (D.R.) banundaridr@yahoo.com (B.R.) edwardksl@fk.undip.ac.id (E.K.).

Abstract: Vitamin D [25(OH)D₃] levels are currently associated with clinical inflammatory markers such as C-reactive protein (CRP) and neutrophil-lymphocyte ratio (NLR). They are used to monitor the development of cervical cancer. Aim of this study was analyze the correlation of vitamin D [25(OH)D₃] levels with NLR values and CRP levels in cervical cancer patients. Vitamin D is expected to be an alternative treatment for cervical cancer. This study was analytical observational with a cross-sectional design conduct at Dr. Kariadi Hospital Semarang in January-March 2024. [25(OH)D₃] level was measured using enzyme-linked immunosorbent assay (ELISA) method, CRP levels were checked with the ADVIA centaur device using the principle of chemiluminescence and combined with immunoassay technology and NLR value was calculated manually. Total sample was 40 cervical cancer patients aged 35-60 years old. Mean±SD results of vitamin D [25(OH)D₃] levels, NLR values, and CRP levels were 12.0±5.5 ng/ml; 5.0±1.2; 5.5±2.0 mg/dL. Correlation tests between vitamin D [25(OH)D₃] levels with NLR values and CRP levels were sequentially obtained (p=0.036; r=-0.333) and CRP (p=0.000; r=-0.588). Result in this study conclude that Vitamin D [25(OH)D₃] levels and NLR values showed a weak negative correlation. A moderate negative correlation exists between vitamin D [25(OH)D₃] and CRP level.

Keywords: Cervical cancer, NLR, CRP, Vitamin D [25(OH)D3].

1. Introduction

Cervical cancer is the second most common malignancy found in women and can increase the risk of death in women globally (Zhang et al, 2020). The incidence rate of cervical cancer worldwide is quite high. Diagnoses in 36 countries found that cervical cancer was one of the leading causes of death in women of productive age (Sung et al, 2021). World Health Organization (WHO) in 2020 stated that there were 4 cases per 100,000 women in each country diagnosed with cervical cancer (Burmeister et al,2022). Data Global Observatory Cancer (GLOBOCAN) in 2020 stated that the incidence of cervical cancer ranks 4th highest among women in the world. Based on Indonesia database, total number of cervical cancer in 2020 was 36,633 cases (Susilawati, 2022).

Cervical cancer is a type of malignancy of the cervical epithelial lining caused by the Human papillomavirus (HPV) (Bhatla et al, 2021). Long-lasting and persistent HPV infection is the cause of cervical cancer. Cervical cancer stages according to the Federation of Gynecology and Obstetrics (FIGO) one of the most important prognostic factors Marth et al, 2017). Data shows that 9% of women under the age of 35 have invasive cervical cancer at the time of diagnosis, while 53% of women under the age of 35 have cervical cancer. Carcinoma In Situ (CIS) was found in women over the age of 35

(Kashyap et al, 2019). The most commonly observed type of histopathology in young women is Squamous cell carcinoma (SCC) where the number of cervical cancer patients, especially stages II and III, is the most dominant stage (56.3% and 15.8%). (Shruthi et al, 2014) (Drokow et al, 2021) (Tian et al, 2020)

Recent studies have found an association between tumour progression and systemic inflammatory response to treatment. Inflammation caused by the tumour microenvironment promotes the initiation, proliferation, haematogenous spread and survival of tumour cells. Neutrophil-to-lymphocyte ratio (NLR) is one of the most commonly used inflammatory markers in cancer patient. Neutrophil lymphocyte ratio (NLR) is a combination of inflammatory markers, consisting of neutrophils as non-specific markers of inflammation and lymphocytes as regulatory marker. (Limijadi et al, 2020) NLR has been linked with poor survival in several cancers, including gastrointestinal and ovarian cancer. (Morelli et al, 2022)

C-Reactive Protein (CRP) is a widely observed as indicator of acute inflammation. (Winarno et al, 2023) CRP is an acute-phase protein produced in the liver and its levels will increase when inflammation occurs and is sensitive to systemic inflammatory responses. (Liu et al, 2021) An increase in plasma CRP levels occurs from about 1 µg/mL to more than 500 µg/mL within 24-72 hours after tissue damage. (Winarno et al, 2021) Sangjeong *et al.*, 2021 explained that there was an increase in hs-CRP levels which showed a negative relationship with regression Low-grade Squamous Intraepithelial Lesion (LSIL), as well as high sensitivity CRP can be used as a biomarker of systemic inflammation is used to predict groups at risk of developing cervical carcinogenesis malignancies. (Sproston et al, 2018)

Research in Japan explains that there is an inverse relationship between calcium and vitamin D intake and the risk of cervical neoplasia. (Koc et al, 2021) Vitamin D [$25(OH)D$]$_3$] and vitamin D receptor (VDR) play an important role in the development of gynecological cancer.(Askandar et al, 2020) Increased circulating vitamin D is associated with a reduced risk of developing cervical cancer. (Deuster et al,2017) Vitamin D and playing important role as a receptors in the inflammatory process, and have an excellent anti-inflammatory effect. (Koc et al, 2021) Suardi *et al.*, 2021 explained that vitamin D levels were significantly lower in the cervical cancer group compared to non-cervical cancer group. Coach *et al.*, 2021 stated that vitamin D has various anti-cancer effects, but study that explained about relationship between vitamin D and cervical neoplasia is still limited.

Previous study describe that there was negative correlation between vitamin D level with CRP level. Research cross-sectional others mentioned that low levels of 25(OH)D were inversely correlated with inflammatory markers such as CRP, IL-6, and TNF-α. (Akbas et al,2016) Correction of low vitamin D status may reduce chronic inflammation and decrease CRP level.

There has been no research that directly analyzes the relationship between vitamin D levels [$25(OH)D_3$] with clinical inflammatory parameters (NLR and CRP) in patients with stage IIB and IIIB cervical cancer, so this is what prompted the researchers to analyze whether there is a relationship between these parameters.

2. Method

Analytical observational research with a cross-latitude approach at Dr. Kariadi Semarang Hospital in January – March 2024. The study was conducted on married patients aged 35 – 60 years with a normal body temperature ($36.5^{o}C$ - $37.5^{o}C$). Patient has been diagnosed with cervical cancer with histopathological results of stages IIB and IIIB. Examination of liver function (SGPT) and kidneys (urea and creatinine) within normal limits. Patients who are taking medications (NSAIDs, statins, broad-spectrum antibiotics, and vitamin D supplements) with obesity status (BMI >30 kg/m2) and have other properties were excluded from this study. This research has received approval from the Health Research Ethics Commission of Dr. Kariadi Semarang Hospital with ethical clearance number: No.1661/EC/KEPK-RSDK/2023.

Vitamin D levels [$25(OH)D3$] were measured using the enzyme-linked immunosorbent assay (ELISA) method. The NLR value is calculated manually. CRP levels were checked with the ADVIA

centaur tool using the principle of chemiluminescence and combined with immunoassay technology. Univariate analysis was carried out on each variable to determine the characteristics of the sample. The Shapiro-Wilk test to determine the normality of the data. Transform tests for abnormally distributed data. The relationship test uses the Pearson test because the data distribution is normal.

3. Result

This is an observational study with cross-sectional method that were carried out from January to March 2024. Research sample was taken from patients of the Gynecology-Oncology Polyclinic at Dr. Kariadi Hospital. Total sample in this study was 40 patients with subject characteristics is seen in Table 1.

Table 1.
Characteristics of the data of the research subjects.

Variable (n= 40)	Mean ± SD	Median (Min.-Max.)
Age (Years)[#]		$53\ (36-59)$
Height (cm)[#]		$156,5\ (140-165)$
Weight (Kg)[#]		$54,5\ (33-64)$
Body mass index[#] (kg/m^2)		$22,9\ (15,1-24,9)$
Leukocyte count $(x10^3/\ uL)$[#]		$10,32\ (8,13-10,92)$
Number of neutrophils[#]		$76\ (71-80)$
Number of lymphocytes	15.9±3.3	
NLR value	5.0±1.2	
CRP level (mg/dL)	5.5±2.0	
Vitamin D levels (ng/ml)	12.0±5.5	

Note: CRP, C reactive protein; NLR, neutrophil-lymphocyte ratio; SD (Standard deviation); min (Minimum); max (Maximum); [#]Abnormal data distribution.

Correlation between vitamin D levels and NLR values was analyzed using the Pearson test. The results of the relationship test showed a weak negative relationship between vitamin D levels and NLR values ($p=0.036$ and $r=-0.333$). The distribution of vitamin D level data with NLR values can be seen in Figure 1.
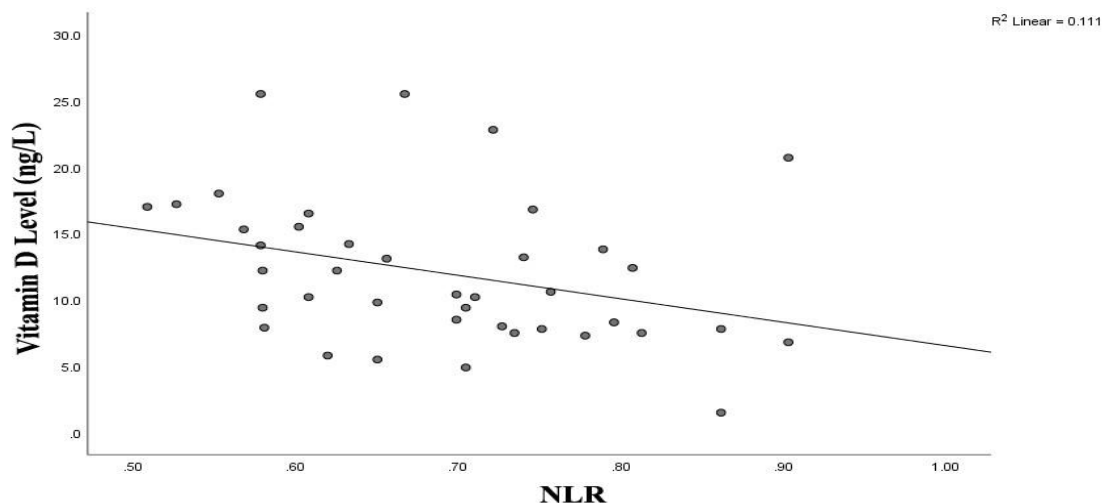

Figure 1.
Scatter plot of the relationship between vitamin D levels and NLR values in cervical cancer.

Corralation between vitamin D levels and CRP levels was analyzed using the Pearson test. The results of the relationship test showed a moderate negative relationship between vitamin D levels and CRP levels (p=0.000; and r=-0.588). The distribution of data on vitamin D levels with CRP levels can be seen in Figure 2.
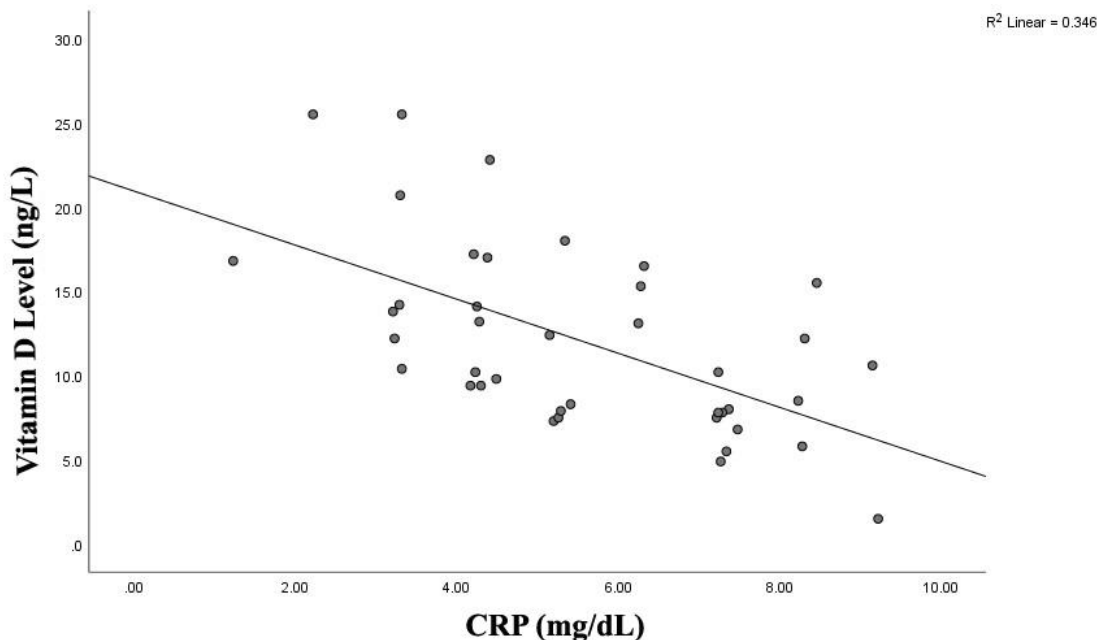

Figure 21.
Graphs scatter plot relationship between vitamin D and CRP levels in cervical cancer.

4. Discussion

There were 40 patients who had signed Informed consent and met the inclusion and exclusion criteria. Median age value of respondent was 53 years old with the youngest in 36 years old and the oldest was 59 years old. Most of the patients in this study were over 50 years of age. Age becoming one of the risk factor for cervical cancer because continuous development of precancerous lesions that increase the invasive process of cervical cancer takes 10-30 years from the beginning of the lesion. (Murillo et al, 2016) This result was accordance with previous study in 2014 which stated that cervical cancer is a malignancy of epithelial metaplasia in the area of transition between the cervical canal mucosa and the vaginal mucosa, usually affecting women aged 35-55 years. (Eva S, 2017)

Respondent in this study had a median BMI value of 22.9 kg/m^2 with the smallest one was 15.1 kg/m^2 and the largest one was 24.9 kg/m^2. The results of this study show that all patients in this study were not obese. Umami *et al.*, 2018 stated that there was a relationship between BMI and an increase in the incidence of cervical cancer. Other research has also stated that obesity is associated with an increased risk of cervical adenocarcinoma because it was hormone-responsive cancer.

Mean NLR value in this study was 5.0±1.2 that categorized as high value. An increase in NLR value ≥ 2.47 indicates an increase in histopathological and metastatic activity metastases of lymph nodes that can worsen the prognosis. Result in this study are in line with the research by Dong *et al.*, 2019 which says that a high NLR value indicates the presence of a poor prognosis in cervical cancer associated with the FIGO stage. Result of this study also describe that average CRP levels was 5.5±2.0 mg/dl. This result is in line with the theory of interpretation of CRP levels of 1.0-10.0 mg/dL which indicates a moderate increase in malignancy cases. (Nehring S, 2023)

Correlation test between vitamin D levels and NLR values in this study showed significant negative correlation between two variable p=0.036 with weak correlation strength r=-0.333. These results conclude that low vitamin D levels are associated with increased NLR values. Previous study that states that lower level of $25(OH)D_3$ were significantly correlated with increase NLR values in cervical cancer patients. Tanaka *et al.*, 2014 mention that the production of Tumor necrosis factor-α (TNF-α), Interleukin (IL-6), and nitric oxide (NO) activity inhibited by vitamin D, which indicates an anti-inflammatory role from vitamin D. Vitamin D levels and values of Neutrophil-lymphocyte ratio (NLR) are observed as a marker of inflammation with an increased number of neutrophils. (İşbilen et al, 2022) Low vitamin D levels was associated with increased concentrations of TNF-α, IL-6, and CRP. Serum concentration of vitamin D $[25(OH)D_3]$ in the circulation of blood flow is associated with an increased prevalence of cervical cancer. (El-Zein et al, 2021) Vitamin D affects the inflammatory state through the interaction between immune cells and tumor cells followed by increased production of various inflammatory cytokines. (Liu et al, 2018) Results of an observational study evaluating the relationship between $[25(OH)D levels]_3$ with the incidence and death from cervical cancer still varying.

Vitamin D levels with neutrophil and lymphocyte counts are related to metabolism and activation of VDR against inflammatory responses. Sufficiency of vitamin D in the body is essential in providing optimal IFN-γ-mediated feedback control at $1,25(OH)D synthesis_2$ by macrophages and antimicrobial responses that enter the body. (Chun et al, 2019) An increase in NLR values as a result of an increase in the number of neutrophils may contribute to the stimulation of cancer development in cervical cancer patients. Bag *et al.*, 2019 stated that the increase in NLR values was related to the metabolism of vitamin D which is active in local inflammatory conditions. (Tas et al, 2019)

Correlation test between vitamin D and CRP levels in this study showed that there was a significant negative relationship between two variable p=0,000 with moderate correlation strength r=-0.588. These results showed that low vitamin D levels were associated with increased CRP level. Akbas *et al.*, 2016 stated that levels of inflammatory cytokines such as CRP, TNF-α, and IL-6 were found to be inversely proportional to vitamin D levels. Low vitamin D levels are associated with inflammation and the development of cervical cancer. Vitamin D has anti-inflammatory activity and is negatively correlated with serum levels of CRP and IL-8 in malignancy patients. (Yang et al, 2017)

Cervical cancer-related molecules caused by inflammation include TNF-α, chemokines, interleukins, and ROS levels. Inflammatory factors such as IL-6, IL-1, and TNFα can induce the production of CRP in the liver as part of an inflammatory response with its potential mechanism in cervical cancer. TNF-α can also induce the production of Amphiregulin, which further leads to the proliferation of cervical cancer cells, while IL-6 and IL-1 play a role in the growth and metastasis of cervical cancer cells. (Wang et al, 2020) The results of this study are to the existing theory, where an increase in CRP levels can predict tumor aggressiveness. Further research takes into account the increase in neutrophil and lymphocyte counts as well as CRP from the beginning of the patient's diagnosis of cervical cancer until the study is conducted.

5. Conclusion

There was a weak negative association between vitamin D $[25(OH)D3]$ levels and NLR values. There was a moderate negative association between vitamin D $[25(OH)D3]$ levels and CRP levels.

6. Limitation

The study did not take into account the increase in neutrophil and lymphocyte counts as well as CRP from the beginning of the cervical cancer diagnosis until the study was conducted.

Copyright:

References

[1]   Akbas, E.M., Gungor, A., Ozcicek, A., Akbas, N., Askin, S., Polat, M. (2016). Vitamin D, and inflammation: Evaluation with neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio. Arch Med Sci.12(4), 721–7.

[2]   Askandar, B., Ekaputra, V.G., Iskandar, T.M. (2020). Comparison of VDR Expression and Blood Vitamin D 1.25 (OH)2 Level between Cervical Cancer Patients and Normal Women. Indones J Cancer. 2020;14(3):80.

[3]   Bhatla, N., Aoki, D., Sharma, D.N., Sankaranarayanan, R. (2021). Cancer of the cervix uteri: 2021 update. Int J Gynecol Obstet,155(S1),28–44.

[4]   Burmeister, C.A., Khan, S.F., Schäfer, G., Mbatani, N., Adams, T., Moodley, J., et al. (2022). Cervical cancer therapies: Current challenges and future perspectives. Tumour Virus Res, 13.

[5]   Chun, R.F., Shieh, A., Gottlieb, C., Yacoubian, V., Wang, J., Hewison, M., et al. (2019). Vitamin D Binding Protein and the Biological Activity of Vitamin D. Front Endocrinol (Lausanne), 10(October):1–15.

[6]   Deuster, E., Jeschke, U., Ye, Y., Mahner, S., Czogalla, B. (2017). Vitamin D and VDR in gynecological cancers-A systematic review. Int J Mol Sci. 18(11).

[7]   Drokow, E.K., Xu, L., Akpabla, G.S., Ahmed, H.A.W., Song, J., Neku, E.A., et al. (2021). Prognostic Variables of Younger-Aged Cervical Carcinoma Patients: A Retrospective Study. J Oncol.

[8]   El-Zein, M., Khosrow-Khavar, F., Burchell, AN., Tellier, PP., Eintracht, S., McNamara E, et al. (2021). Association of serum 25-hydroxyvitamin D with prevalence, incidence, and clearance of vaginal HPV infection in young women. J Infect Dis, 224(3):492–502.

[9]   Eva, S, A.M.S..(2017). Knowledge of Behavioral Risk Factors and Early Detection of Cervical Cancer with IVA in Women in Bogor. Peneliti Kesehat, 53(9), 1689–99.

[10]   İşbilen, E., Karuserci, Kömürcü.Ö. (2022). Evaluation of the Anti-Inflammatory Impact of Vitamin D on Polycystic Ovary Syndrome and Endometriosis. Experimed, 12(2), 80–4.

[11]   Kashyap, N., Krishnan, N., Kaur, S., Ghai, S. (2019). Risk Factors of Cervical Cancer: A Case-Control Study. Asia-Pacific J Oncol Nurs, 6(3), 308–14.

[12]   Koc, S., Kurt, S., Ilgen, O., Timur, H.T., Uslu, T. (2021). The effect of vitamin D on the regression of human papillomavirus infection and metabolic parameters: A retrospective study. Eur J Gynaecol Oncol, 42(2), 340–5.

[13]   Limijadi, E.K.S., Setyadi, A., Utami, S.B., Puruhito, B., Sofia, S.N. (2020). The Correlation between Neutrophil Lymphocyte Ratio, C-reactive Protein, and Serum Amyloid a with the Degree of Stenosis in Acute Coronary Syndrome. Open Access Maced J Med Sci, 8(B), 1234-1239.https://doi.org/10.3889/oamjms.2020.5232.

[14]   Liu, Y., Tian, L., You, J., Li, Y. (2021). The predictive value of postoperative C-reactive protein (CRP), procalcitonin (PCT), and triggering receptor expressed on myeloid cells 1 (TREM-1) for the early detection of pulmonary infection following laparoscopic general anesthesia for cervical cancer. Ann Palliat Med, 10(4), 4502–8.

[15]   Liu, W., Zhang, L., Xu, H.J., Li, Y., Hu, C.M., Yang, J.Y., et al. (2018). The anti-inflammatory effects of vitamin D in tumorigenesis. Int J Mol Sci, 19(9).

[16]   Marth, C., Landoni, F., Mahner, S., McCormack, M., Gonzalez-Martin, A., Colombo, N. (2017). Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up. Ann Oncol [Internet], 28(Supplement 4), iv72–83. Available from: https://doi.org/10.1093/annonc/mdx220

[17]   Morelli, C., Rofei, M., Riondino, S., Fraboni, D., Torino, F., Orlandi, A., Tesauro, M., Del Vecchio Blanco, G., Federici, M., Arkenau, H.T., et al. (2022). Immune Response in Vitamin D Deficient Metastatic Colorectal Cancer Patients: A Player That Should Be Considered for Targeted Vitamin D Supplementation. Cancers , 14, 2594. https://doi.org/10.3390/cancers14112594.

[18]   Murillo, R., Herrero, R., Sierra, M., Forman, D. (2016). Etiology of cervical cancer (C53) in Central and South America. Cancer Cent South Am Lyon Int Agency Res Cancer [Internet], 1–11. Available from: http://www-dep.iarc.fr/CSU_resources.htm,accessed[date].

[19]   Nehring, S.. (2023). C Reactive protein (Japanese). JapJClinPath, 31(5 sup), 1896–904.

[20]   Shruthi, P.S., Kalyani, R., Kai, L.J., Narayanaswamy, M. (2014). Clinicopathological correlation of cervical carcinoma: A tertiary hospital-based study. Asian Pacific J Cancer Prev,15(4), 1671–4.

[21]   Sproston, N.R., Ashworth, J.J. (2018). Role of C-reactive protein at sites of inflammation and infection. Front Immunol, 9(APR), 1–11.

[22]   Suardi, D., Renita, F., Kurniadi, A., Pramatirta, A.Y., Judistiani, R.T.D., Hidayat, Y.M., et al. (2021). The comparison of 25-hydroxyvitamin d3 between patients with and without cervical cancer. Open Access Maced J Med Sci, 9(B), 564–8.

[23]   Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021).Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin, 71(3),209–49.

[24]   Susilawati, D. (2022). Kanker Serviks Penyebab Kematian Tertinggi No 2 Perempuan Indonesia [Internet]. Republica.co.id, p. 1–2. Available from: https://ameera.republika.co.id/berita//r6sfav414/kanker-serviks-penyebab-kematian-tertinggi-no-2-perempuan-indonesia?#:~:text=Di Indonesia%2C setiap hari ada,meninggal dalam waktu dua tahun

[25]   Tanaka, T., Narazaki, M., Kishimoto, T. (2014). Patterns (DAMPs), released from damaged or dying cells in noninfectious inflammations such as burns or trauma, directly or indirectly promote inflammation. During sterile

surgical operations, an increase in serum IL66 levels precedes elevation, 6(Kishimoto 1989), 1–16.

[26]   Tas, M., Yavuz, A., Ak, M., Ozcelik, B.. (2019). Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in discriminating precancerous pathologies from cervical cancer. J Oncol.

[27]   Tian, T., Gong, X., Gao, X., Li, Y., Ju, W., Ai, Y. (2020). Comparison of locally advanced cervical cancer survival outcomes by histopathological types in the surveillance, epidemiology, and end results (SEER) database: A propensity score matching study. Infect Agent Cancer, 15(1):1–10.

[28]   Umami, A., Rahardjo, S.S., Murti, B. (2018). The Biopsychosocial Factors and Genital Hygiene Affecting Cervical Cancer at Dr. Moewardi Hospital, Surakarta, Central Java, 86–86.

[29]   Wang, H., Wang, M.S., Zhou, Y.H., Shi, J.P., Wang, W.J.. (2020). Prognostic values of LDH and CRP in cervical cancer. Onco Targets Ther, 13, 1255–63.

[30]   Winarno, N.S., Arkhaesi, N., Mulyono, Hardaningsih, G., Limijadi, E.K.S. (2023) Relationship Between CRP, Procalcitonin, I/T Ratio and The Incidence of Sepsis in Children. Modern Medicine, 30 (3), 227-233. https://doi.org/10.31689/rmm.2023.30.3.227.

[31]   Yang, B.B., Chen, Y.H., Zhang, C., Shi, C.E., Hu, K.F., Zhou, J., et al. (2017). Low vitamin D status is associated with advanced liver fibrosis in patients with nonalcoholic fatty liver disease. Endocrine. 2017;55(2):582–90.

[32]   Zhang, S., Xu, H., Zhang, L., Qiao, Y. (2020). Cervical cancer: Epidemiology, risk factors and screening. Chinese J Cancer Res, 32(6),720–8.