

The Level of Serum Selenium in Patients with Cervical Intraepithelial Neoplasia in Medical City/ Baghdad Teaching Hospital

Wasan Wajdi¹, Ishraq Mohammed¹, Zaenb Mohammed Abdulhussein¹

¹Department of obstetrics and gynecology, college of medicine, university of Baghdad, Baghdad, Iraq.

KEYWORDS

Selenium, cervical intraepithelial neoplasia, cervical cancer

ABSTRACT

Introduction: Selenium is an essential trace element involved in different physiological functions of the human body. An inverse relationship between serum selenium levels and cervical intraepithelial neoplasia has been reported. cervical intraepithelial neoplasia is regarded as a potentially premalignant transformation of squamous cells of the cervix. **Objectives:** To evaluate the relationship between the serum level of selenium and cervical intraepithelial neoplasia. **Methods:** A case-control study was conducted at Baghdad Teaching Hospital and Iraqi National Cancer Research Center in the University of Baghdad during the period from July 2021 to July 2022. A convenient sample of 100 women was enrolled in the current study and included case group which consisted of 50 women who were diagnosed with cervical intraepithelial neoplasia and control group which consisted of 50 women who did not have cervical intraepithelial neoplasia as confirmed through histological examination after punch biopsy by colposcopy. **Results:** The serum level of the selenium was significantly lower in the case groups compared to the control groups (P-value=0.001). In addition, the proportion of the participant with abnormal levels of selenium was significantly higher in the case group compared to the control group (P-value=0.001). In the case group, there was a significant difference between the cervical intraepithelial neoplasia grade regarding the serum level of selenium, the level of selenium significantly decreased with the progression of the disease (P-value=0.001). **Conclusions:** Selenium deficiency may significantly increase the incidence of cervical intraepithelial neoplasia. In addition, it significantly impacts the transformation to a higher degree as there was a significant difference between cervical intraepithelial neoplasia I, II, and III regarding the level of serum selenium.

1. Introduction

Human papillomavirus (HPV) infection, especially high-risk human papillomavirus persistent infection, is a prerequisite for the development of CIN and cervical cancer (1). According to the Ministry of Health and Environment/Iraqi Cancer Board, 392 women had cervical cancer in 2020 with an incidence of 1.22/100000 women (2).

Persistent infection of HPV is the direct cause of the vast majority of CINs and increases the risk of CIN progression to cervical cancer (3). A large majority of cervical cancer (more than 95%) is due to HPV which is the most common viral infection of the reproductive tract. Most sexually active women and men will be infected at some point in their lives, and some may be repeatedly infected (4). Thus, cervical cancer has become the only gynecological malignant tumor with a clear etiology in the world (5).

HPV is a double-stranded deoxyribonucleic acid (DNA) virus belonging to the Papovaviridae family, with a genome of approximately 8000 base pairs. High-risk types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, and 59. Others are classified as potential high-risk (which are 53, 66, 70, 73, and 82) (6). Currently, it is well-known and proven that HPV16 and 18 are the most virulent high-risk genotypes, causing about 70% of all invasive cervical cancer in the world (7).

Age, parity, smoking, sexual behavior, oral contraceptives, impaired immunity, the concomitant presence of sexually transmitted infections, and socioeconomic status have been reported as potential factors mediating persistent infection by HPV (3).

There are two types of cervical cancer screening tests broadly used which are the Papanicolaou test (also known as the Pap smear) and the HPV test (8). Screening programs using conventional cytology and conventional Pap smear have successfully reduced cervical cancer, but newer tests like liquid-based cytology and HPV testing might enhance screening (9).

The overall sensitivity of the Pap smear in detecting CIN III is 70.80%, but when it is done in

association with an HPV, the sensitivity for early detection of precancerous lesions is increased (10).

Colposcopy represents the second step of the diagnostic approach to CIN. the benefits of selenium for humans were revealed in landmark studies by Klaus Schwartz and Calvin Foltz who demonstrated that dietary selenium protected rats against liver necrosis. Since then, the role of selenium as a trace mineral nutrient in human health and the mechanisms by which it exerts its biological effects have become better understood (11). As selenocysteine, it is incorporated into 25 human selenoproteins with a wide range of health effects, most notably the ability to reduce oxidative stress and inflammation (12).

The supplementation with low doses seems to be beneficial not only for cancer prevention, but can positively influence many other functions in an organism by reducing inflammations, and heart diseases and regulating blood pressure (13).

Higher selenium status or selenium supplementation is essential for successful male and female reproduction (14). Selenium deficiencies may lead to gestational complications, miscarriages and the damaging of the nervous and immune systems of the fetus. A low concentration of selenium in blood serum in the early stage of pregnancy has been proven to be a predictor of the low birth weight of a newborn (15).

The beneficial effects of selenium supplementation were documented in many studies. The serum selenium levels differ significantly between women with a normal cervix and those with high-grade CIN III. This difference may be a pointer to the existence of other biomarkers especially antioxidant micronutrients that may be important cofactors that modulate the progression of HPV infection to CIN and invasive cervical cancer (16).

Objectives

The importance of selenium supplementation in the process of malignant transformation and proliferation in cervical carcinogenesis (17). To achieve aim of the study, evaluate the relationship between the serum level of selenium and CIN.

2. Methodology

Study design and setting

A case-control study was conducted in Iraq / Baghdad / Baghdad Teaching Hospital and Iraqi National Cancer Research Center in the University of Baghdad during the period from July 2021 to July 2022.

Ethical approval

The current study was proposed and subsequently approved by the Scientific Council of Obstetrics and Gynecology Specialization of the Arabic Board of Medical Specialization. All the information was kept anonymous and used for scientific purposes only.

Study sampling

A convenient sample of 100 women who attended the Gynecology /Oncology Outpatient Clinic in Baghdad Teaching Hospital and met inclusion criteria was enrolled in the current study.

Inclusion criteria

1. Age of 18-65 years' old
2. Women with abnormal pap smear test (mild to moderate or severe inflammation)
3. Women with three consecutive inadequate cervical samples in pap smear
4. Women with an abnormal cervical cytology
5. Women with an abnormal cervical appearance
6. Women with post-coital bleeding,

7. Women with inter-menstrual bleeding
8. Persistent vaginal discharge that can be explained by an infection or other causes.

These women were invited to have a colposcopic examination. Written consent was taken from them after a detailed explanation about the procedure, purposes, and expected adverse events with confidentiality ensured regarding their personal and medical information. After histological results, the participants were submitted into two groups, including:

Case group: It consisted of 50 women who were diagnosed with CIN. The diagnosis of CIN was confirmed through punch biopsy by the colposcope.

Control group: It consisted of 50 women who did not have CIN as confirmed through histological examination after punch biopsy by colposcope.

Exclusion criteria

1. Pregnant women
2. Women with chronic illnesses such as hypertension, diabetes mellitus, chronic liver disease, chronic kidney disease, and cancer.
3. Women who were using multivitamins supplementation
4. For social concerns, single women were excluded from the study.

Data collection

A structured questionnaire was adopted for data collection; this questionnaire was prepared by the researcher after a review of many similar articles with revision by the supervisor. The data collection was done through three steps, including: Step one: Sociodemographic, medical and obstetrical history including age, occupation, education, residency, marital state, history of chronic disease, parity, miscarriage, age.

Step two: Colposcopic examination: The woman was asked to lie in a modified lithotomy position on the examination table after she has emptied their bladder. Inspection of the external genitalia was done for the presence of any lesions followed by a full pelvic examination was done in the usual way. A sterile Cusco's speculum was carefully inserted in the vagina without using antiseptic solution for sterilization of the genitalia. Normal saline solution was applied to the cervix with cotton balls or a syringe and excess water is removed to examine the detail of cervical capillaries and surface blood vessels, then 5% acetic acid was applied to the cervix with a cotton-tipped swab or cotton balls and wiping the cervix a few times with it to assist in the coagulation and removal of mucus, which in turn helps the acetic acid to penetrate to the epithelium in full strength, the acetowhitening effect of acetic acid develops gradually over 60 seconds and fade afterwards, Areas of premalignant lesions and invasive cancer turned densely white and opaque after application of acetic acid, due to their higher concentration of abnormal nuclear protein and the presence of large numbers of dysplastic cells in the superficial layers of the epithelium.

Finally, with applying Lugol's iodine solution, abnormal transformation zones, such as those with CIN or invasive cancer appeared as mustard or saffron yellow areas because they contain very little or no glycogen.

Once an abnormal transformation zone was detected, the biopsy was obtained from the area of the lesion with the worst features and closest to the squamocolumnar junction. The biopsy was taken by oncologists or gynaecologists and sent for histopathology. If the woman had premalignant lesions (CIN I, CIN II, or CIN III), she submitted to the case group, but if she had normal cervical histology then submitted to the control group.

Step three: Measurement of serum selenium Venous blood samples were taken by the researcher and sent to the Teaching Laboratories in the Directorate of the Medical City where they were centrifuged

for 10 minutes at 3500 rpm. Afterwards, the serum was separated and stored at -20 and -80° and sent to a private laboratory for investigation.

Statistical analysis

The data was collected and analyzed using Microsoft Excel software, version 2016, and Statistical Package for the Social Sciences (SPSS), version 22. Categorical data were expressed as frequencies and percentages while continuous data were presented as mean (\pm Standard deviation (SD)). The Chi-Square test, t-test, and ANOVA test were used to assess the significance of the difference between the study groups. A P-value of 0.05 or less was considered statistically significant

3. Result and Discussion

A total of 100 women were enrolled in the current study. There were no significant differences between the study groups regarding age, residency, occupation, education, or marital state (P-values were 0.460, 0.405, 0.159, 0.259, and 0.640, respectively). Among the case group, the largest age group was 30-39 years followed by 40-49 years. As shown in table1.

Table 1: Sociodemographic distribution according to the study groups.

Sociodemographic characteristics		Groups		Total	P-value
		Case N (%)	Control N (%)		
Age group	<30	14 (28.0)	11 (22.0)	25 (25.0)	0.460
	30-39	19 (38.0)	15 (30.0)	34 (34.0)	
	40-49	15 (30.0)	19 (38.0)	34 (34.0)	
	≥ 50	2 (4.0)	5 (10.0)	7 (7.0)	
Residency	Urban	30 (60.0)	34 (68.0)	64 (64.0)	0.405
	Rural	20 (40.0)	16 (32.0)	36 (36.0)	
Occupation	Housewife	19 (38.0)	26 (52.0)	45 (45.0)	0.159
	Employed	31 (62.0)	24 (48.0)	55 (55.0)	
Education	Illiterate	6 (12.0)	5 (10.0)	11 (11.0)	0.259
	Primary	7 (14.0)	15 (30.0)	22 (22.0)	
	Secondary	21 (42.0)	19 (38.0)	40 (40.0)	
	College	16 (32.0)	11 (22.0)	27 (27.0)	
Marital state	Married	39 (78.0)	37 (74.0)	76 (76.0)	0.640
	Widow or divorced	11 (22.0)	13 (26.0)	24 (24.0)	

Chi-Square test

There were no significant differences between the study groups regarding parity, miscarriage, history of the COCP, and age at first pregnancy (P-values were 0.444, 0.704, 0.086, and 0.088, respectively), as shown in table 2.

Table 2: Obstetrical history of the participants

Sociodemographic characteristics		Groups		Total	P-value
		Case (N=50) N (%)	Control (N=50) N (%)		
Parity	<3	31 (62.0)	25 (50.0)	56 (56.0)	0.444*
	4-6	14 (28.0)	17 (34.0)	31 (31.0)	
	>6	5 (10.0)	8 (16.0)	13 (13.0)	

Miscarriage	0	32 (64.0)	29 (58.0)	61 (61.0)	0.704*
	1	11 (22.0)	13 (26.0)	24 (24.0)	
	2	1 (2.0)	3 (6.0)	4 (4.0)	
	3	6 (12.0)	5 (10.0)	11 (11.0)	
OCP	Yes	14 (28.0)	7 (14.0)	21 (21.0)	0.086*
	No	36 (72.0)	43 (86.0)	79 (79.0)	
		Case (N=50) Mean (\pm SD)	Control (N=50) Mean (\pm SD)		
Age at first pregnancy		24.8 (\pm 5.3)	23.2 (\pm 3.8)		0.088**

Within the case group, 31 (62%) have CIN I, 17 (34%) have CIN II, while only 2 (4%) have CIN III, as shown in **figure 1**.

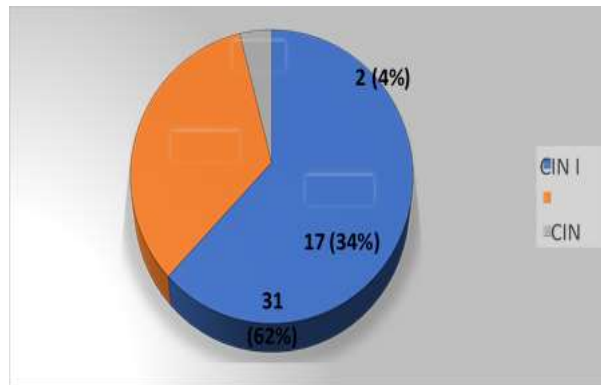


Figure 1: Distribution of the patients according to the grades of cervical intraepithelial neoplasia. The serum level of the selenium was significantly lower in the case groups compared to the control group (P-value=0.001). In addition, the proportion of the participant with abnormal levels of selenium was significantly higher in the case group compared to the control group (P-value=0.001), as shown in **table 3**.

Table 3: Distribution of the serum level of selenium according to the study groups.

Serum selenium		Groups		Total	P-value
		Case N (%)	Control N (%)		
Serum selenium level (110-165 mcg/L)	Abnormal	33 (66.0)	14 (28.0)	47 (47.0)	0.001*
	Normal	17 (34.0)	36 (72.0)	53 (53.0)	
		Case Mean (\pm SD)	Control Mean (\pm SD)		
Serum selenium level (mcg/L)		103.7 (\pm 18.6)	123.3 (\pm 20.8)		0.001*
SN=66%, SP=72%, PPV=70.2%, NPV=67.9%, Accuracy=69%					

*Chi-Square test. **t-test.

In the case group, there was a significant difference between the CIN grade regarding the serum level

of selenium, the level of selenium decreased with the progression of the disease (Table 4 and figure 2)

Table 4: Distribution of selenium according to the cervical intraepithelial neoplasia grades

CIN grade	N	Serum selenium (mcg/L)	P-value
		Mean (\pm SD)	
CIN I	31	112.8 (\pm 16.6)	0.001
CIN II	17	89.5 (\pm 10.4)	
CIN III	2	83.0 (\pm 4.2)	
Total	50	103.7 (\pm 18.6)	

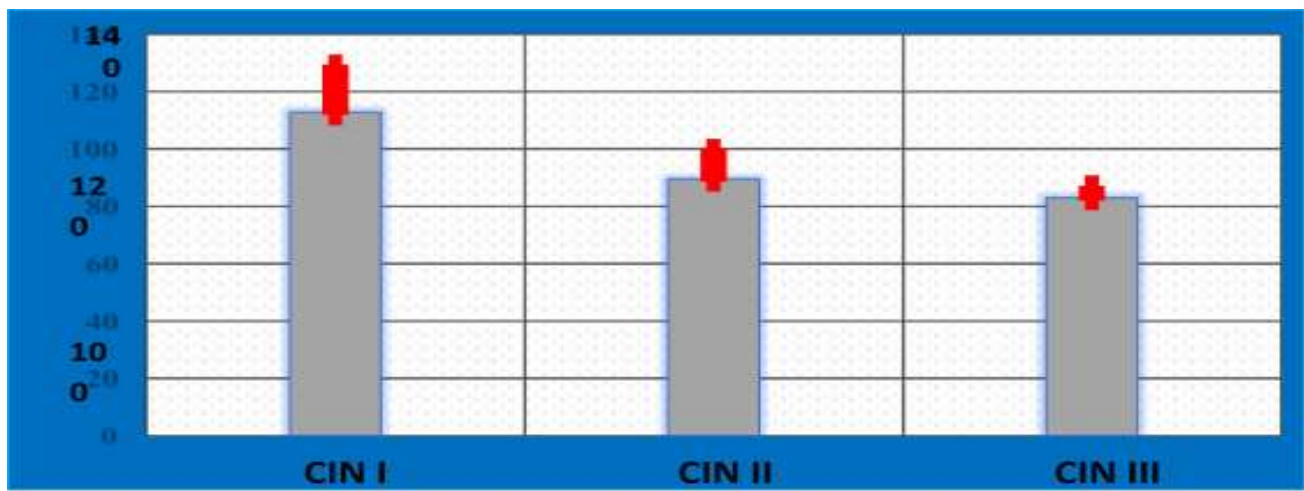


Figure 2: Trend of the serum selenium level with the progression of cervical intraepithelial neoplasia.

Discussion

Nutritional antioxidants such as selenium, a component of glutathione peroxidase have been demonstrated to be involved in the anti-carcinogenic processes of a variety of human cancers (18). Best to our, this was the first study in Iraq to assess the role of antioxidants in the development of cancer, specifically, the role of selenium in the development of CIN.

In the current study, women with age 30-39 constituted the largest age group, followed by those aged of 40-49 years. In the United States, Massad et al. concluded that women over the age of 30 years are more likely to have a persistent infection and warrant more aggressive follow-up to rule out CIN (19). In China, a study was done by Yang J et al. revealed that the mean age of the patients with CIN was 49.2 (\pm 9.1) years, most of them aged 30-50 years (20).

There were no significant differences between the study groups regarding the parity, miscarriage, and age of women at first pregnancy. In Nigeria, Obhielo et al. obtained the same results (18). In China, Yang et al. agreed with these results and concluded an insignificant association between CIN and parity (20).

According to the results of the current study, no significant association was obtained between CIN and the duration of use of COCP. In comparison, Hui Jun Chih et al. concluded that the use of oral contraceptives appeared to be inversely associated with CIN. Prolonged use of oral contraceptives demonstrated its benefits of reducing the risk of CIN (21). In contrast, Putri et al. postulated that the use of COCP for more than ten years was considered a risk factor associated with cervical cancer incidence (22).

The main finding of the current study was that patients with CIN were significantly associated with low selenium levels compared to the control. Soo Yeon Kim et al. concluded that the selenium level was significantly lower in patients with CIN or cancer compared to the controls. In addition, lipid

peroxidation, decreased selenium, decreased zinc levels, and impaired serum antioxidant system were involved in the pathogenesis of cervical dysplasia (23). Bae et al. concluded the same findings were obtained (24).

Leri septiani et al. revealed that the serum selenium concentration was significantly lower in cervical cancer patients and had an important role in the carcinogenesis of cervical cancer (25). Karamali et al. revealed an inverse relationship between serum selenium levels and CIN or cervical cancer, there were significantly lower concentrations of selenium in cervical cancer tissues compared with normal tissues. Daily supplementation with 200 µg of selenium for women with CIN1 for six months was associated with higher CIN1 regression (88% vs. 56% in the placebo group). Additionally, selenium was also shown to exert beneficial antioxidant and metabolic effects in women with CIN1(17).

Jing Ji et al. suggested that the concomitant use of selenium could be a potential therapeutic approach to improve the efficacy of therapy for uterine cervical cancer (26). Another important finding of the current study was a significant decrease in the selenium level in patients with CIN III compared to patients with CIN II and CIN I. which agreed with... Obhielo et al. who revealed a statistically significant difference between patients with normal cervical cytology, CIN I, II and III regarding the levels of serum selenium (18).

4. Conclusion and future scope

Selenium deficiency may significantly increase the incidence of CIN. In addition, it significantly impacts the transformation to a higher degree as there was a significant difference between CIN I, CIN II, and CIN III regarding the level of serum selenium. Assessment of antioxidants, including serum selenium, should be done during the routine investigation for patients with suspected or confirmed CIN. Other studies can be done during the next time to assess the changes of the serum selenium in other types of cancers and assess the changes of other antioxidants in patients suspected or confirmed to have CIN

Acknowledgments

The authors express gratitude to Baghdad Teaching Hospital and Iraqi National Cancer Research Center in the University of Baghdad for their assistance with the accomplishment of the research.

Conflict of interest

None

Funding

None

Reference

- [1] Liang Y, Chen M, Qin L, et al. A meta-analysis of the relationship between vaginal microecology, human papillomavirus infection and cervical intraepithelial neoplasia. *Infectious Agents and Cancer*. 2019;14(1):29.
- [2] Board MoHaEIC. Annual Report of Iraq Cance Registry 2020 2020 [Available from: <https://moh.gov.iq/>].
- [3] Ouh Y-T, Cho HW, Kim SM, et al. Risk factors for type-specific persistence of high-risk human papillomavirus and residual/recurrent cervical intraepithelial neoplasia after surgical treatment. *ogs*. 2020;63(5):631-42.
- [4] WHO. Cervical cancer 2022 [Available from: <https://www.who.int/news-room/fact-sheets/detail/cervical-cancer>].
- [5] Zhang J, Cheng K, Wang Z. Prevalence and distribution of human papillomavirus genotypes in cervical intraepithelial neoplasia in China: a meta- analysis. *Archives of Gynecology and Obstetrics*. 2020;302(6):1329-37.
- [6] Chan CK, Aimagambetova G, Ukybassova T, et al. Human Papillomavirus Infection and Cervical Cancer: Epidemiology, Screening, and Vaccination—Review of Current Perspectives. *Journal of Oncology*. 2019;2019:3257939.
- [7] Zhao X-L, Hu S-Y, Zhang Q, et al. High-risk human papillomavirus genotype distribution and attribution to cervical cancer

- and precancerous lesions in a rural Chinese population. *Journal of gynecologic oncology*. 2017;28(4).
- [8] kareem Aledan NA, Hussien WAL. The Cervical pap smear cytological changes with clinicopathological correlation in a sample of Iraqi patients. *Iraq Medical Journal*. 2021;5(3):75-80.
 - [9] Singh VB, Gupta N, Nijhawan R, et al. Liquid-based cytology versus conventional cytology for evaluation of cervical Pap smears: experience from the first 1000 split samples. *Indian Journal of Pathology and Microbiology*. 2015;58(1):17.
 - [10] Sachan PL, Singh M, Patel ML, et al. A Study on Cervical Cancer Screening Using Pap Smear Test and Clinical Correlation. *Asia-Pacific Journal of Oncology Nursing*. 2018;5(3):337-41.
 - [11] Avery JC, Hoffmann PR. Selenium, Selenoproteins, and Immunity. *Nutrients*. 2018;10(9):1203.
 - [12] Rayman MP, Winther KH, Pastor-Barriuso R, et al. Effect of long-term selenium supplementation on mortality: Results from a multiple-dose, randomised controlled trial. *Free Radical Biology and Medicine*. 2018;127:46-54.
 - [13] Sanmartín C, Plano D, Sharma AK, et al. Selenium Compounds, Apoptosis and Other Types of Cell Death: An Overview for Cancer Therapy. *International Journal of Molecular Sciences*. 2012;13(8):9649-72.
 - [14] Rayman MP. Selenium and human health. *The Lancet*. 2012;379(9822):1256- 68.
 - [15] Pieczyńska J, Grajeta H. The role of selenium in human conception and pregnancy. *Journal of Trace Elements in Medicine and Biology*. 2014;29.
 - [16] Obhielo E, Ezeanochie M, Olorok O O, et al. The Relationship between the Serum Level of Selenium and Cervical Intraepithelial Neoplasia: A Comparative Study in a Population of Nigerian Women. *Asian Pac J Cancer Prev*. 2019;20(5):1433-6.
 - [17] Karamali M, Nourgostar S, Zamani A, et al. The favourable effects of long- term selenium supplementation on regression of cervical tissues and metabolic profiles of patients with cervical intraepithelial neoplasia: a randomised, double- blind, placebo-controlled trial. *British Journal of Nutrition*. 2015;114(12):2039-45.
 - [18] OBHIELO EI. COMPARISON OF SERUM SELENIUM LEVELS IN NIGERIAN WOMEN WITH AND WITHOUT PRE-INVASIVE CERVICAL LESIONS. *FACULTY of OBSTETRICS AND GYNAECOLOGY*. 2016.
 - [19] Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis*. 2013;17(5 Suppl 1):S1-s27.
 - [20] Yang J, Yang A, Wang Z, et al. Interactions between serum folate and human papillomavirus with cervical intraepithelial neoplasia risk in a Chinese population- based study. *The American Journal of Clinical Nutrition*. 2018;108(5):1034-42.
 - [21] Chih HJ, Lee AH, Colville L, et al. Condom and oral contraceptive use and risk of cervical intraepithelial neoplasia in Australian women. *jgo*. 2014;25(3):183- 7.
 - [22] Putri AR, Khaerunnisa S, Yuliati I. Cervical cancer risk factors association in patients at the gynecologic-oncology clinic of Dr. Soetomo Hospital Surabaya. *Indonesian Journal of Cancer*. 2019;13(4):104-9.
 - [23] Kim SY, Kim JW, Ko YS, et al. Changes in lipid peroxidation and antioxidant trace elements in serum of women with cervical intraepithelial neoplasia and invasive cancer. *Nutrition and cancer*. 2003;47(2):126-30.
 - [24] Bae H-S, Lee G-J, Ahn H-S. Selenium, Manganese, Copper and Zinc Contents in Serum of Patients with Cervical Intraepithelial Neoplasia. *Korean Journal of Community Nutrition*. 2005;10(5):700-7.
 - [25] Septiani L, Hidayat YM, Mutakin M, et al. Serum selenium concentration in cervical cancer patients: Preliminary study and literature review. *International Journal of Integrated Health Sciences*. 2016;4(2):56-61.
 - [26] Ji J, Liu J, Liu H, et al. Effects of Fermented Mushroom of *Cordyceps sinensis*, Rich in Selenium, on Uterine Cervix Cancer. *Evidence-Based Complementary and Alternative Medicine*. 2014;2014:173180.