Retinol as a micronutrients related to cervical local immunity: The expression of tumor necrosis factor-alpha specifically stimulated with E6 epitope of human papillomavirus type-16 and ratio of CD4+/CD8+ T cell in natural history of cervical cancer

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Abstract. Retinol is one of the antioxidant micronutrients that plays essential roles in the immune system, by preventing the persistence of modulating CD4+ and CD8+ T cells and cytokines production. Tumor Necrosis Factor-Alpha (TNF-α) is an acute pro-inflammatory cytokine which has many crucial roles in controlling HPV. In contrast, when persistent infection occurs, TNF-α induces carcinogenesis. The ratio of CD4+ cells to CD8+ T cells and adequate TNF-α production in acute HPV infection are key points for clearance. The aim of this research is to analyze the sufficiency level of retinol deposit, the expression of TNF-α, and the ratio of CD4+/CD8+ T cells in a normal cervix, clearance and persistent HPV subclinical infection, and cervical cancer group. The sufficiency level of retinol deposit was analyzed from peripheral blood using the ELISA method. The cervico-vaginal secretions, which were incubated for 24 hours, were stimulated specifically by E6 epitope HPV type-16, measuring TNF-α expression semi-quantitatively by the ELISpot method and CD4+/CD8+ T cells quantitatively by flowcytometry method. The sufficient level of retinol deposit in a normal cervix, clearance HPV subclinical infection, persistent, and cervical cancer group was 85%, 75% (OR 1.89), 33.3% (OR 11.33), and 75% (OR 1.89), respectively. The expression of TNF-α in normal cervix group was 10%, while for cervical cancer it was 75% (OR 27.00; p < 0.001). There was no expression in clearance and persistent HPV subclinical infection groups. A high ratio of CD4+: CD8+ T cells in the normal cervix and cervical cancer group was 10% and 25% (OR 0.33). There was no high ratio of CD4+: CD8+ T cells in clearance (OR 1.22) and persistent (OR 0.95) HPV subclinical infection groups. This study was able to prove that the normal cervix group has the highest retinol deposit sufficiency level and the cervical cancer group has the highest TNF-α expression (OR 27; p < 0.001). The lowest of retinol deposit sufficiency level was not in cervical cancer, but in the persistent HPV subclinical infection group (OR 11.33). There was significant correlation in TNF-α expression between cervical cancer and normal cervix (p < 0.001), cervical cancer and clearance subclinical HPV infection (p = 0.024), and between clearance and persistent group (p = 0.007).

1. Introduction
Cervical cancer is ranked as the second most common cancer experienced by women in the world. Eighty-three percent of cases occur in developing countries [1]. This cancer is highly preventable
because of the known cause. The main causative factor is persistent infection with high-risk Human Papillomaviruses (HPVs) (HPV-RT), which is oncogenic and a malignant precursor. Although HPV has been known to cause cervical cancer, 70-90% of cases will have clearance. Only very few HPV infections become persistent, later developing into cervical cancer. This raises the premise that there are other factors contributing to the occurrence of cervical cancer besides HPV infection [2]. An effective individual immune response is a determinant factor of susceptibility to HPV infection and its progression to cervical cancer. In patients with a good immune system, most HPV infections occur subclinically, and only a small proportion of these infections will progress into pre-cancerous lesions or invasive cervical cancer. Nutritional cofactors associated with immunity play a crucial role in defense against HPV. Retinol as an antioxidant micronutrient plays an important role in non-specific and specific immunity to HPV and tumor cells [3] which have a central role in the growth, development, and differentiation of B and T lymphocytes and the activation of the immune system’s main regulatory cells [4].

A competent and effective local immune response against HPV is a determinant factor in achieving clearance, thereby preventing the persistence of HPV infection and its progression into cervical cancer. Retinol as an essential nutrient cofactor in cervical mucosa immunity. It is able to modulate CD4+ and CD8+ T cells, as well as potent TNF-α production against the control of HPV infection and tumor cells. However, there has been no recommendation of retinol to prevent HPV infection and to improve the clinical cervical cancer treatment response.

TNF-α has two opposing roles; these are immunomodulation and tumorigenesis. The role of TNF-α at the beginning of acute infection and the ratio of CD4+: CD8+ T cells determines HPV clearance and regression of lesions. Adequate retinol deposits will increase the ratio of CD4+: CD8+ T cells and TNF-α production. Therefore, how does TNF-α expression, CD4+ T cell ratio: CD8+ cervix is associated with a sufficient level of retinol deposits when performed simultaneously on the natural course of cervical cancer. No studies have demonstrated a local immune response through semi-quantitative TNF-α expression, especially stimulated cervico-vaginal secretions with 16-cell HPV antigens in vitro, CD4+ T cell ratio: CD8+ in the cervix, and the adequacy of retinol deposits simultaneously in normal cervix, infection subclasses of persistent and persistent HPV, and cervical cancer. This study aims to understand the role of nutritional cofactors and local immunity in the cervix on the natural course of cervical cancer.

2. Materials and Methods
In this study, data for normal cervical groups and cervical cancers were taken by cross-section. Data for cervical groups with subclinical HPV-RT clearance and persistent infections were taken from the cohort population with positive HPV-RT DNA test results, examined between 12 and 24 months later. The study was conducted in the Department of Obstetrics and Gynecology, Women Health Center, Kencana Hospital in Central Jakarta and Gynecology Fatmawati Hospital in South Jakarta. The target demographic for the study was the affluent population that attends the Gynecology Oncology Polyclinic Department of Obstetrics and Gynecology Cipto Mangunkusumo and Fatmawati Hospital, Jakarta. Thus, the subjects of the study was the affluent population that attends the Gynecology Oncology Polyclinic Department of Obstetrics and Gynecology Cipto Mangunkusumo and Fatmawati Hospital, Jakarta. The exclusion criteria were malnutrition, pregnancy, history of sharing needles, having multiple sexual partners, using intrauterine contraceptives (IUD), having an infection of the genitourinary tract, the age of marriage for the first time in less than 20 years, or engaging with sexual partners, while on long-term steroid therapy.

The research subjects were divided into two groups: potential subjects, and newly diagnosed patients with cervical cancer (Cipto Mangunkusumo Hospital, Fatmawati Hospital) with pathological anatomy (PA) results of Squamous Cell Carcinoma. These groups were then divided into two subgroups: 1) cytology/LBC (-) and HPV-RT (-) groups during the last two months and 2) cytology/ LBC (-) and HPV-RT (+) DNA groups for 12 to 14 years. All subjects were recalled for the filling of the consent form. The subjects were then divided into 4 small groups, namely group A for subjects with cytology/LBC (+) and HPV-RT DNA (-) over the past two months; Group B for cytology/LBC (-) and
HPV-RT DNA (-) or referred to as HPV-RT clearance; Group C for cytology/LBC (-) subjects and HPV-RT DNA (+) or referred to as persistent HPV-RT and group D for newly diagnosed cases of cervical cancer.

All subject groups A, B, C, D were asked to fast for 8 hours. Afterward, blood samples were obtained for laboratory tests in groups A, B and C in WHC, while group D was in polyclinic or hospital wards. The test performed included the following stages: 1) 5 ml peripheral blood was sampled (fast RBP4 examination), 2) 5 ml peripheral blood was sampled (RBP4 2 h after retinol palmitate 6000 IU per oral), 3) cervico-vaginal secretion A & B: 2 tubes (TNF-α, CD4+:CD8+) and C & D cervico-vaginal secretions: 3 tubes (TNF-α, CD4+:CD8+, HPV DNA). Each tube was labeled. Tubes for TNF-α and peripheral blood for RBP4 were sent to the PRVKP / IHVCB laboratory for the ELISpot test. Tubes for CD4+ cell examination: CD8+ were sent to RSCM Clinical Pathology laboratory for Flowcytometric test. The tube for the detection and genotyping of HPV DNA was sent to the KALGEN lab for Xpress Matrix™ testing. After the lab results were obtained, the data analysis was performed.

3. Results and Discussion

3.1 Level of Adequacy of Retinol Deposit

RBP4 levels before and after the oral administration of retinol palmitate 6000 UI in each of the studied groups are presented in Figure 1 below:

![Figure 1](image_url)

**Figure 1.** RBP4 levels before and after the oral administration of retinol palmitate 6000 UI. A: Normal cervix (A1-A20) B: Cervix with subclinical HPV-RT clearance (B1-B4) and persistent (C1-C3) C: Cervical cancer group (E1-E20)

In this study, it was found that retinol deposit adequacy rates were lower in the normal cervical group, subclinical HPLC-RT clearance, persistent, and cervical cancers were 15%, 25%, 66.7%, and 25%, respectively (OR - 1, 89; 11,33; 1.89). The highest retinol deposits were found in the normal cervical
group (85%); the lowest were found in the persistent HPV-RT group. HPV-RT clearance and cervical cancer groups had the same adherence of 75%. The level of adequacy of retinol deposits in each group is presented in Table 1 below.

### Table 1. The adequacy of retinol deposits

| Groups                  | The adequacy of retinol deposits | OR     | CI (95%)     | p-value |
|-------------------------|----------------------------------|--------|--------------|---------|
|                         | Deficient                        | Adequate |              |         |
| Cervical cancer         | 5                                | 15     | 1.89         | 0.39-9.27 | 0.433   |
| HPV-RT persistent       | 2                                | 1      | 11.33        | 0.76-167.97 | 0.078   |
| HPV-RT clearance        | 1                                | 3      | 1.89         | 0.14-24.79 | 0.628   |
| Controls (normal)       | 3                                | 17     | Ref          |         |

Chi-Square Test Continuous Correction

The cervical cancer group obtained results that diverged from clinical justification. The relationship between the groups with the level of retinol deposit adequacy is presented in Table 2.

### Table 2. Relationship between groups with sufficient levels of retinol deposits

| Groups                         | OR     | CI (95%)     | p-value |
|--------------------------------|--------|--------------|---------|
| Cervical cancer vs HPV-RT clearance | 1.00   | 0.08-11.93  | 1.000   |
| Cervical cancer vs HPV-RT persistent  | 0.17   | 0.01-2.26   | 0.430   |
| HPV-RT persistent vs HPV-RT clearance | 6.00   | 0.22-162.54 | 0.740   |

Chi-Square Test Continuous Correction

Tables 1 and 2 demonstrate that there is no statistically significant difference in retinol deposit adequacy rates between all study groups. Between normal cervical groups with cervical cancer, normal cervix with persistent HPV-RT, and normal cervix with HPV -RT clearance, p = 0.433; 0.078; 0.628 respectively. Similarly, among cervical cancer groups with persistent HPV-RTP, p = 1.000; 0.430 respectively, and between persistent HPV-RT groups with clearance, p = 0.740. This study was able to demonstrate that the highest levels of retinol deposit adequacy were found in the normal cervical group and fewer retinol deposits were found in the persistent subclinical HPV infection group. However, the study did not succeed in proving that the cervical cancer group had a sufficient level of retinol deposits. These results provide important data contributions to some previous studies on the role of vitamin A, which is still inconsistent as a chemopreventive agent in the natural course of cervical cancer. The significance of the results obtained in this study may still be due to the many factors affecting endogenous retinol levels, including the individual’s age, race, contraceptive pill use, pregnancy number, in addition to climatic seasons [5,6].

This study demonstrated that there was no statistically significant difference in the adequacy of retinol deposits between the persistent groups, the normal cervical groups, the cervical with clearance group, and the cervical HPV infection group. The results of this study concur with the results of research conducted by Palan et al. [7] and Siegel et al. [8]. In Siegel et al study, there was no significant association between endogenous retinoic acid with clearance of HPV infection and cervical lesion regression. Palan et al. stated that there was no significant difference in mean plasma retinol levels in 235 subjects, some with normal cervical conditions, some with cervical intraepithelial, some with cervical cancer. In another study, Giuliano et al. [9], point out that persistent HPV infection can be inhibited with several micronutrients of antioxidants. Goodmann et al. [10], found a significant association between high plasma antioxidant levels and NIS risk. Siegel et al. concluded that retinol is not protective against persistent oncogenic HPV infection in Brazilian female populations [8]. The
The present invention is supported by Alvarez et al. [11], which showed no significant difference in regression rates of cervical lesions between placebo groups, groups with low doses of retinoids, and groups with high doses of retinoids.

3.2 TNF-α expression in cervico-vaginal secret cells stimulated with Epitope E6 HPV type 16

The spot view on the plate depicting TNF-α expression in cervico-vaginal discharge cells stimulated by E6 HPV type 16 epitope can be seen in Figure 2.

![EliSpot examination plate](image)

**Figure 2.** EliSpot examination plate. A: Negative results expressed in the absence of spots or less than 2 spots, B: Positive results as 2 spots appear, C: Positive results as 14 spots appear, D: Positive results as there are 214 spots from the calculation result with EliSpot reader

The TNF-α expression data recapitulation in each group is presented in Table 3 below:

| TNF-α expression groups | OR (95%)       | p-value |
|-------------------------|----------------|---------|
| Positive                | Negative       |         |
| Cervical cancer         | 15             | 5       | 27.00 | 4.57-159.66 | <0.001   |
| HPV-RT persistent       | 0              | 3       | 1.06* | 0.04-27.11  | 0.99     |
| HPV-RT clearance        | 0              | 4       | 0.82* | 0.03-20.25  | 0.99     |
| Controls (normal)       | 2              | 18      | Ref   |           |          |

Chi-Square Test Continuous Correction
*Calculated by adding 0.5 to each table cell
TNF-α expression is positive with spot

From Table 3, it can be seen that TNF-α expression in the cervical cancer group is 75% (OR 27.00; p < 0.001), compared to 10% in the normal cervix group. There was no TNF-α expression in the subclinical clusters of persistent HPV-RT subclinical groups. This study proved that TNF-α expression in cervico-vaginal cervical cells stimulated with the HPV type 16 antigen was highest in the cervical cancer group. In this study, there were significant differences in TNF-α expression, both clinically and statistically, between normal cervical (control) groups and cervical cancer (OR = 27.00; CI 95% = 4.57-
There were statistically significant differences between cervical cancer group and HPV-RT clearance, and persistent HPV-RT with clearance (OR = 25.36; CI 95% = 1.17-551.59; p = 0.024) and (OR = 1.29; CI 95% = 0.02-82.50; p = 0.007). However, in the control group with persistent HPV-RT and clearance, and persistent cervical cancer group, there were no significant differences in TNF-α expression, either clinically or statistically (p = 0.99, 0.99, and 0.058). TNF-α expression was not found on all HPV-RT persistent and clearance subjects. Thus, the results of this study support previous studies, confirming that TNF-α plays a significant role in cervical cancer tumorigenesis. The statistical calculation of the relationship between the groups with TNF-α expression can be seen in Table 4.

| TNF-α expression groups                  | OR     | CI (95%)          | p-value |
|-----------------------------------------|--------|-------------------|---------|
| Cervical cancer vs HPV-RT clearance     | 25.36* | 1.17-551.59       | 0.024   |
| Cervical cancer vs HPV-RT persistent    | 19.72* | 0.87-446.23       | 0.058   |
| HPV-RT persistent vs HPV-RT clearance   | 1.29*  | 0.02-82.50        | 0.007   |

3.3 TNF-α Expression Associated HPV with -RT DNA Examination

The HPV-RT type detected in the cervical cancer group and subclinical HPV infection associated with TNF-α expression is presented in Table 5.

| Type of HPV-RT | TNF-α expression groups |
|---------------|-------------------------|
|               | Cervical cancer (n=20)   |
|               | HPV-RT persistent (n=3)  |
|               | HPV-RT Clearance (n=4)   |
| 16            | 2/3                     |
| 18            | 6/8*                    |
| 31            | 0/1                     |
| 33            | ½                       |
| 35            | 1/1                     |
| 51            | -                       |
| 52            | 2/2                     |
| 58            | -                       |
| 59            | 3/3*                    |
| 66            | 1/1                     |
| * Multiple infections on the same subject (type 18,59) |

- Description: 2/3 interpreted that there were 3 specific sample subjects that were positive for certain HPV-RT types and 2 of them gave positive results on TNF-α expression.
This study showed that all subjects in the cervical cancer group had a positive result on HPV DNA detection, all of which included high-risk types (HPV-RT). Distribution of HPV-RT types detected in cervical cancer can be seen in Table 6.

Table 6. Distribution of HPV-RT types detected in cervical cancer

| Type of HPV | Cervical cancer (n=20) | % (100) |
|-------------|------------------------|---------|
| HPV 16      | 3                      | 15%     |
| HPV 18      | 8*                     | 40%     |
| HPV 31      | 1                      | 5%      |
| HPV 33      | 2                      | 10%     |
| HPV 35      | 1                      | 5%      |
| HPV 52      | 2                      | 10%     |
| HPV 59      | 3*                     | 15%     |
| HPV 66      | 1                      | 5%      |

* 1 subject has multiple HPV types 18 and 59

The histopathologic type of cervical cancer in this study was entirely Kearns-Sayre syndrome (KSS), but the type of HPV detected in this study was dominated by type 18 (8/20 = 40%), followed by types 16 and 59 (3/20 = 15% respectively), types 33 and 52 (2/20 = 10%), and types 31, 35, and 66 (1/20 = 5%). The distribution of HPV types in cervical cancer obtained from this study is relatively inconsistent with previous studies, that most KSS cervical cancers are dominated by HPV infection type 16 (59.3%), type 18 (12.4%) and type 59 (5.1%). In the cervical cancer group, it was found that 15 of the 20 samples of cervical cancer gave positive results of TNF-α expression with HPV types 16, 18, 31, 33, 35, 52, 59, and 66. The 5 cervical cancer subjects whose expression was negative all had a single infection of HPV types 16, 18 (2 subjects), 31, and 33.

3.4 The ratio of CD4\(^+\) T cells: CD8\(^+\)

The manual count results of the ratio of CD4\(^+\):CD8\(^+\) T cells from the flowcytometric examination are presented in Figure 3.

A.

B.
The count results of the ratio of CD4$^+$ T: CD8$^+$ T cells from flowcytometric examination in each group are presented in Figure 4. The CD4$^+$ T cell CD4$^+$ recapitulation data is presented in Table 7.
Figure 4. Number of CD4+ T cell counting: CD8+, A: Normal cervix (A1-A20), B: Cervix with subclinical HPV-RT clearance (B1-B4) and persistent (C1-C3), C: Cervical cancer (E1-E20)
Table 7. Ratios of CD4+ T cells: CD8-

| Groups of CD4+ : CD8+ Cells T Ratio | OR   | CI (95%)  | p-value |
|------------------------------------|------|-----------|---------|
| Low                                | High |           |         |
| Cervical cancer                    | 15   | 5         | 0.33    | 0.06-1.97 | 0.226 |
| HPV-RT persistent                  | 3    | 0         | 0.95*   | 0.04-24.26 | 0.999 |
| HPV-RT clearance                   | 4    | 0         | 1.22*   | 0.05-30.03 | 0.999 |
| Control (normal)                   | 18   | 2         | Ref     |           |       |

Chi-Square Test Continuous Correction
* Calculated by adding 0.5 to each table cell CD4+ T cell ratio: CD8+ expressed with value <2

Table 7 shows a low CD4+ T: CD8+ cell ratio in all subject groups. In the normal cervical group, only 10% had a high ratio. Most surprisingly, the ration was also low in the HPV subclinical infection group. In the cervical cancer group, 75% of subjects had low CD4+ T cell ratio: CD8+. This result diverges from the results of previous studies. The proportion of T cells in tumor microenvironment, CD4+ T cell count, and the inverse ratio of CD4+: CD8+ T cells were significantly associated with rapid tumor growth and lymph node metastasis in cervical cancer [12]. Walayat S et al. support the findings that the reverse CD4+ T cell: CD8+ TIL ratio and decreased proportion of TIL provide poor clinical outcomes in patients with cervical cancer [13]. This study cannot prove that normal cervical groups have high CD4+ T-CD8+ T cell ratios, but it is able to prove that cervical cancer groups have low CD4+ T cell: CD8+ T cell ratios. This supports previous studies suggesting that the ratio of CD4+: CD8+ T cells in most cases of cervical cancer is low.

Table 8. Relationships between groups with CD4+ T cell ratio: CD8+

| Groups (Ratio of CD4+:CD8+ Cells T) | OR    | CI (95%)  | p-value |
|------------------------------------|-------|-----------|---------|
| Cervical cancer vs HPV-RT clearance | 0.31* | 0.01-6.81 | 0.653   |
| Cervical cancer vs HPV-RT persistent | 0.40* | 0.02-9.11 | 0.819   |
| HPV-RT persistent vs HPV-RT clearance | 0.78* | 0.01-49.91 | -       |

Chi-Square Test Continuous Correction

There was no statistically significant difference in the ratio of CD4+ T: CD8+ between the normal cervical group with cervical cancer, the persistent HPV-RT subclinical infection and clearance group (p = 0.226; 0.999; 0.999), the cervical cancer group with clearance and persistence (p = 0.653; 0.819), and as the persistent group with clearance. Based on these results, the ratio of CD4+:CD8+ T cells cannot be considered a significant factor affecting the natural course of cervical cancer (Table 8).

3.5 Relation of Retinol Deposit Sufficiency Level with TNF-α Expression

Based on the statistical analysis of the results of this study showed that there is no significant relationship between retinol deposit adequacy rate and TNF-α expression (OR = 2.73; CI 95% = 0.68-10.87; p = 0.147). The extent of the relationship between retinol deposit adequacy rates and TNF-α expression can be seen in Table 9.

Table 9. Relation of retinol deposit level adequacy with TNF-α expression

| The adequacy of retinol deposits | TNF-α Expression | OR (CI 95%) | p-value |
|----------------------------------|------------------|-------------|---------|
| Deficient                        | Positive         | 6 (54.5%)   | 2.73(0.68-10.87) | 0.147 |
| Adequate                         | Positive         | 11 (30.6%)  | 25 (56.4%)       |       |

Chi-Square Test Continuous Correction
The results obtained from this study differ from previous epidemiological studies showing an inverse relationship between cancer progression and nutrients containing vitamin A. Systemic retinol significantly reduced the thickening of the arterial intima layer after endothelial injury in vivo. In vitro and in vivo, retinol has pro-inflammatory properties and can increase TNF-α expression. Between 14 and 16 pharmacologic concentrations of vitamin A can decrease the incidence of tumors by experimental chemistry. Both natural and synthetic retinoids have been shown to inhibit the growth and development of different types of tumors [14-17].

3.6 Relation Level of Retinol Deposit Adequacy with Ratio of CD4+ T cells: CD8+

The relationship between the retinol deposit rate and the ratio of CD4+:CD8+ T cells can be seen in Table 10.

| Level of adequacy | Retinol deposits | Ratio of CD4+: CD8+ T Cells | OR (CI 95%) | p-value |
|-------------------|------------------|-----------------------------|-------------|---------|
| Deficient         |                  | Low                         | 9(22.5%)     | 2(28.6%) | 0.73 (0.12-4.39) | 0.726 |
| Adequacy          |                  | High                        | 31(77.5%)    | 5(71.4%) |                     |       |

Chi-Square Test Continuous Correction

This study found no statistically significant relationship between retinol deposit and the CD4+ T:CD8+ ratio (OR = 0.73; CI 95% = 0.12-4.39; p = 0.726). This result is contrary to some previous studies which show that retinol significantly increases the myeloid / lymphoid dendritic cell ratio and the ability of the patient's mononuclear cells to stimulate allogenic T cells. Retinoic acid is a differentiation regulator. HPV activity is closely related to epithelial cell differentiation, retinoic acid can be a preventive agent for the development of HPV-induced cervical lesions through its role in HPV-specific cellular immune response. CD4+ and CD8+ T cells have strong potential as anti-viral and anti-tumor. Codifferentiation is considered a very attractive therapeutic modality because it is more specific and less toxic that conventional chemotherapy [18].

3.7 Relationship between CD4+ T Cell Ratio: CD8+ with TNF-α Expression

Based on the statistical analysis in Table 11, there was no significant relationship between CD4+ T cell ratio: CD8+ with TNF-α expression (OR = 0.72; CI 95% = 0.14-3.67; p = 0.690).

| Ratio of CD4+ : CD8+ T Cell | Ekspresi TNF-α | OR (CI 95%) | p-value |
|-----------------------------|----------------|-------------|---------|
| Low                         | Positive       | 14 (35%)    | 26 (65%) | 0.72 (0.14-3.67) | 0.690 |
| High                        | Negative       | 3 (42.9%)   | 4 (57.1%)|                     |       |

Chi-Square Test Continuous Correction

Again, the results of this study differ from the findings of several previous studies. Previous studies have suggested that TNF-α is able to activate T cells and dendritic cells, thereby enhancing the adaptive anti-tumor immune response. The anti-tumor effect of TNF-α is enhanced by IFN-γ [19]. IFN-γ is secreted by CD8+ T cells that may play a role in viral infection elimination and apoptotic induction [20,21]. CD8+ T cells are cells that play an important role in tumor immunity by killing tumor cells. His ability in anti-tumor immunity is evident in animal experiments induced by carcinogens or in tumors induced by the virus. CD8+ T cells are tasked with recognizing and killing tumor cells expressing peptides, which are derived from the proteins of normal cells undergoing mutations or oncogenic viral
proteins presented with the help of MHC class I molecule [22]. TNF polymorphisms are associated with an increased risk of cervical cancer. TNF-α may increase or decrease the incidence of cervical cancer depending on the type [23]. In terms of microsatellite polymorphism, the HLA-DQB allele SNP-237, TNF-α is associated with infection with HPV type 16, NIS, and susceptibility to cervical cancer [23].

4. Conclusion
This study has proven that the highest level of retinol deposit sufficiency is owned by normal cervical groups. Furthermore, this study has demonstrated that TNF-α expression in cervico-vaginal cervical cells stimulated by 16-cell HPV antigens in the cervical cancer group was higher than in the persistent subclinical HPV-RT infection and normal cervical and cervical groups.

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