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Original Article

Correlation of CD4+ levels and caspase-3 in condyloma acuminata with HIV reactive patients

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A B S T R A C T

Background: Human papillomavirus is an infection that causes malignancy because of persistency and modulation of apoptotic pathways, especially caspase-3. Factors that can increase persistency, recurrency, and malignancy of HPV infection include HIV infection with low CD4 levels. There is disagreement or deep molecular understanding of the induction and modulation of apoptosis in HIV-mediated CD4+ T cell depletion, especially in CA. However, it is necessary to see how CD4+ levels can influence caspase-3, so it may open up new avenues for supporting investigation to consider the presence of malignancy or therapeutic strategies regarding CD4+ can induce apoptosis.

Purpose: This study aimed to determine whether there is a correlation between CD4+ levels and caspase-3 expression in condyloma acuminata with HIV reactive patients.

Methods: This is an analytic observational study with a cross-sectional design. The research was conducted at Dr. Moewardi General Hospital from August to December 2023. Nineteen patients with condyloma acuminata and HIV reactive were included in this study with a consecutive sampling technique. The expression of caspase-3 was assessed using immunohistochemical staining, looking at the percentage of stained cell nuclei and cytoplasm and CD4+ levels with flow cytometry examination—data analysis using Pearson correlation.

Results: Respondents in this study were primarily men, self-employed with heterosexual orientation and genito-genital-oral sexual intercourse. The statistical analysis showed no significant relationship (p: 0.300, r: -0.251) between CD4+ levels and caspase-3 expression.

Conclusions: While not statistically significant, CD4+ level is reduced in correlation with increased caspase-3 expressions.

INTRODUCTION

The Human Papillomavirus (HPV) is the source of the sexually transmitted disease Condyloma Acuminata (CA). 200 HPV genotypes have been identified based on the structure of the viral genome and its tropism in human epithelial tissue. World Health Organization (WHO) estimates that 35.3 million have Human Immuno-deficiency Virus (HIV) infection, and 15% of the general population have CA infection.[1] The prevalence of CA is 44%, and it is the most common Sexually Transmitted Infection (STI) in Moewardi Hospital.[2,3] HPV is known to cause cancer with prevalence of 5%.[4] Malignancy due to HPV is caused by persistent infection and abnormal epithelial changes.[5] Risk factors associated with malignancy in CA include immunosuppression or immunocompromise such as HIV.[6] High incidence of HPV-associated cancer in patients with low T cell counts. HIV patients show decreased levels of Langerhans cells, CD4+ cells, macrophages, and natural killer cells, thereby reducing the ability of the general system to eradicate HPV and increasing recurrence.[7] Recurrence is predicted to occur in 48.5% of CA patients.[1]

HIV reactive patients have a 3-10 times greater risk of suffering from CA and a 15-25 times higher risk of carcinoma compared to patients without HIV.[8] HPV infection is a viral

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infection that can modulate apoptosis. It inhibits the caspase activation cascade, thereby reducing apoptosis activity and deoxyribonucleic acid (DNA) damage, activating various proteins to prevent apoptosis. Dysregulation of apoptosis is a characteristic feature of cancer lesions and causes the progression of benign lesions to malignant lesions.[9] HPV infections develop into benign lesions if there is an effective immune cell response so the lesions can regress. Regression of HPV infection is dominated by the role of T helper (Th) 1 subset of CD4+ T cells.[10] There has been debate on the specific mechanism by which the HIV envelope causes apoptosis; reports have indicated both Fas-dependent and Fas-independent pathways or the interaction of the envelope with CD4+ cells and the production and secretion of apoptosis proteins.[11]

Caspase is a group of enzymes that regulate apoptosis.[12] Caspase-3 is the primary executor of caspase in the apoptosis process. One prominent theory for the loss of CD4+ T cells is caused by apoptosis.[13] A small percentage of infected CD4+ cells die due to caspase-3-mediated apoptosis.[14] There has never been any research linking the immunocompromised state with the modulation of apoptosis carried out by HPV. However, some studies stated that there is a correlation between CD4+ and condyloma acuminata. There is a significant relationship between CD4 count and giant condyloma acuminata.[15] Other studies stated that there is a significant relationship between the incidence of HIV and high-risk HPV. These results indicate that condyloma acuminata patients with HIV reactive should be examined for high-risk CA.[16] Low CD4+ levels have a 1.16 times risk of contracting STIs.[2] Some review articles mention the role of apoptosis in HIV pathogenesis, but no studies have examined the role of CD4 on caspase-3 expressions, especially in CA. The study aims to know the impact of CD4+ levels on caspase-3 expression in condyloma acuminate so it can predict CA malignancy and new therapeutic strategies in CA.

METHOD

Study Design

This is an analytic observational study with a cross-sectional design.

Setting and Respondent

This study was conducted at Dr. Moewardi General Hospital, Surakarta, from August to December 2023. The population in this study were condyloma acuminata with HIV reactive patients who were treated at the Dermatology and Venereology Polyclinic at Dr. Moewardi General Hospital, Surakarta. This study involved 19 samples that were taken using consecutive sampling. Inclusion criteria for this study include patients aged ≥18 years who had not received therapy or had been washed out for six weeks and were willing to participate by signing informed consent. Exclusion criteria were the patients with giant condyloma and a history of squamous cell carcinoma.

The Variable, Instrumen, and Measurement

The variables examined in this study were CD4+ levels and caspase-3 expressions. CD4+ levels were measured by flow cytometry and reported in cells/µl. Caspase-3 expressions were measured by immunohistochemical examination by looking at the percentage and color of stained cell nuclei and cytoplasm.[17] The experiments were conducted in the pathology anatomy section of the Faculty of Medicine, Sebelas Maret University.

Data Analysis

Pearson correlation was chosen as the statistical method for correlating CD4+ levels and caspase-3 expressions.[18]

Ethical Consideration

This study has received ethical approval from the Health Research Ethics Committee Dr. Moewardi General Hospital with No: 1.659/VIII/HREC/2023.

RESULTS

Table 1 outlines the characteristics of patients included in this study. Most patients were male, with an age range of 21-30 years old, self-employed, heterosexual sexual orientation, genito-oral-genital sexual intercourse, perianal lesions, and multi-partner. The statistical analysis showed that low CD4+ levels cause strong expression of caspase-3, although the relationship is insignificant (p: 0.300; r: -0.251) (Table 2).

Figure 1 shows the strong, moderate and low caspase-3 expressions. Figure 1A shows strong caspase-3 expressions as seen by the dark brown cytoplasm and nucleus of >60-100% of keratinocyte cells, a moderate expression which is visible as brown in the cytoplasm and nucleus of 20-60% shown of keratinocyte cells cytoplasm and nucleus of 20-60% of keratinocyte cells (Figure 1B) and weak expressions which is seen as light brown in the cytoplasm and nucleus of < 20% (Figure 1C).

DISCUSSION

The study's findings show no correlation between CD4+ levels and caspase-3 expressions in condyloma accumulation in HIV reactive patients, but low CD4+ levels increase caspase-3 expressions. There is no previous study about CD4+ levels and caspase-3 expressions, but a study revealed that caspase-1 and caspase-3 plasma levels in the CD4 low group increased after one year of HIV infection.[14] Some studies stated that the primary forms of HIV infection-related cell death as well as the impact of-

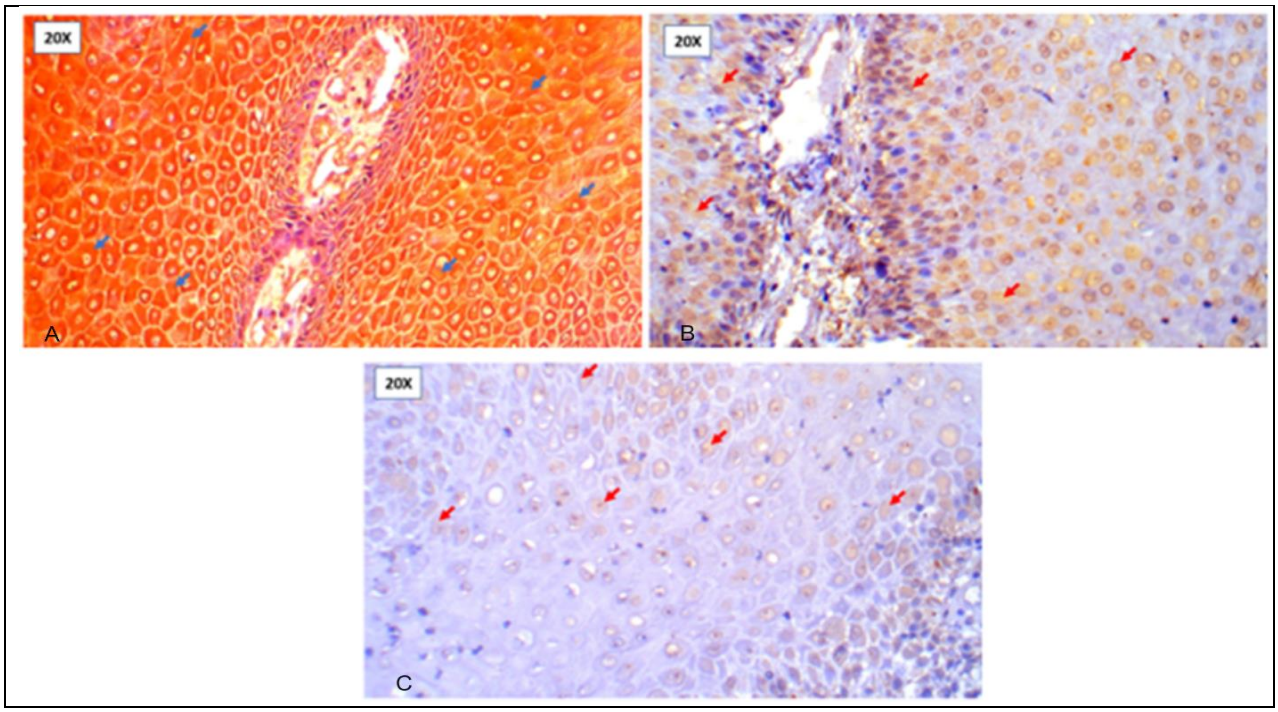

Figure 1. A. Strong expressions; B. Moderate expressions; C. Low expressions

Table 1. Characteristics Respondent

Characteristics	Result
Sex	
Male	13 (68.4%)
Female	6 (31.6%)
Age	
≤20 years old	3 (15.8%)
21-30 years old	11 (57.9%)
31-40 years old	3 (15.8%)
≥41 years old	2 (10.5%)
Occupation	
Worker	2 (10.5%)
Housewife	1 (5.3%)
University student	2 (10.5%)
Student	2 (10.5%)
Self-employed worker	12 (63.2%)
Education	
Primary School	4 (21.1%)
Secondary School	3 (15.8%)
High School	12 (63.2%)
Marital Status	
Unmarried	11 (57.9%)
Married	5 (26.3%)
Divorced	3 (15.8%)
Sexual Relationship	
Heterosexual	15 (78.9%)
Homosexual	4 (21.1%)
Sexual Intercourse	
Genito-genital-oral	10 (52.6%)
Genito-anal-oral	9 (47.4%)
Number of Sexual Partner	
1-5 Partners	13 (68.4%)
6-10 Partners	5 (26.3%)
>10 Partners	1 (5.3%)
Lesion Site	
Perianal	8 (42.1%)
Anal	7 (36.8
Genital	4 (21.1%)

Table 2. Correlation of CD4+ Levels with Caspase-3 Expression in Condyloma Acuminata (n=19)

Variable	r	p-value
CD4+ - Caspase-3	-.251	0.300

TNF peptides on latent infection. The results of this investigation indicate that apoptosis induction in HIV-positive cells contributes to the prevention of HIV transmission.[19] Through its activation of Nuclear Factor Kappa Beta (NF-κB), Tumor Necrosis Factor/ Tumor Necrosis Factor (TNF/TNFR) signaling may play a role in memory CD4+ T-cells' formation and maintenance of latent HIV-1 reservoirs.[20] Furthermore, TNF activation of HIV-positive T-cells that express and prevent CD4+ T-cell death may expand the T-cell reservoir. Therefore, TNF-based treatment may be utilized to eradicate HIV-1 reservoirs in individuals living with HIV.[21] HIV-encoded proteins also alter TNF signaling pathways, which allows HIV-positive cells to survive and kill uninfected ones. Anti-TNF treatment has been applied to several inflammatory illnesses.[22] Activation of TNF

and TNFR will cause activation of caspase-3 via the extrinsic pathway so that it can predict apoptosis pathway activation and dysregulation of cell homeostasis, leading to malignancy.[23]

Low CD4+ levels, especially CD4+ levels <200 cells/µl, are associated with HPV infection, especially high-risk HPV types. HPV infection increased by 3.5 times in patients with HIV due to changes in the regulation of T cells and macrophages.[24] The increase in HPV infections at low CD4+ levels is due to reduced activity in clearing HPV infections, especially high-risk types.[25] There was a relationship between CD4+ levels and CA especially at CD4+ levels <200 cells/µl. The incidence of HPV infection also increases in people living with HIV who do not take antiviral drugs regularly, namely 13.2%.[26,27] HPV infection can increase susceptibility to HIV infection due to increased tissue microvasculature, fragility, involvement of CD4+ T cells, and dendritic cells in response to HPV infection. HIV infection increases HPV replication, whereas HPV reduces CD4+ levels, cytokine, and macrophage production, decreasing immune function to eradicate the HPV virus.[28]

Reactive HIV occurs due to hyperproliferation of keratinocyte cells and decreased Langerhans cells, which causes the accumulation of apoptotic cells. Dendritic cells, T cells, macrophages, and the suppression of Langerhans cells are necessary for HPV transmission. Because HPV infection downregulates antimicrobial and cell attachment proteins, it impairs immunity and mucosal integrity. The immune system's impaired status can promote HPV infection, persistence, and spread. Initial infection in the basal layer of keratinocytes also involves immune system components such as interferons and cytokines. Approximately three weeks after the initial infection, virus multiplication, and immune system response mechanisms occur.[29]

Another study mentions that the death of most productively infected cells is not caused by caspase-3-mediated apoptosis. A viral infection that ends in the abortive phase causes caspase-1-mediated pyroptosis and kills the remaining >95% of quiescent lymphoid CD4+ T-cells.[30] Pyroptosis is a highly inflammatory type of programmed cell death in which pro-inflammatory cytokines, such as IL-1β, are released together with the contents of the cytoplasm. Thus, this death pathway establishes a vicious pathogenic cycle whereby dying CD4+ T-cells emit inflammatory signals that draw in additional cells to die, so linking the two hallmark processes of HIV infection: CD4+ T-cell depletion and chronic inflammation. Caspase-3-dependent apoptosis causes silent cell death when HIV infects permissive, activated CD4+ T cells. On the other hand, nonpermissive, quiescent CD4+ T cells from lymphoid tissue die by caspase-1-dependent pyroptosis, a highly inflammatory type of programmed cell death.[13]

Caspases -1, -4, -5, and -12 are the caspases that cause inflammation. Pyroptosis, a mechanism leading to programmed cell death, is also connected to inflammatory caspases.[31] During HIV infection, caspase-1-mediated pyroptosis accounts for more than 95% of CD4+ cell death. A small percentage of infected CD4+ cells die due to caspase-3-mediated apoptosis. Within two years of infection, CD4+ levels should be below 250 cells/µl and above 450 cells/µl. During the early stages of HIV-1 infection, the CD4+ above 450 cells/µl caspase-1 and caspase-3 increase quickly and then decline. On the contrary, following a year of HIV infection, there was an apparent increase in the levels of caspase-1 and caspase-3 in the CD4+ below 250 cells/µl.[32] This study's limitation is the small sample size. Due to the many differences in study results and the limitations of this study, further research is needed.

CONCLUSIONS AND RECOMMENDATION

CD4+ levels were not related to caspase-3 expression, especially in CA, but low CD4+ levels have the potential to increase strong expression of caspase-3. Further research is needed, but this research can be used as a reference to provide deep molecular understanding. We can consider using caspase-3 as an additional examination in CA patients with HIV reactive disease to predict malignancy.

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