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Term Pregnancy Women with Human Immunodeficiency Virus Infection Receiving Antiretroviral Threapy as A Risk Factor for Low Expression of Mitochondiral Deoxyribose-Nucleic Acid in Placenta

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ABSTRACT

Antiretroviral therapy (ART) given to pregnant women with Human Immunodeficiency Virus (HIV) has the effect of mitochondrial DNA (mtDNA) depletion through several steps, either directly or indirectly. This decrease may trigger the occurrence of reactive oxygen species (ROS) and mitochondrial toxicity which are characterized by energy insufficiency, cell dysfunction, and maternal and placental apoptosis. This study aimed to determine whether term pregnant women with HIV infection receiving antiretroviral therapy are risk factors for low mtDNA expression in the placenta. This cross-sectional analytic study was conducted at Sanglah Hospital Denpasar and affiliation hospitals. Inclusion samples were termed pregnant women with HIV (+) who received ART ≥ 6 months as a risk group and pregnant women with HIV (-) as a non-risk group. Expression of mtDNA was assessed by real-time Polymerase Chain Reaction (rtPCR) examination of placental samples. The estimated relative amount of mtDNA was calculated by dividing the number of mtDNA to the number of nDNA, as a reference. The cut-off value of mtDNA expression was determined by plotting a Receiver Operating Characteristics (ROC) curve. The results of the analysis found that pregnant women with HIV who received ART≥6 months had a prevalence of low mtDNA expression by 2.83 times higher than pregnant women without HIV (95% confidence interval = 1.42 – 5.67; p-value = 0.000). The conclusion of this study is that term pregnant women with HIV infection who receive ART is a risk factor for low mtRNA expression in the placental.

Keywords: Antiretroviral therapy, human immunodeficiency virus, mitochondrial DNA, term pregnancy.

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I. Introduction

Infection Human Immunodeficiency Virus (HIV) is a global pandemic, which it can find in almost all countries worldwide. The problem that happened is not only limited to how governance healing to the sufferer but also how it impacts baby born HIV infected mothers.

Based on the Joint United Nations Program on HIV/AIDS (UNAIDS) in 2018, around 37.900,000 people were infected with HIV worldwide, and as many as 18,800,000 of them were women. Directorate General of Disease Control (Ditjen PP) and Healthy Environment (PL) The Indonesian Ministry of Health reported that HIV cases in Indonesia cumulatively from April 1, 1987 - September 30, 2014, as many as 150,296 people, while for cases of Acquired Immune Deficiency Syndrome (AIDS) there were 55,799 people. Amount HIV infection in Bali until December 2017 reached 1,739 cases,

and AIDS got 734 cases [1]-[3].

Suspected HIV infection relates to happening outside bad pregnancy resulting from insufficiency placenta through an apoptotic mechanism. Apoptosis of the placenta in the mother with suspected HIV infection is caused by the viral factor alone and the impact of gift ART drugs. There is an interaction Between HIV infection and mitochondria. Several studies show that HIV RNA is found in mitochondria from infected cells, so dysfunctional mitochondria occur in HIV patients. Mitochondria hold a vital role in viability cells because of their role in various metabolic processes. A lot of research reports different levels of the evidence enhancement toxicity mitochondria and, in some cases, maternal, fetal, and placental apoptosis. But with a total small sample in studies, then becomes difficult could look for a connection Among toxicity mitochondria or the occurrence of apoptosis with outside lousy pregnancy, so it still required research similar to support results from previous studies has there [4], [5].

Change macroscopic assessed placenta covers weight, length rope center, rope diameter center, sum vessels blood rope center, insertion membrane, presence meconium, maternal surface completeness, maternal surface blood clot6. In women who do not become pregnant, known HIV causes comprehensive disturbance mitochondria _ with method promote Dead cells through apoptosis triggered by specific viral proteins. There is an interaction Between HIV infection and mitochondria. Some studies show that HIV RNA is found in mitochondria from infected cells, so dysfunctional mitochondria occur in HIV patients. Besides, the Tat protein, a gene product of HIV, can trigger the expression of the known DNA polymerase beta role in the process of DNA replication.

II. Discussion

Cross-sectional study This study was conducted on 20 HIV (+) term pregnant patients with ART > 6 months and 20 HIV (-) term pregnant patients. The characteristics of the research sample are presented in Table I.

TABLE I: CHARACTERISTICS SAMPLE BASED ON MOTHER'S AGE, GESTATIONAL AGE, BMI AND PARITY

Characteristic	HIV with ART 6 months	Without HIV	P-value
Maternal age	28 (7)	25 (8)	0.05 [a]
Gestational age	1 (1)	1 (2)	0.14 [a]
BMI	20.65 (3.07)	21.80 (2.49)	0.21 [a]
Parity	38 (1)	38 (2)	0.60 [a]

This study used the *Shapiro Wilk normality test,* with the analysis results showing that the data on age, parity, BMI, and gestational age in both groups were not normally distributed with each P-value < 0.05. Comparative analysis of research sample characteristics in both groups using the *Mann-Whitney* test. The median age of pregnant women at term HIV (+) with ART treatment ≥ 6 months is 28 with *an Inter Quartile Range* (IQR) of 7 years, while pregnant women at the time of HIV (-) were 25 with an IQR of 8 years, not statistically significant (P=0.05). The median parity of HIV (+) term pregnant women with ART treatment ≥ 6 months is 1 with IQR 1, while pregnant women at time HIV (-) was 1 with IQR 2, not statistically significant (P=0.14). The median BMI of pregnant women with HIV (+) term on ART ≥ 6 months is 20.65 with an IQR of 3.07 kg/m2, while pregnant women at term HIV (-) were 21.80 with an IQR of 2.49 kg/m2, not statistically significant (P=0.21). The median gestational age of pregnant women with HIV (+) term with ART treatment ≥ 6 months is 38 with an IQR of 1 week, while pregnant women at the time of HIV (-) were 38 with an IQR of 2 weeks, not statistically significant (P=0.60). Expression of mtDNA in the placenta of pregnant women with HIV (+) term on ART ≥ 6 months and on HIV (-) term pregnant women are presented in Table II.

TABLE II: CHARACTERISTICS SAMPLE BASED ON MOTHER'S AGE, GESTATIONAL AGE, BMI AND PARITY

Variable	HIV with ART 6 months	Without HIV	P-value
Expression of mtDNA Mean (±SD)	11.34±4.67	18.69 ± 5.92	0.00 [a]

The results of the analysis of the normality test for mtDNA expression data in both groups using Saphiro Wilk , showed that the data were normally distributed with each P value > 0.05 . The results of the analysis of the homogeneity of the mtDNA expression data in the two groups using the Levene Test , showed that the data homogeneous with P value > 0.05 . Comparative analysis of the mean mtDNA expression in the two groups using the T-Independent test. Average The mtDNA expression of HIV (+) term pregnant women with ART treatment > 6 months was 11.34 ± 4.67 fg/µl, while pregnant women at term HIV (-) was 18.69 ± 5.92 fg/µl, statistically significant difference (P=0.00).

The cut-off value of the mtDNA expression of HIV (+) term pregnant women with ART treatment > 6 months and HIV (-) term pregnant women was obtained using the curve receiver operating characteristics (ROC) (attached). Value of area under curve (AUC) = 0.83 with Confidence Interval (CI) 95% = 0.71 – 0.9 6, P value = 0.00 . The cut off value of mtDNA selected was 15.44 fg / µl with a sensitivity of 70% and a specificity of 85%. mtDNA expression is less than 15.44 fg/µl and high mtDNA expression were more or equal to 15.44 fg/µl. The distribution of mtDNA expression in HIV (+) term pregnant women on ART > 6 months and HIV (-) term pregnant women using 2x2 tabulation is presented in Table III. Analysis using Chi-square Test based on the tabulation, it was found that the Prevalence Ratio (RP) was 2.83 with a 95% Confidence Interval (CI) = 1.42 – 5.67, P value = 0.00. RP > 1 is interpreted that mother pregnant term with HIV infection who gets ART treatment > 6 months is factor risk happening expression mtDNA low in the placenta. Characteristics base sample study This study found no significant age difference between pregnant women with and without HIV. Researchers got 40 samples that fit the inclusion criteria and exclusion criteria. Based on the results of the analysis, it is known that the characteristics of the sample include maternal age, gestational age, BMI, and parity in aterm pregnant women with HIV infection and receiving ART treatment with term pregnant women without HIV infection, in this study, there were no statistically significant differences in characteristics.

TABLE III: DISTRIBUTION OF MTDNA EXPRESSION IN HIV (+) TERM PREGNANT WOMEN ON ART > 6 MONTHS AND HIV (-) TERM PREGNANT WOMEN

		mtDNA expression		RP	95% CI	P value
		Low	High			
Pregnant Aterm	HIV (+) on ART > 6 months	17	3	2.83	1.42 – 5.67	0.00 [a]
	HIV (-)	6	14			

In this study, the median age of pregnant women at term HIV (+) with ART treatment > 6 months was 2-8 with an IQR of 7 years, while for pregnant women at term HIV (-) it was 25 with an IQR of 8 years, not statistically significant (P =0.05). These results are in accordance with the prevalence of cases at Sanglah Hospital, where the majority (68.86%) of pregnant women who took part in the HIV Prevention of Mother to Child Transmission (PMTCT) program in the period 2005-2014 were aged 20-29 years [7]-[9].

This study also showed that there was no difference in gestational age, Body Mass Index (BMI), and parity between the at-risk group [HIV (+) term pregnant women on ART > 6 months] and the no-risk group [HIV term pregnant women (-)] In this study, median gestational age of pregnant women with HIV (+) term with ART treatment > 6 months is 38 with an IQR of 1 week, while pregnant women at time HIV (-) were in the HIV group (-), not statistically significant (P=0.60). This result is like a previous study by [10] which showed that the gestational age at delivery in women with HIV was 37.5 (32.2-41.2) weeks, not significantly different from women without HIV, which was 38.6. (38.3-40.3) weeks [10].

Median BMI in this study in HIV (+) term pregnant women with ART treatment > 6 months was 20.65 with an IQR of 3.07 kg/m2, while pregnant women at the time of HIV (-) were 21.80 with an IQR of 2.49 kg/m2, not statistically significant (P=0.21). Previous studies have shown that mothers with HIV have a reasonably even BMI, around 30% in each category, including underweight, normal, and overweight /obese.

In this study, the median parity of HIV (+) term pregnant women with ART treatment > 6 months is 1 with IQR 1, while pregnant women at time HIV (-) was 1 with IQR 2, not statistically significant (P=0.14). These results are like previous studies at Sanglah Hospital, which showed that the majority of HIV patients had a parity of 1 child (42.8%) [8], [11].

HIV infection can Become triggered by abnormal conditions in the mitochondria. This thing showed through alteration mitochondria, ok in vivo or in vitro, through Activation HIV protein-induced apoptosis mechanism. Moreover, increased apoptosis has also been showing through exposure in vitro against NRTIs and in-network patients who use ART drugs [10].

Expression of mtDNA in the placenta Mother pregnant in this study, it was found that the mean mtDNA expression in the placenta of pregnant women at term HIV (-) was significantly higher than the group of pregnant women at term HIV (+) with ART treatment > 6 months. In addition, it was found that HIV (+) term pregnant women with ART treatment > 6 months were the risk of low mtDNA expression in the placenta with 95% confidence interval (CI) = 1.42 – 5.67, P-value = 0.00. Reference [10] also reported that there was a significant decrease in the amount of mtDNA in the placenta in the group of pregnant women with HIV (+) when compared to the HIV control group (-) ($p < 0.001$). In addition, Hernandez also found a significant increase in placental oxidative stress levels in the group of pregnant women with HIV (+) when compared to the control group ($p < 0.01$).

ART treatment as factor risk happening the expression of low mtDNA in the placenta; antiretroviral therapy (ART) has transformed HIV from a lethal disease to a treatable chronic disease. ART has been shown to improve long-term prognosis and significantly increase the life expectancy of HIV-infected patients. ART is used over a long period, generally consisting of two or more Reverse Transcriptase Inhibitors (RTIs) and protease inhibitors.

Nucleoside Reverse Transcriptase Inhibitors work by joining the growing viral DNA chain during the reverse transcription phase, causing termination of the viral DNA chain.

Depletion in mtDNA can occur long before clinical signs appear, so it is often used as a tool to evaluate mitochondrial toxicity in HIV (+) patients on ART. A study conducted by Heijden et al. (2021) reported that there was a significant decrease in mtDNA copy (mtDNApl) in platelets of HIV (+) people receiving long-term ART when compared with HIV (-). The study found a significant reduction in the mean mtDNA copies/cells in the ART pregnancy group compared to the control group ($p < 0.001$). Comparison of mean mtDNA copies in ART-exposed pregnancies did not show significant differences in the first, second, or third trimesters [12].

Decrease total mtDNA in the placenta in the mother pregnant with HIV who get ART treatment was also reported in a study conducted by Shiramizu et al (2003). In his research, Shiramizu et al use sample consisting of from Mother pregnant women taking NRTIs and mothers pregnant without HIV. From research this obtained significant difference in the amount mtDNA Among Mother pregnant women taking NRTIs with Mother pregnant without HIV ($p = 0.0026$) [13].

Research conducted by [14] regarding apoptosis and the function of mitochondrial with examined the levels of Mfn2/β-actin (mitofusin 2) and Drp1/β-actin (dynamin-related protein 1), which are proteins that play a role in in activity fusion and fission from mitochondria, in research this found that these two proteins decrease by significant on the sample placenta [14].

Another study conducted by [15] Work reported that maternal mtDNA was significantly higher in HIV (+) mothers ($p = 0.016$, using the Mann-Whitney U test), while this was not found in placental mtDNA from the infant side ($p =0.45$). This could be an indication for future research that the combination of ART can affect the mitochondria of the placenta from the maternal side more than the baby side which may be due to the exposure to the mother being greater

than to the baby, but this still needs to be done further research [15]. Based on the discussion above, it can be concluded that term pregnancy mothers with HIV infection who gets ART treatment is a factor risk happening the expression of mtDNA low in the placenta.

III. Conclusion

Based on the discussion above, it can be concluded that term pregnant women with HIV infection who receive ART treatment are a risk factor for low mtDNA expression in the placenta.

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