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# THE EFFECT OF ACTIVE HEXOSE CORRELATED COMPOUND (AHCC®) ON LEVELS OF CD4+ AND CD8+ LYMPHOCYTE IN PULMONARY TUBERCULOSIS PATIENTS

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## Keywords:

Active Hexose Correlated Compound, CD4+ cell, CD8+ cell, pulmonary tuberculosis

## ABSTRACT

Cellular immunity plays an important role in the protection against Mycobacterium tuberculosis. The AHCC supplementation concomitant with anti-tuberculosis drugs is expected to increase levels of CD4+ and CD8+ lymphocytes as protective lymphocytes. This study aims to evaluate levels of CD4+ and CD8+ T cell before and after AHCC supplementation. The study design was a double-blind randomized clinical trial, in which AHCC and placebo were administered for 3 months. The levels of CD4+ and CD8+ T cell lymphocytes were collected at baseline and third month after AHCC and placebo administration. The Mann-Whitney test was used to analyze the levels of CD4+ and CD8+ T cell lymphocytes statistically. Sixty subjects (30 AHCC group and 30 placebo group) showed the CD4+ and the CD8+ levels after AHCC supplementation were better than placebo. Absolute CD4+ cell levels AHCC vs placebo were mean±se, baseline (519±39.4 vs 473±47.5 cells/μl, p=0.506) and after 3 months (695±52.1 vs. 637.5±50.4 cells/μl, p=0.574). Absolute CD8+ cell levels in the AHCC group vs placebo were baseline (488.8±46.9 vs 367.5±39.8 cells/μl, p=0.071) and after the 3rd month (628.7±101.9 vs 480.2±42.2; p=0.501 cells/μl). Percentage of CD4+ cells in AHCC group Vs placebo group, baseline (32.1±1.8 vs 32.5±1.6; p=0.391), and after 3 months (36.1±1.8 vs 34.7±1.8; p=0.701). While the percentage of CD8+ cells in the AHCC vs placebo was baseline (30.3±1.7 vs 25.2±1.4; p=0.044), and after the 3rd month (28.8±1.8 vs. 25.3±1.3; p=0.144). This study concluded that increased levels of CD4+ and CD8+ cells after AHCC administration was better than placebo in TB patients.



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## 1. INTRODUCTION

Tuberculosis (TB) is one of the oldest infectious diseases throughout history and remains a public health problem in the world even though control efforts with the DOTS strategy have been implemented in many countries since 1995 [1- 5]. The World Health Organization (WHO) has declared TB as a Global Emergency [6], [7]. Based on the Global Tuberculosis Report 2020 published by WHO, it is estimated that the highest number of cases is in the Southeast Asia region (44%), Africa (25%) and the West Pacific region (18%). There are 8 countries with the highest number of cases. Meanwhile, Indonesia ranks second after India [8]. Tuberculosis infection involves the cellular immune system caused by Mycobacterium tuberculosis (M. TB) [9], [10]. TB infection occurs when the immune system is compromised and enters through T cell nuclei (2,4). CD4+ Th1 lymphocytes (Cluster differentiation) have an important protective function by producing interferon (IFN- $\gamma$ ), tumor necrosis factor (TNF- $\alpha$ ), and interleukin-2 (IL-2). In addition, CD8+ T lymphocytes also play their role as cytotoxic cells that can directly kill the mycobacteria [11- 14]. Active hexose correlated compound (AHCC) is a nutritional supplement produced from Shiitake micelles containing a mixture of polysaccharides, amino acids, and minerals [15]. According to Sun et al [2009], the active ingredient of AHCC is the most  $\beta$ -glucan of polysaccharides [16]. Alpha-glucan can stimulate the phagocytic system, enhance the general immune system defense mechanism, increase tumor resistance and significantly stimulate the defense reaction against infection [17], [18]. So far, we have not found any research on this topic. Therefore, this study aimed to assess the changes in CD4+ and CD8+ T lymphocytes before and after AHCC administration compared to placebo.

## 2. METHODS

This study was a randomized control trial, double-blind, that was conducted from March to December 2020 at the pulmonology polyclinic at Labuang Baji Hospital, Makassar. AHCC supplementation and placebo were given for 3 months. Patients were divided into 2 groups, group 1 consisted of 30 TB patients who were given AHCC1 supplementation (Placebo), and Group 2 consisted of 30 TB patients who were given AHCC2 (original AHCC) in capsule form every 3 grams/day. The inclusion criteria for this study were male or female aged 18 years, new cases of pulmonary TB patients who would receive 1st category of Anti-tuberculosis therapy and agreed to participate in the study. While the exclusion criteria were impaired liver function, ATD resistance, and severe malnutrition. Each subject underwent anamnesis, physical examination, and anthropometry, as well as blood levels of CD4+ and CD8+ T lymphocytes. This research has been approved by the Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Informed consent was collected from the subjects. Anthropometric measurements (weight and height, as well as CD4+ and CD8+ T lymphocyte levels, were performed on subjects before and after 3 months of AHCC and placebo administration (after the initial phase of ATD administration). If there are side effects or not taking AHCC/placebo then the subject will be excluded. Three mL of the subject's blood sample was taken and put into a tube containing EDTA. The levels of CD4+ and CD8+ T lymphocytes were measured using flow cytometry and the results were stated in cells/ $\mu$ L, percentage, and the ratio of CD4+/CD8+. This step was repeated after the 3rd month of AHCC and placebo administration. The level of CD4+ T lymphocytes and CD8+ cells were analyzed statistically using the Mann-Whitney test. The analysis was performed using SPSS version 21.0. The statistical significance of the results was based on the p-value <0.05.

## 3. RESULTS

The basic characteristics of this study consisted of age, sex, and nutritional status of pulmonary TB patients, as shown in table 1. The median age in the AHCC group (42.5 years) with a minimum limit of 21 years and a maximum of 60 years. Meanwhile, the median age for the placebo group was (37.5 years) with a minimum age limit of 18 years and a maximum age of 69 years. Based on gender, males were dominantly

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found in the AHCC and placebo groups (66.7% in the AHCC group and 50% in the placebo group). None of the study subjects were severely malnourished, although there were several underweight patients in both the AHCC and placebo groups.

**Table 1.** Basic characteristics of pulmonary TB patients before receiving supplementation

Characteristic		AHCC	Placebo
		n=30, (%)	n=30, (%)
Age (year-old)	<25	5 (16,7)	4 (13,3)
	25-35	9 (30)	6 (20)
	36-45	8 (26,7)	7 (23,3)
	>45	8 (26,7)	13 (43,3)
	Median	42,5 years	37,5 years
	Range	21-60 years	18-69 years
Sex	Male	20 (66,7)	15 (50)
	Female	10 (33,3)	15 (50)
Nutritional Status	Underweight	12 (40)	10 (33,3)
	Normal	16(53,3)	18 (60)
	Overweight	2 (6,7)	2 (6,7)

\*AHCC, active hexose correlated compound

Table 2 shows the changes in CD4+ and CD8+ T cell lymphocyte levels before and after administration of AHCC and placebo (also patients while on Anti-tuberculosis therapy). Overall, it can be seen in the table that there was an increase in CD4+ and CD8+ levels in both the AHCC group and the placebo group. However, if we look at Table 2, the increase in CD4+ and CD8+ levels in the AHCC group was much better than in the placebo group. Statistically, the administration of AHCC and placebo did not significantly increase the levels of CD4+ and CD8+ cells ( $p>0.05$ ), although there was a significant difference in the percentage of CD8 ( $p = 0.044$ ) before treatment.

**Table 2.** Levels of CD4+ and CD8+ T cell lymphocytes before and after AHCC and placebo administered

Variable	Baseline		P-value	After 3rd Months		P-value
	AHCC	Placebo		AHCC	Placebo	
CD4 absolute	519.3±39.4	473.7±47.5	0.506	695±52.1	637.5±50.4	0.574
CD8 absolute	488.8±46.9	367.5±39.8	0.071	628.7±101.9	480.2±42.2	0.501
CD4%	35±1.8	32.5±1.6	0.391	36.1±1.8	34.7±1.8	0.701
CD8%	30.3±1.7	25.2±1.4	0.044	28.8±1.8	25.3±1.3	0.114
CD4:CD8	1.3±0.11	1.4±0.10	0.367	1.4±0.14	1.4±0.1	0.594

\*Mann Whitney test, SE, standar error, AHCC, active hexose correlated compound, CD, cluster of differentiation

#### 4. DISCUSSION

This study aimed to evaluate immunological factors in pulmonary TB patients before and after administration of AHCC and placebo. We evaluated two CD4+ and CD8+ markers (absolute CD4, absolute CD8, CD4%, CD8% and CD4/CD8 ratio). In this study, we did not take malnourished patients because it

can affect cellular immunity which plays an important role against Mycobacterium Tuberculosis. As a result, there will be a decrease in CD4, CD8 lymphocytes, and the CD4/CD8 ratio in the periphery [17], [18]. This can be seen in our study that CD4+ and CD8+ T lymphocytes increased after treatment in both the AHCC group and the placebo group. The improvement in the AHCC group was better than the placebo group. The possible explanation is because there were no malnourished patients. Another factor, AHCC is a nutritional complex that can combat infection and improve the patient's immune system [15], [16]. Another study explained that administering AHCC to infected people has shown to be effective in improving the immune system. Lee et al. reported that AHCC improved CD4+ cell counts in HIV patients. As we know that HIV patients are often accompanied by pulmonary TB [15- 20]. Several other studies have shown that the autophagy process plays an important role at the end of M. tuberculosis infection. The autophagy process is relevant to the ability of endogenous cytokines and pharmacological agents to eradicate M.TB. In the natural immune response, autophagy is associated with membrane receptor mechanisms, such as pathogen-associated molecular patterns (PAMPs) and toll-like receptors (TLR). In the adaptive immune response, autophagosomes will make the transfer of intracellular substrates, including pathogens that invade macrophages, to the MHC class II complex for CD4+ T cell stimulation. This is following one of the workings of the AHCC effect [21]. Mallet et al (2015) described that AHCC is an immune system-modulating compound that activates natural immunity by binding to natural pattern recognition receptors (PRRs) [22]. The limitation of this study is that this study was an initial study that was conducted for 3 months. So the data only show the immunological features before and after the initial phase of ATD and AHCC/placebo administration and was not seen until the end of TB treatment (TB disease outcome). In addition, the supporting variables are also limited so that it is difficult to analyze the patient characteristics that might influence changes in CD4+ and CD8+ cell levels.

## 5. CONCLUSION

In this study, there was an increase in the levels of CD4+ and CD8+ T cell lymphocytes after administration of AHCC and placebo. The increase of CD4+ and CD8+ T cell lymphocyte levels in the AHCC group was superior to the placebo group. Nutritional intake in the form of AHCC supplements, concomitant with ATD, supports to improve body protection, especially in terms of cellular immunity.

## 7. ACKNOWLEDGMENTS

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