

# COMPARISON OF TOTAL ANTIOXIDANT CAPACITY (TAC)'S PATIENTS OF CHRONIC KIDNEY DISEASE UNDERGOING HEMODIALYSIS AND NON- HEMODIALYSIS AND NON- HEMODIALYSIS

*by Zaky Firmawan El Hakim*

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**COMPARISON OF TOTAL ANTIOXIDANT CAPACITY (TAC)'S PATIENTS OF CHRONIC KIDNEY DISEASE UNDERGOING HEMODIALYSIS AND NON-HEMODIALYSIS**Zaky Firmawan El-Hakim<sup>1</sup>, Mochammad Thaha<sup>2</sup>, Yeti Hernaningsih<sup>3</sup><sup>1</sup>Medical Study Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia<sup>2</sup>Department of Interna, Dr. Soetomo General Hospital, Universitas Airlangga, Surabaya, Indonesia<sup>3</sup>Department Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia**ABSTRACT**

**Background:** Total antioxidant capacity (TAC) is one of the biomarkers to determine a person's health condition through antioxidant and oxidant activity or oxidative stress so that it can help in determining the therapy needed. However, currently, TAC levels in patients with chronic kidney disease (CKD) undergoing hemodialysis (HD) and non-hemodialysis (non-HD) are still controversial, so further research is needed. **Objective:** To analyze the comparison of TAC in HD and non-HD CKD patient. **Material and Method:** This was an analytical cross-sectional study with clinical observation in CKD patient. A total of 71 CKD patients consisting of 28 CKD HD patients and 43 non-HD CKD patients were enrolled in this study. Data about the characteristics of the subjects were taken using the anamnesis method, while data about TAC were taken using the colorimetric method with COBAS C-501 0837-19 series and 0835-13 series to measure TAC levels in the serum of CKD patients. The data that has been obtained were analyzed using independent sample t-tests. **Result:** TAC levels were significantly higher in CKD patients undergoing HD compared to non-HD CKD patients ( $p < 0.05$ ). There are data on albumin as an antioxidant and MDA as an oxidative stress biomarker that affects TAC levels. **Conclusion:** TAC levels found to be higher in hemodialysis CKD patient compared to non-hemodialysis CKD patient.

**Keywords:** Chronic kidney disease, total antioxidant capacity, hemodialysis, antioxidant, oxidative stress

Correspondence: Zaky Firmawan El-Hakim, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen. Prof. Moestopo no. 47, Surabaya 60131, East Java, Indonesia, [zelhakim@gmail.com](mailto:zelhakim@gmail.com)

**Background**

Total antioxidant capacity (TAC) is a serum examination that is used to measure the capacity and total antioxidant activity found in the body, this examination will later provide data of levels of total antioxidant capacity which is one of the important biomarkers in monitoring the person's health condition. In patients with chronic kidney disease (CKD), structural damage and kidney dysfunction occur, this can trigger the emergence of oxidative stress caused by increased oxidant activity and decreased antioxidant systems which will later have an impact on the TAC (Putri and Thaha, 2014). A number of studies have reported TAC levels of CKD patients who undergo hemodialysis (HD) and non-hemodialysis (non-HD) that are quite varied and controversial (Jackson et al., 1995; Fiorillo et al., 1998; Mayer et al., 2003; Montazerifar et al., 2012 ). In addition, in Indonesia, especially in Surabaya, there is still no data on TAC CKD patients who undergo HD and non-HD.

**Objective**

The purpose of this study was to analyze the differences in TAC CKD patients who undergo HD and non-HD as well as to obtain data on TAC levels in CKD HD and non-HD patients

**Material and Method**

This study was a cross sectional analysis study with clinical observations in CKD patients. A total of 71 CKD patients consisting of 28 CKD patients who undergo HD procedures and 43 non-HD CKD patients in Universitas Airlangga Hospital, Royal Surabaya Hospital, and Premier Surabaya Hospital. Subjects were selected using inclusion criteria namely, adult subjects, aged  $\geq$  21 years; diagnosis of CKD is based on the

medical record of the subject; no clinical signs of infection (afrebis in the last 3 days); no signs of dehydration; no clinical signs of excess fluid causing edema; hemodialysis subjects were categorized as CKD patients who had undergone hemodialysis for  $\geq$  3 months and were stable; while for the exclusion criteria, namely, the patient received Fe preparation. Then, the data was collected using anamnesis method for patient profiles in the form of age, history of disease, history of dialysis, and history of subject habits. To measure TAC levels in the serum of CKD patients, the colorimetric method with the COBAS C-501 0837-19 series and 0835-13 series was used to obtain data on the levels of total antioxidant capacity of the subjects. In this study, it also measured levels of albumin which is an antioxidant and malondialdehyde (MDA) which is a patient's oxidative stress biomarker as a comparison between antioxidant and oxidant levels to strengthen data on total antioxidant capacity. Furthermore, the data collected from several examinations would be analyzed for comparison using the Statistical Package for the Social Sciences (SPSS) application. To analyze the TAC comparison of CKD HD and non-HD patients, we conducted independent sample t-test.

**Results****General Characteristics and Profile of Research Subjects**

Table 1.1 presented data on general characteristics and profile of research subjects which contained gender, age, CKD stage, history of disease, as well as history of research subjects' habits.

Table 1.1 Characteristics of research subjects

Variable		HD	Non-HD
Gender [n (%)]	Male	16 (22.5)	25 (35.2)
	Female	12 (17)	18 (25.3)
Age [n (%)]	Early adulthood (26-35 tahun)	2	-
	Late adulthood (36-45 tahun)	2	-
	Early elderly (46-55 tahun)	8	13
	Late elderly (56-65 tahun)	11	25
	Seniors ( $\geq 65$ tahun)	10	4
CKD Stage [n (%)]	5	28 (39.5)	2 (2.8)
	4	-	16 (22.5)
	3	-	17 (24)
	2	-	5 (7)
	1	-	3 (4.2)
History of Disease [n (%)]	Hypertension	27 (38)	38 (53.5)
	Diabetes Mellitus	16 (22.5)	40 (56.3)
	Dyslipidemia	16 (22.5)	27 (38)
	Heart Failure	15 (21.1)	9 (12.7)
	Coronary Heart Disease	2 (2.8)	3 (4.2)
History of Habits [n (%)]	Smoking	7 (9.9)	10 (14.1)
	Alcohol Consumption	2 (2.8)	1 (1.4)

Table 1.2 Levels of total antioxidant status

Category	N	Average	Lowest limit	Highest limit
TAC HD	28	2,2032	1,61	2,5
Non-HD	43	1,6814	1,52	1,71

Table 1.2 presents data in the form of TAC levels in patients with chronic kidney disease who undergo HD and non-HD procedures. The results of independent sample test conducted on

these data indicate that TAC levels were significantly higher in CKD patients undergoing HD compared to CKD non-HD patients ( $p < 0.05$ ).

Table 1.3 Types of antioxidants and oxidative stress biomarkers

Variable			HD	Non-HD
Antioxidant	Serum Albumin	Lowest limit	2,5	2,8
		Highest limit	5,1	5
		Average	4,2	4,1
	Albumin Urine	Lowest limit	3,6	0,1
		Highest limit	438	814
		Average	149	100,4
Biomarker stres oksidatif	Serum MDA	Lowest limit	2,55	1,82
		Highest limit	7,25	3,78
		Average	3,46	2,42
	MDA Urine	Lowest limit	2,24	2,04
		Highest limit	10,87	19,41
		Average	4,58	5,9
Variabel			HD	Non-HD
Antioksidan	Albumin Serum	Lowest limit	2.5	2.8
		Highest limit	5.1	5
		Average	4.2	4.1
	Urine Albumin	Lowest limit	3.6	0.1
		Highest limit	438	814
		Average	149	100.4
Oxidative stress biomarkers	MDA Serum	Lowest limit	2.55	1.82
		Highest limit	7.25	3.78
		Average	3.46	2.42
	Urine MDA	Lowest limit	2.24	2.04
		Highest limit	10.87	19.41
		Average	4.58	5.9

From laboratory tests conducted on patient's TAC levels, there are also data on albumin and MDA (table 1.3) which shows one of the antioxidant components and oxidative stress biomarkers found in the subject's serum (Thaha, 2017).

### Discussion

#### Total Antioxidant Status Levels in Chronic Kidney Disease Patients Undergoing Hemodialysis and Non-Hemodialysis

As discussed in table 1.1, some subjects in this study have a history of diseases such as hypertension, diabetes mellitus, dyslipidemia, heart failure, and coronary heart disease. This is

in accordance with research by Putri, A. Y. and Thaha, M. (2014) and Sundaram, M. S. et al. (2014) that CKD is influenced by risk factors in the form of the disease. Some subjects also have bad habits such as smoking and consuming alcoholic drinks, according to Ladi-Akinyemi, and Ajayi (2017), this can be a trigger or even worsen the condition of CKD patients.

The results of the analysis in this study showed that CKD patients who underwent HD procedures had a significantly higher TAC than CKD patients who did not undergo HD procedures. This is caused by antioxidant activity and oxidative stress (Ryan, et al., 1997;

Buico, et al., 2009); in addition, indirectly TAC is also influenced by a person's history of disease related to oxidative stress itself (Putri and Thaha, 2014; Sundaram, et al., 2014). In the case of this study, the subjects were divided into 2 groups, according to Sundaram, M. S. et al. (2014) TAC levels of individuals who do not undergo HD will be lower than healthy individuals due to factors of oxidative stress arising from a history of CKD in the group's subjects. On the other hand, TAC in the group of CKD patients who underwent the HD procedure was influenced by a history of CKD, and also affected by the HD procedure done, it was strengthened by Liakopoulos, V. et al., (2017) who stated that stress oxidative that appear even in the early stages of CKD will increase frequently with worsening the kidney's function, it will also be worsen by HD procedures done.

#### **Factors Affecting Total Antioxidant Status Levels in Chronic Kidney Disease Patients**

Several studies have suggested that oxidative stress in individuals with CKD is influenced by several factors such as increased oxidation activity, decreased protective mechanisms of oxidants, diabetes mellitus type 2, dyslipidemia, increased phagocytic activity, inflammation, and failure of ROS removal, and factors associated with uremia (Putri and Thaha, 2014; Sundaram, et al. 2014; Ladi-Akinyemi, and Ajayi, 2017).

An article written by Tangvarasittichai, S. (2015) stated that oxidative stress increases in metabolic syndrome and type 2 diabetes and is most likely a cause of developing cardiovascular disease, diabetes mellitus type 2, and other diabetes complications. Increased oxidative stress is a damaging factor that leads to insulin resistance, dyslipidemia,  $\beta$ -cell dysfunction, impaired glucose tolerance; oxidative stress, hyperglycemia. This causes decreased insulin production, fasting hyperglycemia and ultimately causes the development of type 2 DM which is getting worse.

Sundaram MS et al. (2014) stated that hyperglycemia can cause vascular damage through several mechanisms, and there are four mechanisms; all of these mechanisms will lead to the formation of oxidative stress that comes from inflammation, and coagulopathy which can also cause atherosclerosis. In the body of patients with CKD, there will be an accumulation of Advanced Glycation End-

products (AGEs) which are biomarkers of oxidative stress, a product of carbohydrate oxidation.

Dyslipidemia has a relationship with the incidence of CKD and the prognosis of CKD, through several inflammatory mechanisms and increased oxidative stress, which will lead to endothelial damage and atherosclerosis (Sheen, Y.-J. and Sheu, WH-H., 2011). According to Delaney MP (2006), an increase in serum triglycerides (TGL), which is the predominance of very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), will lead to hypertriglyceridemia and low high density lipoprotein (HDL).

Research conducted by Tangvarasittichai, S. (2015) showed that in the group of subjects with higher lipid concentrations, there was an increase in the concentration of malondialdehyde (MDA).

Malondialdehyde is a biomarker of oxidative stress which is a by-product of lipid peroxidation. In the early stages of dyslipidemia, the cytotoxic effects of ROS are likely to have affected the body, then MDA will continue to increase along with the progression of the disease, and there will be an overproduction of free radicals causing lipid peroxidation and cell damage due to oxidation (Stocker, and Keaney, 2004). All of these changes will eventually lead to inflammation, oxidative stress, and coagulopathy, and can cause atherosclerosis which will further aggravate the patient's condition (Goldberg, 2000). In this study, it was proven by significantly higher MDA levels in CKD patients who did not undergo HD compared to patients who underwent HD (Thaha, 2017).

Inflammation and oxidative stress are two things that are interrelated with each other and are factors in the occurrence of kidney failure triggered by mechanisms such as endothelial dysfunction and general complications such as cardiovascular disease and death; as previously known that CKD is an inflammatory disease or inflammation where ROS formation is the center of several inflammatory diseases (Shankar, SK et al., 2005; Sundaram, MS et al., 2014; Liakopoulos, V. et al., 2017).

Several studies have shown that oxidative stress can cause inflammation, CKD patients are in the stage of chronic inflammation which will lead to increased oxidative stress through the activation mechanisms of inflammatory cells such as polymorphonuclear (PMNs) and monocytes; these cells will increase the

secretion of NADPH oxidase and myeloperoxidase (MPO) which will cause an increase in ROS (Cohen, G. and Hörl, WH, 2012; Putri and Thaha, 2014; Sundaram, MS et al., 2014; Liakopoulos, V. et al., 2017).

In patients suffering from CKD in a state of uremia, LDL will be more easily oxidized compared to normal people. Low density oxidized lipoprotein (Ox-LDL) is a molecule that can trigger inflammatory processes in blood vessels, this will lead to the expression of leukocyte adhesion molecules and binding to inflammatory cells in the circulation, this shows that uremia indirectly affects the formation of stress oxidative via inflammatory pathways (Putri and Thaha, 2014). Some proofs presented by Sundaram MS (2014) also showed that antioxidant enzymes will change in response to decreased kidney function and will get worse in patients with uremia.

In addition, many studies state that aging, antioxidant diets, smoking, and alcohol consumption can also influence oxidative stress which will also affect SAT levels (Liguori, et al., 2018; Boaz, et al., 2000; Thaha et al. 2010; van der Vaart, et al., 2005; Nsonwu-Anyanwu, et al., 2018; de Boer, et al., 2007; Sun et al., 1997; Bosch-Morell i, 1998; Ramachandran et al., 2003; Almansa et al., 2013; Flores-Bellver et al., 2014).

#### **Factors Affecting Total Antioxidant Status of Chronic Kidney Disease Patients who Underwent Hemodialysis**

In research subjects undergoing HD procedures, oxidative stress is also influenced by HD procedure. Basically HD will indeed provide benefits for individuals suffering from CKD, especially those who have reached terminal stage (Bems, 2017). Nevertheless, behind these benefits, HD also has side effects caused by compatibility of dialyzer membranes, dietary restrictions of vegetables and fruits during HD procedures, loss of antioxidants, and accumulation of oxidative products during HD procedures (Sundaram, et al., 2014; Liakopoulos, et al., 2017).

Bioincompatibility in HD systems has an important role in the production of ROS through the activation of neutrophils PMN, there are two components that greatly influence the formation of oxidative stress in HD procedures, namely dialyzer membrane and the amount of endotoxins in the dialysate. At the time of dialysis process, there is a possibility of disappearance of vitamins and antioxidants, this

is proven by the findings that showed plasma vitamin E and selenium are reduced in patients undergoing HD procedures; but also found significant deficiencies and downregulation of glutathione peroxidase, copper-zinc superoxide dismutase, and manganese superoxide dismutase in kidney disorders (Kuo and Targ, 2010; Sundaram, et al. 2014). Hemodialysis (HD) which uses cellulose and semi-cellulose membranes also contributes to increasing oxidants through the mechanism of activating the complement pathway and by stimulating inflammation which then leads to increased oxidant production and ultimately will increase oxidative stress (Coombes, and Fassett, 2012; Putri and Thaha, 2014).

In addition to bioincompatibility in dialyzer membrane, it is known that white blood cells and platelet activation that lead to oxidative responses are significantly increased by dialysate microbial contaminants. Even if a small amount of dialysate is contaminated, it will worsen the biocompatibility of HD therapy and will also aggravate amyloidosis, inflammation, atherosclerosis caused by HD, and accumulation of oxidative products will form (Lonnemann, 2000; Masakane, 2006).

However, oxidative stress that is formed during HD process can be reduced by decreasing the activity of inflammatory cells and eliminating inflammatory mediators. To reduce the activity of inflammatory cells can be done by using synthetic biocompatible membranes such as polysulfone and using ultrapure dialysate, there is increasing evidence that the use of ultrapure dialysate in HD patients can reduce the level of serum inflammatory mediators,  $\beta$ 2-microglobulin, carbonyl, and oxidative stress biomarkers and increase anemia status (Liakopoulos, 2017). Meanwhile, to eliminate inflammatory mediators, hemolipodialysis can be done, namely by using a dialysis membrane that has been coated with vitamin E and the use of reduced water electrolysis (Hacisevki, 2008). According to Putri, AY and Thaha, M. (2014) vitamin E coatings performed on cellulose membranes can reduce oxidative stress and prevent damage to endothelial function caused by HD procedures.

#### **Comparison of Total Antioxidant Status of Chronic Kidney Disease Patients who Underwent Hemodialysis and Non-Hemodialysis**

In this study higher SAT levels in HD patients

were caused by accumulation of endogenous antioxidants that were formed. From Table 1.3, it is known that urine albumin levels of HD patients are higher than those of non-HD patients and normal people, where the normal limit of urine albumin in the body is <30 mg (24 hour urine), while serum albumin levels in both groups of subjects are still within normal limits, there are even some subjects who have serum albumin levels below normal, where the normal limit of serum albumin is 3.5 - 5.5 g / dl (Medscape, 2018; NIDDK, 2018).

High levels of albumin in the urine are caused by inflammation that occurs in the glomerular kidney, this condition is often called glomerulonephritis or nephritis; moreover, diabetes and high blood pressure are also the main risk factors for the occurrence of albuminuria because both of these can also interfere with kidney function; older age, weight gain and certain races can also increase the risk of albuminuria; Cardiovascular disease is also believed to have a relationship with albuminuria (Kidney Health Australia, 2015). Thus, the data obtained about history of disease suffered by research subjects such as diabetes mellitus, cardiovascular disease is stronger in proving its effect on the levels of antioxidant status indirectly in CKD patients.

In addition to high albumin levels, high SAT levels most likely were also affected by gout, nevertheless in this study no gout was examined. High levels of uric acid in the body are known as hyperuricemia, this often happens in individuals with CKD. Hyperuricemia is caused by decreased ability to remove uric acid by the kidneys; data obtained by Filiopoulos V. et al. (2012) showed that hyperuricemia and hypertension had a strong association with cardiovascular disease, CKD progression, and mortality where oxidative stress had an impact. The main benefit provided by gout is its ability to act as an antioxidant, there is even a claim that gout may be one of the most important antioxidants in plasma. Uric acid works in the form of urate, which is a form of uric acid that dissolves in the blood, urate will react with various oxidants, such as hydrogen peroxide, hydroxyl radicals, and nitric oxide (NO) derivatives, and can neutralize the toxic effects of these oxidants (Kuzkaya, et al., 2005). This further reinforces that high SAT in CKD patients is affected by gout.

High SAT in HD patients can also be influenced by extrinsic factors such as the type

of dialyzer membrane used and antioxidant interventions before HD, during HD, and after HD. In this study, dialyzer membrane used was a bioincompatible dialyzer membrane, thus the total antioxidant status would decrease due to its exposure to blood which would result in an increase in oxidative stress. In the study, there was no data search on what interventions were given to the two groups of subjects, it was because the non-HD group subjects were not given specific directions on their diets, whereas in HD subjects there were no data on antioxidant interventions whether they were food or supplement.

Serum taking factors also influence high SAT in patients with CKD HD. In this study, serum subjects with CKD undergoing HD were taken before routine HD was performed, whereas routine HD was performed 2 times / week. According to Mayer B. et al. (2003), serum intake time affected SAT in patients with HD CKD, which in his study it was found that SAT levels during the HD process would decrease dramatically and only about 28% of the total antioxidant would occur during the initial HD procedure. This supports that serum intake time affects SAT levels.

Although there are many factors that can exacerbate oxidative stress, when viewed from data on the types of antioxidants and oxidative stress biomarkers found in both groups of subjects, it is found that antioxidants in the form of albumin are still higher when compared to oxidative stress biomarkers in the form of MDA, this might also apply to other antioxidants found in the bodies of both groups of subjects, thus the amount of all antioxidants would be higher than the amount of all oxidative stress, thus SATs obtained would be of high value, especially in CKD patients undergoing HD procedures, this was due by the treatment of these HD procedures and the interventions given.

### Conclusion

Total antioxidant status in CKD patients undergoing HD is higher compared to non-HD CKD patients. This is caused by the treatment received by CKD patients who underwent HD before, during, and after the HD procedure was performed, as well as random serum intake time. In this study, serums were taken before routine HD, which allowed an accumulation of antioxidants in the body before HD. Further research by examining antioxidants and



oxidative stress as a whole that can affect SAT levels in CKD patients; serum extraction is done before, during and after the HD procedure; as well as conducting a comprehensive data collection starting from the antioxidant intervention obtained by the patient until all components regarding hemodialysis need to be done to be able to further confirm which SAT levels are higher.

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