



وزارة التعليم العالي والبحث العلمي  
جامعة ميسان  
كلية التربية الاساسية

Ministry of Higher Education and Scientific  
Research  
University of Misan  
College of Basic Education

Misan Journal for Academic Studies  
Humanities, social and applied sciences

# مجلة ميسان للدراسات الأكاديمية العلوم الانسانية والاجتماعية والتطبيقية

ISSN (Print) 1994-697X  
(Online)- 2706-722X

المجلد 24 العدد 54 حزيران 2025

Vol 24 Issue 54 June 2025



# مجلة ميسان للدراسات الأكاديمية

العلوم الإنسانية والاجتماعية والتطبيقية

كلية التربية الأساسية / جامعة ميسان / العراق

Misan Journal for Academic Studies

Humanities, social and applied sciences

College of Basic Education/University of Misan/Iraq

ISSN (Print) 1994-697X ( Online ) 2706-722X

حزيران 2025

العدد 54

المجلد 24

June 2025

Issue 54

Vol 24



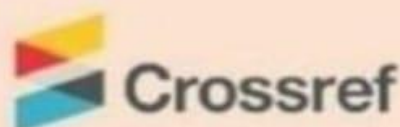
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رقم الأيداع في دار الكتب والوثائق بغداد 1326 في 2009

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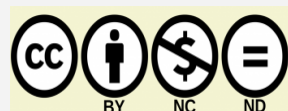
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ISSN (Print) 1994-697X  
ISSN (Online) 2706-722X

DOI:  
<https://doi.org/10.5463/3/2333-024-054-005>

Received:22/Apr/2025  
Accepted:5/May/2025  
Published online:30/June/2025



## Salivary biomarkers of oxidants and antioxidants for chronic renal disease in patients undergoing maintenance hemodialysis

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### Abstract:

The steady decrease in glomerular filtration rate is a hallmark of chronic kidney failure, culminating in elevated serum creatinine concentrations and blood uric nitrogen. Chronic renal failure patients, especially those on hemodialysis, are increasingly diagnosed with oxidative stress. This cross-sectional study sought to estimate oxidative and antioxidative salivary biomarkers (malondialdehyde and catalase) in chronic renal disease patients and compare them to healthy control subjects. This research was performed at Al-Sader Medical City in Al Najaf. There were 60 participants separated into two groups: 30 patients made up the first group, with chronic renal failure, who had hemodialysis. The second group comprised 30 healthy patients with no severe disease in their medical history. It was noted that there were more females than males in this study. The current results indicated a considerable disparity between the MDA value in the control and uncontrolled groups. The MDA value exhibited a greater magnitude in the uncontrolled group. Conversely, the CAT value indicated a statistically significant distinction between the uncontrolled and control groups, with the control group exhibiting a higher CAT value than the uncontrolled group. The primary cause of free radical production and enzymatic antioxidant losses is hemodialysis; this indicates that HD intensifies oxidative stress rather than alleviating it.

**Keywords:** MDA, CAT, saliva flow rate, oxidative stress, hemodialysis.

### Introduction

Chronic kidney disease is a global health issue that leads to cardiovascular disease and early mortality. It can be categorized into five stages based on the severity of kidney damage or the level of decline in kidney function. The last stage of chronic kidney disease is referred to as end-stage renal disease (Hatem and Mohammad, 2015).

There is accumulating evidence that chronic renal failure patients, especially those undergoing hemodialysis, are afflicted with oxidative stress. This appears to result from a combination of factors, including a decline in antioxidant defenses and more harmful substances that come from oxidative metabolism (oxygen-derived substances made by active leukocytes, transition

metal compounds, and other contaminants with different molecular weights. Furthermore, the use of low biocompatible membranes and the purity of dialysis water (Canaud et al., 1999, Morena et al., 2000) has an impact on oxidative stress. The physiological or pathological production of OS damages cellular components such as membrane lipids, proteins, and DNA(Rico et al., 2006).

Chronic renal failure (CRF) is correlated with oxidative stress, which plays a role in the pathogenesis of various immediate and prolonged complications. These complications comprise neurological disorders, arteriosclerotic cardiovascular disease, hypertension, anemia, hemostatic abnormalities, and compromised immunity. Reactive oxygen species (ROS) interact with many functional or structural molecules in the plasma and tissues of people with CRF and animals. Several investigations have indicated higher plasma concentrations of malondialdehyde, a lipid peroxidation product, in humans and animals with CRF (Salman, 2025) (Kishore et al., 1983, Trznadel et al., 1989, Paul et al., 1991).

Because free radicals are very reactive and only last for a short time, the products that are made when ROS/RNS combine with large molecules in cells are often used to show that cells have been damaged by oxidation (Mahmood, 2024a). To measure oxidative stress, products of lipid peroxidation, oxidized and fragmented DNA, and damaged proteins are utilized. The primary function of antioxidants is to counteract the harmful effects of free radicals by neutralizing them, thus lowering their ability to cause damage (Sapakal et al., 2008, Al-Souz and Al-Obaidi, 2015) Analyzing the redox status may also be accomplished by quantifying various antioxidants or the total antioxidant status.

Oxidative stress markers are commonly examined in plasma or serum, which are relatively stable environments for examining systemic biomarkers. However, the acquisition of blood may create significant stress for the patient. As a result, new biofluids are being investigated as alternatives to plasma. Patients should be capable of producing substantial amounts of these biofluids, making them easier to collect (Čolak, 2008).

Different chemicals enter saliva through transcellular or paracellular pathways (passive intracellular diffusion and active transport or extracellular ultrafiltration, respectively), making saliva filter of the blood. Saliva is hence comparable to serum and so reflects the physiological status of the body (Bagalad et al., 2017, Salim and Diajil, 2022).

The need for accurate measurements of lipid peroxidation is growing as the significance of oxidative damage in the development of many diseases is better understood. Malondialdehyde (MDA) is a compound that is produced when certain primary and secondary lipid peroxidation products break down. It is one of several small molecules that are created in this process (Janero, 1990, Abbas et al., 2014).

The enzyme catalase converts the chemical compound  $H_2O_2$  into its component molecules, water, and molecular oxygen.; it's a part of the antioxidant defense system(Scaglione et al., 2016). This enzyme protects cells from the harmful effects of hydrogen peroxide and is present in nearly all eukaryotic cells, particularly in their cytoplasm and peroxisomes(Labiós et al., 2009).

The aims of the study estimation of oxidative and antioxidative salivary biomarkers (malondialdehyde and catalase) in chronic kidney disease patients and compare them with healthy control subjects.

## Materials and methods:

### Study design:

This case-control research consisted of 60 patients divided into two groups: 30 with chronic renal failure undertaking hemodialysis and 30 healthy people with no significant illness in the past taken from the Ethical Committee (The study protocol was approved by the Ethics Committee of the college of Dentistry University of Baghdad, and All participants provided their informed assent by signing a consent form.

**Criteria for inclusion:** Patients with CKD on maintenance hemodialysis.

**Exclusion criteria:** Pregnancy, smoking, patients with radio or chemotherapy. Patients with cancer. All the exclusion criteria were negative in the history of the two groups.

### Saliva sample collection:

The unstimulated saliva we analyzed was collected using the spitting method in this study. Samples from patients and the control group samples were taken throughout 8:00 AM to 11:00 AM to reduce the impact of diurnal fluctuation on salivary composition. At the same time, the participants do not eat for at least one hour before saliva collection, eliminating the effect of diet on the collected samples. First, the saliva pH was measured immediately after collecting pH indicator strips (litmus paper). Saliva was collected on a timed basis so that the volume of collected saliva (ml) could be divided by the time (min) to get the flow rate (ml/Min). The saliva sample was centrifuged at a speed of 4000 revolutions per minute for 10 minutes. This process separated the clear liquid portion, known as the supernatant, which was then stored in a deep freezer at a temperature of  $-80^{\circ}\text{C}$ . Finally, MDA and catalase were measured by using spectrophotometry.

Salivary MDA levels were quantified using a method based on the reaction of MDA with Thio barbituric Acid, which produces a pink complex that can be detected spectrophotometrically.

catalase activity measurement using UV spectroscopy relies on detecting the amount of UV light absorbed by  $\text{H}_2\text{O}_2$ . The technique relies on absorbance monitoring to determine catalase activity after hydrogen peroxide has degraded, with a measurement taken at 240 nm. One way to estimate enzyme activity is by looking at the change in absorbance over time.

### Results:

The participant pool exhibited variability in terms of gender, encompassing individuals aged 20 to 70 years. The average age of the female cohort was  $46.37 \pm 2.73$  years, constituting 63.33% of the overall population. On the other hand, the female cohort demonstrated an average age of  $41.2 \pm 11.51$  years, accounting for 36.67% of the total population (Figure 1).

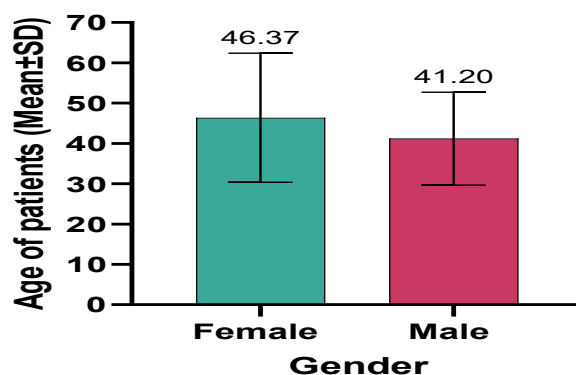


Figure 1. A bar chart illustrates the mean  $\pm$  SD for comparing the study groups based on gender and age

Hypertension caused 40% of chronic renal disease, while joint treatment, hypovolemia, and spinal Bifida contributed 13.33%. Polycystic kidney, nephrotic syndrome, and kidney stones cause 30% of CKD. Diabetes and hypertension caused 16.67% of CKD. Chronic kidney disease duration differed significantly between category durations. High rates of chronic kidney disease 83.3% were observed in individuals with illness durations of 3 months to 5 years, with females being the most prevalent gender  $1.357 \pm 1.15$ . The lowest ratio of over-6s was 16.7. Varying age groups had varying dialysis durations. Three months to four years had the highest dialysis rate, 86.66%. Females averaged  $1.58 \pm 1.24$  dialysis time, the longest across genders. In contrast, the female group had the highest average weekly dialysis rate 56.67%. Approximately 70% of patients had no family history of renal failure, with the female group having the greatest prevalence 43.33%. Males had the lowest family history of renal disease 10% cardiac failure, joint disease, hypertension, and cardiac muscle weakening are the most prevalent dialysis complications. Females had the highest illness rates and dialysis rates; the average was  $3.8 \pm 5.22$  and the percentage was 63.34.

The result of the current study illustrated that there was a significant difference in the Saliva flow rate between the uncontrolled and control groups, as shown in Table (1). The saliva flow rate was higher in the ci=control group at about  $1.440 \pm 0.521$ , while it was lower in the uncontrolled group at about  $0.360 \pm 0.130$ , figure (2). There was a significant difference between the PH rate (control and the uncontrolled group), Table 1. The rate of PH was higher in the uncontrolled group  $0.49 \pm 0.09$  in comparison to the control group  $7 \pm 0.00$ , (Figure 2).

**Table 1: Descriptive and inferential statistics comparing the Saliva flow rate and pH value (uncontrolled and control group)**

Parameters	Control group	Uncontrolled group	Test statistic t-test	P. value
	Mean $\pm$ SEM	Mean $\pm$ SEM		
Saliva flow rate	$1.440 \pm 0.095$	$0.360 \pm 0.024$	11.01	0.001**
pH rate	$7 \pm 0.0$	$7.47 \pm 0.090$	5.208	<0.0001*

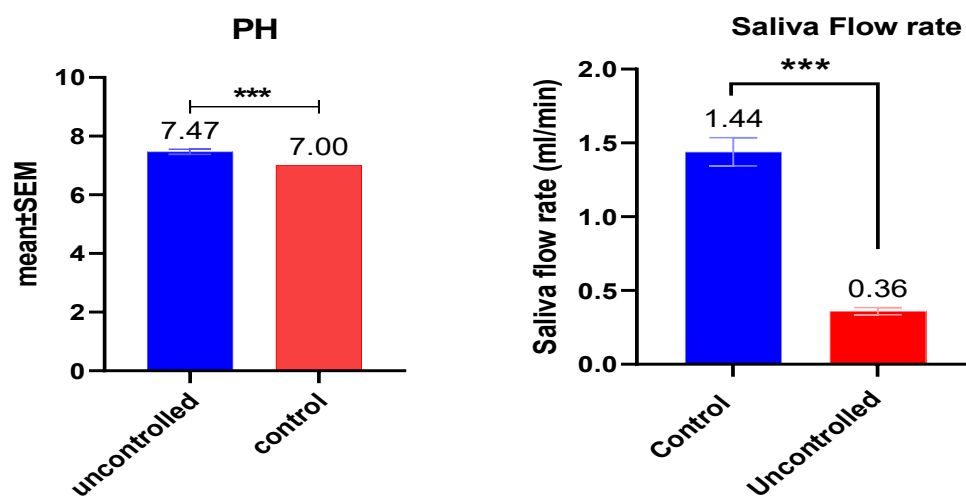


Figure 2: Bar chart representing the value of saliva flow rate and pH rate (uncontrolled and the control group) mean  $\pm$  SEM



A statistically significant difference was observed between the value of MDA in the uncontrolled and control groups, (Table 2). The analysis of the MDA data revealed that the concentration of MDA was significantly greater in the uncontrolled group  $0.048 \pm 0.55$  compared to the control group  $0.216 \pm 0.23$ ), (Figure 3).

**Table 2: Descriptive and inferential Statistics of comparing the MDA value (uncontrolled and control group)**

	Sample size	Mean	SD	SEM	Test statistic t-test	P. value
Control group	30	0.216	0.23	0.042	2.36	0.023*
Uncontrolled group	30	0.477	0.55	0.101		

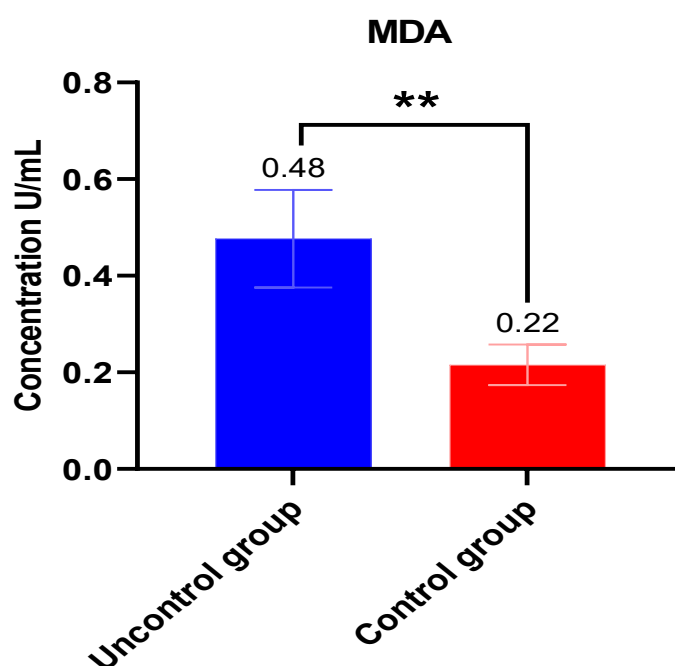


Figure 3: Bar chart illustrating the mean  $\pm$  SEM value of MDA in both the uncontrolled and control groups

#### CAT value:

The findings indicated a significant difference in the CAT Value between the uncontrolled group and the control group, (Table 3). The CAT value concentration was significantly greater in the control group  $13.02 \pm 14.12$  compared to the uncontrolled group  $6.357 \pm 3.681$ , (Figure 4).

**Table 3: Descriptive and inferential statistics of comparing the CAT value (uncontrolled and control group)**

	Sample size	Mean	SD	SEM	Test statistic t-test	P. value
Uncontrolled group	30	6.357	3.681	0.67	2.501	0.015*
control group	30	13.02	14.12	2.58		

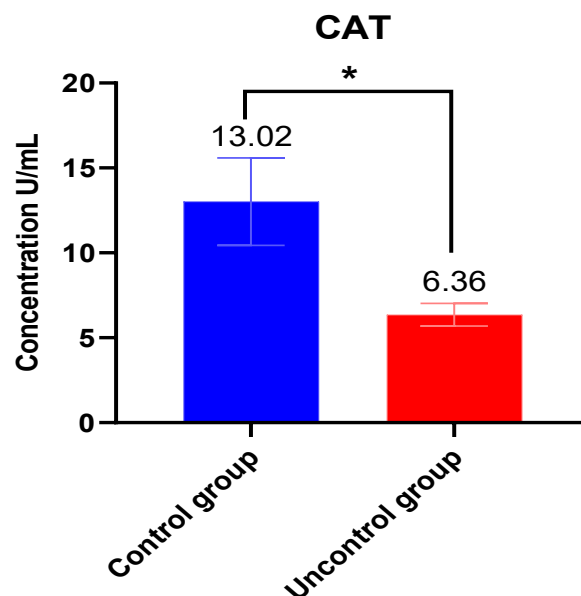


Figure 4: Bar chart illustrating the numerical value of CAT (uncontrolled and the control group) mean  $\pm$  SEM

### Discussion:

This research aims to assess the value of antioxidant and oxidant salivary biomarkers for diagnostic purposes in Iraq. It proves that CKD leads to oxidative damage of salivary lipids being higher and that enzymatic antioxidant mechanisms are disrupted. With the improvement of sensitive analytical tools, there is more interest in using body fluids other than blood to diagnose long-term diseases that affect the whole body. The non-invasive acquisition of test material is crucial in mitigating patients' anxiety related to this process, particularly in the case of children and individuals with disabilities. It may also encourage the administration of control tests more frequently.

The patients' ages ranged from 20 to 70. The ratio of female to male patients was greater. Early diagnosis is critical to the therapeutic process because it enables prompt identification of the disease and application of the most suitable treatment. Saliva is an intriguing alternative to blood, which is frequently used in diagnostic procedures.

Unstimulated saliva plays a vital role in preserving oral health and overall well-being of the mouth. Additionally, it offers a potent safeguard against dental caries, also known as tooth disease. (Van Nieuw Amerongen et al., 2004). Hypofunction is defined as any unstimulated flow rate below 0.1 ml/min (Sreebny and Valadini, 1987). The study's findings show that those receiving dialysis produced less saliva than usual, which resulted in xerostomia. These results align with a study carried out by Kaushik et al., (Burge et al., 1984) Additionally, a notable reduction in both the rate of saliva flow during stimulation and at rest was detected, and this decrease was found to be linked to xerostomia. Another study conducted by Kho et al., (Savica et al., 2008) It was found that the average flow rate of whole saliva that wasn't activated was much slower in the cases than in the controls. This is in line with what this study found. The reduced rate of saliva production in individuals with chronic kidney disease (CKD) is associated with issues such as limited fluid intake, dehydration, imbalance of electrolytes, and the potential effect of severe infection on the salivary

glands. Salivary normally has a pH of 6.7, with an average value between 6.2 and 7.6. The resting the mouth's pH remains above 6.3. Saliva helps maintain a nearly neutral pH (6.7-7.3) in the mouth cavity. Saliva helps maintain pH through two processes. Initially, Saliva flow removes digestible carbs and neutralizes acids produced by bacteria. Furthermore, Saliva's buffering effect neutralizes the acidity of food and drink, as well as that produced by bacteria (Mahmood, 2024b). Elevated salivary pH is consistently observed in patients with chronic renal failure (CRF). Saliva plays a key role, as stated by Bots et al., oral clinical findings are influenced by alterations in saliva flow, pH levels, and biochemical composition (Bots et al., 2007). Salivary ammonia concentrations rise in CKD due to the breakdown of urea by the enzyme urease, which in turn causes an increase in salivary pH (Kaushik et al., 2013). A study conducted by Bayraktar et al. (Bayraktar et al., 2009). The study also observed a rise in saliva pH among individuals who were undergoing dialysis. According to multiple studies, increased ammonia content due to urea hydrolysis is the sole reason for a higher pH (Postorino et al., 2003).

Non-stimulated whole saliva (NWS) is mostly secreted by the submandibular (60%) and parotid glands (20%). However, when stimulated, the proportion of submandibular saliva reduces (to approximately 50%) in favor of parotid gland saliva (40%) (Maciejczyk et al., 2017, Zalewska et al., 2015).

Chemical components found in saliva can be categorized into two groups: those that are solely formed in salivary glands and those that are transferred from plasma to saliva. The latter category is especially significant for laboratory diagnosis, as many constituents of saliva mirror their levels in the bloodstream (Zalewska et al., 2015). Saliva has a longer shelf life than blood, which makes it a more cost-effective, convenient, and non-invasive sample-collecting method. There are more benefits to using saliva in laboratory diagnostic (Abdulrazzaq and Ali, 2025). Saliva is therefore utilized in the identification of cancer as well as in the monitoring of medication and/or intoxicant concentrations and the diagnosis of cardiovascular, autoimmune, viral, and metabolic illnesses (Wang et al., 2015, Zhang et al., 2016).

Reactive oxygen species are produced on the surface of dialysis membranes by the activation of polymorphonuclear leukocytes, leading to the hypothesis that memorialization itself induces oxidative stress (Salem et al., 1993, Hoenich, 1999). It is generally known that hemodialysis, even for just one session, dramatically raises lipid peroxides and lowers antioxidants (Peuchant et al., 1994, Hultqvist et al., 1997). Patients with MHD may be at increased risk for cardiovascular disease due to the pathophysiological involvement of ongoing oxidative stress. Evaluation of oxidative stress markers in end-stage renal disease patients is a common practice. Malondialdehyde (MDA) is a low molecular weight result of lipid hydroperoxide degradation. Lipid hydroperoxides are the hydroperoxide portion of plasma lipids (Nourooz-Zadeh, 1999).

A common way to measure lipid peroxidation is by measuring malondialdehyde (MDA). In hemodialysis, results regarding MDA levels are contradictory; some studies have shown an increase (Rao et al., 2001, Haklar et al., 1995), while others have shown a drop to normal levels following dialysis as a result of clearance (Gerardi et al., 2002, Dalle-Donne et al., 2006).

Maciejczyk et al. (2018) conducted a study to assess oxidative stress markers in the saliva Among children with chronic kidney disease (CKD) and healthy controls. The study examined both non-stimulated saliva (NWS) and stimulated saliva (SWS), including 25 children with chronic kidney disease (CKD) and 25 healthy controls.

The evaluation included salivary antioxidants such as catalase (CAT) and malondialdehyde (MDA) among other measures. The activity of antioxidant enzymes (CAT) in the non-stimulated saliva of patients with CKD was not substantially different from that observed in the healthy controls. The levels of MDA were considerably elevated in both the non-wakeful state (NWS) and the sleep-wakeful state (SWS) in Children with Chronic Kidney Disease (CKD) (Maciejczyk et al., 2018). The study conducted by Maciejczyk et al. (2018) contradicts the current study's findings since it observed a difference in CAT between the control and uncontrolled groups. In addition, the study conducted by Maciejczyk et al. (2018) aligns with the findings of our current investigation, which observed a significant difference in MDA levels between the control and uncontrolled groups.

### Conclusion:

Hemodialysis primarily causes a breakdown of antioxidant enzymes and the generation of free radicals. This indicates that HD does not improve oxidative stress but rather exacerbates it. Chronic kidney disease (CKD) is linked to abnormalities in the salivary antioxidant systems and the harmful effects of oxidative damage on lipids. The salivary parameters associated with oxidative stress, particularly MDA, have the potential to be used as biomarkers for chronic kidney disease (CKD) instead of relying on blood samples. Chronic kidney disease (CKD) causes significant alterations in the characteristics and content of saliva, including a decrease in saliva production and an increase in salivary pH, compared to people without any health issues.

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**Manuscript title:****Salivary biomarkers of oxidants and antioxidants for chronic renal disease in patients undergoing maintenance hemodialysis**

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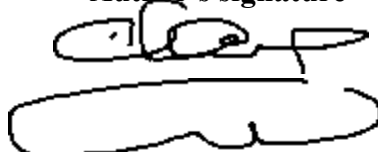
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