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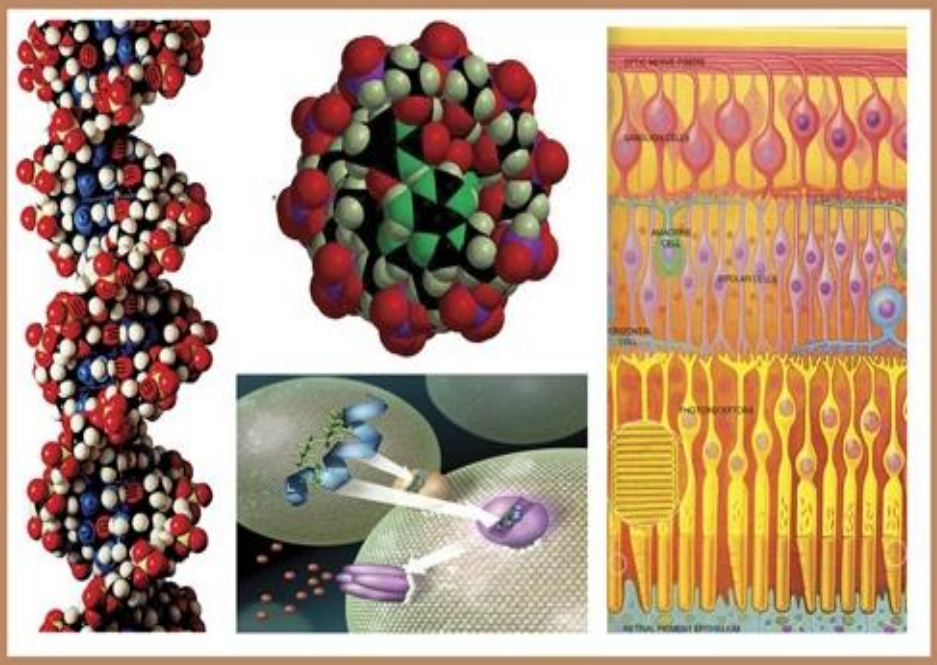

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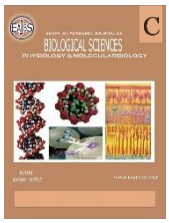

Estimation of Total Antioxidant for The Patients with Diabetic Nephropathy by Spectrophotometer and Microfluidic Sensor

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ABSTRACT

This study included a new, simple, fast, and sensitive new microfluidic sensor for the determination of total antioxidant (TAC) in patients with diabetic nephropathy and control then compare results with the conventional method (spectrophotometer technique). This method is dependent on the reduction of Cu^{2+} into Cu^{1+} by the action of antioxidants that are existing in the sample and chelating factor like neocuproine that produced complex colors. The spectrophotometry method was utilized to measure the absorbance of TAC at 450 nm in patients with diabetic nephropathy and control, also measured of TAC by using a innovative microfluidic system that contain a novel double split ring resonator sensor, after that compare outcomes between both techniques that indicated no significance differences in result of TAC after measured by spectrophotometer and new microfluidic sensor.

INTRODUCTION

Diabetic nephropathy (DN) is a progressive, irreversible disease characterized by hypertension, and microalbuminuria with a progressive decline of glomerular filtration rate (GFR) that lead to end stage of renal disease. The main cause of diabetic nephropathy is chronic hyperglycemia. Resident, as well as non-resident kidney cells, can be triggered by hyperglycemia that generate humoral mediators and cytokines, which can result in functional and phenotypic changes in kidney cells and tissues, interference with cell growth, interacting proteins, and the formation of advanced glycation end products (AGEs), and eventually glomerular and tubular damage and the onset of kidney disease. Therefore, a initial factor in the progression of diabetic nephropathy is poor blood glucose control, as well as various hyperglycemia-induced metabolic and hemodynamic abnormalities that involve diabetic nephropathy involved increased AGEs production, increased reactive oxygen species creation and stimulation of PKC (Protein Kinase C), polyol pathway (Wu *et al.,* 2023). Decreased total antioxidant capacity (TAC) in patients with diabetic nephropathy because increased of oxidative stress and increased consumption of antioxidants (Al-Kufaishi *et al.,* 2020; Etienne *et al.,* 2019).

Traditional methods for the detection and analysis of total antioxidant capacity using a variety of methods like High-Performance Liquid Chromatography (HPLC), micellar liquid chromatography, spectrofluorometry, flow-injection chemiluminescence, fluorescence, thin-layer chromatography,ultraviolet-visible spectrophotometer and flow-injection analysis (Lin *et al.,* 2021; Zhang *et al.,* 2018).

In spite of this technologies have benefits of sensitivity high, efficiency of separation elevated, range of application wide and high qualitative and quantitative accuracy they still have some limitations. For example, optical techniques like spectrophotometers typically make use of laboratory analytical techniques that required costly, large equipment, tedious sample pretreatment, consumption of chemicals high and detection time long (Yi-Qiang *et al.,* 2018).

Flow injection analysis (FIA) is a technology that makes most analytical procedures and processes flexible, fast, repeatable, and economical (Kolev & McKelvie, 2008). Effective flow injection requires planning how sample and reagent solutions are mixed and processed throughout the chemical reaction (Rocha & Zagatto, 2020). In recent years, microwave resonators have grown in popularity as precise instruments for determining the reflect light characteristics of materials at microwave frequencies. The principle of the sensor's work is based on the theory of perturbation (Sulejmanpasic & Ünsal, 2018).

They are also used in many fields including dielectric characterization of microparticles for the study of single cells in medicine and biological applications(A. Abduljabar *et al.,* 2015) and glucose concentration determination (Mondal et al., 2018) and chemical sensing applications (Salim *et al.,* 2018). Microwave split-ring resonators (SRR) have lately shown promise in a variety of sensing applications, including solid, gas, and liquid sensing (Zarifi *et al.,* 2014).

It was recently proved that utilizing tubes or microfluidic channels, such resonators can enable many sorts of liquid sensing (Korostynska *et al.,* 2013). The split ring resonator (SRR) has been developed to detect a wide range of liquids via capillary or microfluidic channels. However, in small quantities and sizes, microfluidics focuses on very accurate fluid management (A. A. Abduljabar *et al.,* 2014; Ali, 2022).

MATERIALS AND METHODS

Sample Collection:

This study is case-control and contains of 90 individuals separated for two groups, 45 with diabetic nephropathy patients and 45 controls, this study start in November 2022 to February 2023 is performed for patients in Dialysis and Kidney Transplant Center both at Al-Najaf Teaching Hospital Al-Sadr Teaching Hospital and as well as Al-Imam Al-Sajjad Hospital. The age of all Individuals from 30 to 75 years old.

Apparatus:

Vector network analysis, plastic valve with multi-use homemade, Disposable syringe, Injection tubes from Teflon, port injection, Double Split-ring resonator homemade, Spectrophotometer. Spectrophotometer utilized to scan and measure the absorption of TAC with a 1cm path-length quartz cell. The design of the new microfluidic System with a novel double split ring resonator home-made design. The many conditions that affect the new system designing have been studied and a final technique evaluation has been chosen. As seen in Figure 1, the developing new microfluidic analysis systems.

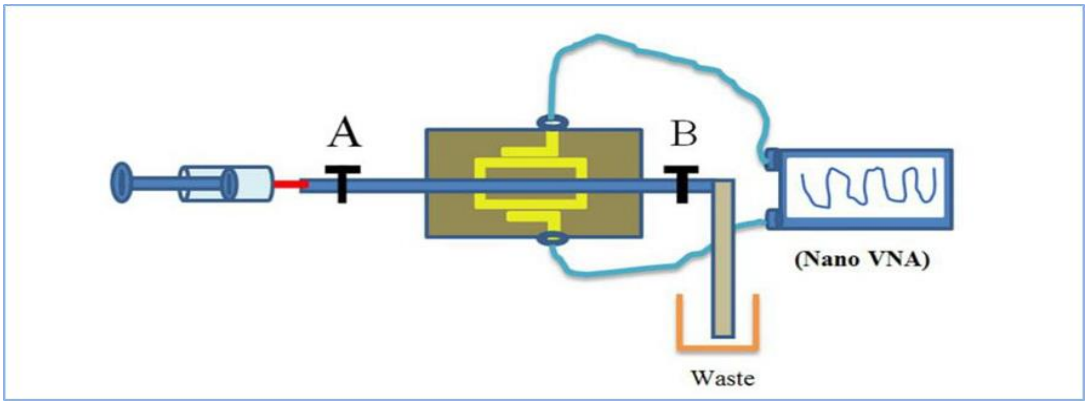

Fig.1: The structure of the new system is desiging to determine TAC.

Reagents Preparation:

1- a solution containing copper (II) chloride at a concentration of (10^{-2}M) used CuC_{l2} to prepare $.2H_2O$ weighting (0.4262) g, dissolving in H_2O, and diluting to (250) ml in water.

2- Ammonium acetate (NH_4Ac) buffer pH=7.0 was prepared by dissolving (19.27) g of NH_4Ac in water and accomplish the volume to (250) ml.

3- Neocuproine (Nc) (2.9-dimethyl-1,1-phenanthroline) solution at a concentration of ($7.5*10^{-3}$) M was prepared by dissolving (0.04) g Nc in 96% EtOH, the volume was completed to (25) ml with ethanol.

4- The standard uric acid solution (1mM) was made by dissolving (16.81) mg of uric acid in (100) ml of distilled water and then adding a few drops of sodium hydroxide to completely dissolve the uric acid.

The Procedure of Total Antioxidants Capacity (TAC) Test:

Test tubes were mixed by vortex and incubated for 30 minutes at 37 ºC and centrifuged at (4500Xg) for two minute then reading outcomes by spectrophotometer and microfluidic sensor.

Table 1: Indicate for Manual Procedure to Determination of Total Antioxidants Capacity (TAC).

Tubes	Serum	Standard	Blank
Copper (II) chloride solution	1mL	1mL	1mL
Serum	50µL	----	----
Working standard solution	----	50µL	----
D.W	----	----	50µL
Neocuproine (Nc) solution	1mL	1mL	1mL
Ammonium acetate (NH_4Ac) buffer	1mL	1mL	1mL

Statistics Analysis:

The P-value used to find the significant difference in two study groups, patients with diabetic nephropathy and controls, and the Probability-values less than 0.05 were considered significant differences. SPSS version 22 was used to complete all statistical analysis and data were provided as (mean ± SD) with upper and lower 95% confidence intervals.

RESULTS AND DISCUSSION

TAC measurements via spectrophotometric in Control and Patients with Diabetic Nephropathy.

Total antioxidant capacity levels in Controls and in patients with diabetic nephropathy are shown in **(**Table 2), findings suggest significance elevated in total antioxidant capacity levels in the control group (20.33 ± 2.67) compare to diabetic nephropathy group (14.78 ± 2.27).

Table 2:(TAC) levels in Control and Patients with DN.

Parameter	Groups Type	Means ± SD	SE	95%Confidence Interval		P-Value
				Lower	Upper	
TAC (µmol/L)	Controls	20.33 ± 2.67	0.39	19.53	21.14	0.001
	Patients	14.78 ± 2.27	0.33	14.10	15.46	

*The significance results when P-Value ≤ 0.05.

Measurements of TAC via spectrophotometric outcomes indicated a decrease in TAC levels because increased of reactive oxygen species in patients with diabetic nephropathy. Increased lipid peroxidation and excessive use of antioxidants against oxidative stress reduce the damage. TAC levels may have decreased due to increased consumption to scavenge the free radicals created in large quantities as a result of increasing oxidative stress (Africa & Town, 2017).

TAC measurements via Microfluidic Sensor in Control and Patients with Diabetic Nephropathy:

Table (3) refer significant differences in levels of TAC in the controls group (20.30 ± 2.65) and in the diabetic nephropathy group (14.79 ± 2.21).

Table 3: (TAC) levels in Control and Patients with DN.

Parameter	Groups Type	Means ± SD	SE	95%Confidence Interval		*P-Value
				Lower	Upper	
TAC (µmol/L)	Controls	20.30 ± 2.65	0.39	19.69	21.22	0.001
	Patients	14.79 ± 2.21	0.33	14.10	15.49	0.001

***The significance results when P-Value ≤ 0.05.

From Table (3) results of Total Antioxidant Capacity (TAC) that measured by newly design microfluidic sensor in control and patients with diabetic nephropathy represent significant differences, that also indicated causes reduction of TAC in patients with diabetic nephropathy is hyperglycemia that lead to produced reactive oxygen species (ROS) via activation several pathway such as advanced glycation end products , protin kinase C , NADPH oxidase all this causes lead to lowering of TAC in DN patients (Volpe et al., 2018).

Study the Microwave Spectra of Total Antioxidants Capacity (TAC):

After applied procedure in **Table (1),** vortex test tubes were mixed and incubated for 30 minutes at 37 ºC and centrifuged at (4500Xg) for two minute, after that injecting the colored product by means of a syringe into the system to indicate microwave spectrum, when the frequency is shown f=2.906 GHz, λ= 0.5921 m and the absolute value of the S_{21} was taken. (Fig. 2) shows this.

Fig. 2: Microwave spectrum of TAC test. At f=2.906 GHz, λ= 0.5921 m give read of S_{21}.

Study of Repeatability:

The effect of repeatability may be evaluated to establish the method's accuracy and precision by executing at least nine repeated injections of the sample. The repeatability of readings was studied using TAC for 3 different concentrations of 13.41 (µmol/L), 14.07 (µmol/L), and 15.96 (µmol/L). Under optimal conditions, the outcomes of nine successive injections were assessed and were summarized in Table (4).

Table 4: The repeatability of responses.

Repeatability Exp.	**TAC (µmol/L)**	14.07	13.41	15.96
	S_{21}	116	109	120
		116	109	120.0
		116.1	109	120.0
		115.8	109.1	119.8
		115.8	108.7	119.8
		115.8	108.7	119.9
		115.9	108.9	119.9
		116	109.1	120.1
		116.0	108.9	120.1
	Mean	115.93	108.93	119.95
	S.D	0.11	0.15	0.11
	R.S.D	0.09	0.13	0.09

The Results of TAC in Controls and Patients with Diabetic Nephropathy by Using Spectrophotometer and Microfluidic Sensor:

Table (5) shown no significant differences in TAC levels of controls group and patients when measured in both spectrophotometer and microfluidic sensor.

Table 5: Results of TAC in Spectrophotometer and Microfluidic Sensor.

Groups Type	Parameter	Means ± SD	*P-Value
Controls	**TAC Spectrophotometer**	20.33±2.67	0.58
	TAC Microfluidic	20.30±2.65	
Patients	**TAC Spectrophotometer**	14.78±2.27	0.51
	TAC Microfluidic	14.79±2.21	

*****The significance results when P-Value ≤ 0.05.

From Table (5) that indicated result of Total Antioxidants Capacity (TAC**)** match in both spectrophotometer and microfluidic sensor techniques, so we can used this new design in determination other biochemical parameters.

Comparison between Spectrophotometry and Microfluidic Sensor:

Through the results obtained when measuring TAC using the two spectrophotometers and the microfluidic techniques, the most important features of the two techniques are briefed in Table (6).

Table 6: Comparison Spectrophotometry and Microfluidic Sensors.

Parameter	Spectrophotometer	Microfluidic Sensor
Sample volume	450 µL	35.32 µL
Consumption of detection reagents	High consumption	Low consumption
Throughput	High	High
Time reaction	7-8 second	3-4 second
Sensitivity	High	High compared to the spectrophotometer
Testing equipment	Some tests large equipment required	Portable handheld and miniaturized devices
Sample pretreatment	Tedious	Module
Cost	Expensive	Moderate

CONCLUSION

The novel systems are distinguished by the presence of a new detector (DSRR sensor) that works with high efficiency and precision and is home-made manufactured, it is characterized by fast reaction speed, efficiency, sensitivity, easy to use, low costs, high sampling rate, portably, microfluidic sensors have been developed with low reagent consumption of microliters and small size in comparison to spectrophotometers, which are costly, large in size, tedious sample pretreatment, high reagent consumption, and have a long detection time.

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