Breakthrough For Diagnosing Acute Renal Failure Using An Embedded System-Based Design Aided With Pragmatic Spectrophotometry

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Abstract: In the modern era, non-communicable diseases are striking the highest rate of premature death worldwide. Apart from cardiovascular disease, diabetics, and hypertension, renal failure (kidney failure) is considered to have a high fatality rate. Creatinine is the waste produced from muscle metabolism which has to be removed from the blood by the kidney. Improper function of the kidney results in the accumulation of this waste in the blood. Embedded system-based design is developed to analyze the creatinine level present in serum and saliva samples. In this research, a standard solution is taken as the reference and with the help of this standard value, the samples are cross-checked. The sole objective of the prototype is to make an amalgamated instrument that helps in not only finding the value but also display them for future reference. The prototype consists of a source (LED), a casing, and a detector (photodiode). The wavelength of LED used in the model is appropriately chosen for the color of the solution. The light from the LED passes through the solution before it hits the detector. It also passes through the sides of the vessels holding the liquids. As more light is absorbed, less light passes through the solution, so the number of photons striking the photodiode varies, the current that gets induced is comparatively different for different light intensities that tend to fall on the photodiode. Finally, the output was processed by the controller and the corresponding values are calculated. The exact creatinine level can be mapped using the basic calculation which perfectly yields the value. Apart from that, the prototype involves IoT (Internet of Things) wherein the creatinine level of the subject with chronic kidney disease (CKD) along with other morbidity gets stored in the cloud using the Wi-fi module ESP-8266 and technicians can access these data anywhere and anytime across the globe.

Index Terms: Absorption coefficient, Creatinine, Chronic Kidney Disease, ESP 8266, Glomerular Filtration Rate (GFR), Renal diseases, Spectrophotometry.

1 INTRODUCTION

Renal disease or any case of disfunction in the kidney can be diagnosed using the level of creatinine. Creatinine biosynthesis has proved to be of prime importance in diagnosis in recent years [1,2]. Jaffe' reaction is one of the common reactions used to determine the creatinine level present in the body. It forms a complex under the alkaline environment when reacts with picric acid [3]. Since the implementation of the original Jaffe' reaction, the procedure has been changed, despite being simple [4-6]. As it was required to ensure the selectivity the procedure had undergone some changes. Different metabolites had interference in the reaction which was discretely explained [4,5,7]. Serum samples and interference were still a challenge even after imposing changes in the Jaffe' reaction. Enzymatic methods dominated Jaffe' procedure much before twenty years. But the principle behind the method was known at the end of 1925 [8]. The enzymatic method comprises of two different groups. The first one is based on the hydrolysis of creatinine with the help of creatininase [9], and the second group of the category involves iminohydrolase of creatinine [10]. The process of separation was included as the third category lately. Some of the advanced and best performance chromatographic methods are discussed in the literature [10-19] and out of which some of them are used as references [16,17].

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In order to analyze protein and nucleic acid zone electrophoresis was used with the help of a capillary approach [20]. And for determining the creatinine in the body fluids micellar electro-kinetic chromatography is also used [21]. A microanalysis in capillary which is conducted electrophoretically promised reduction in interference in the production of the creatinine-picrate complex [22]. Recently, simultaneous measurement of the renal marker such as creatinine using electrophoresis with the capillary chip was developed [23]. Later mass spectrometry and isotope dilution gas chromatography were considered to be an effective method [24]. At present for food analysis and study related to drug metabolism using Tandem Mass Spectrometry (TMS) is in high demand. Over the past 15 years, the error detection with TMS is changing the era of biochemical diagnostics [25,26]. Generally, the samples are high, the mass spectrometry is one of the efficient tools in screening samples as per rate for the sample cost is low. And, also the application has found its way in the field of clinical diagnostics and other paramedical industries [27]. In order to determine the creatinine level along with Cyclosporin A present in the blood was done using liquid chromatography and isotope dilution TMS was used to determine the creatinine serum with high accuracy [28,29].

2 METHODOLOGY

According to the Assay principle, a yellowish red colored compound was formed with the salt picrate after the antibody binding with a specific type of antigen depicting the deproteinization of the creatinine in the presence of the alkaline solution which is shown in the equation (1).

$$C_4H_7N_3O\ (creatinie) + C_6H_3N_3O_7(Picric\ Acid) \xrightarrow{Alkaline\ solution} Creatinine\ Picrate \quad (1)$$

The creatinine concentration and the intensity of the light falling on the photodiode refracted during the reaction are proportional to each other and it was observed that the

absorbance at the rate of 500-560 nm wavelength.

2.1 Reagent Preparation

The reagents used are mentioned in Table 1. The reagents were stored at 15-30^0 C before it was used for preparation it was needed to be stabilized.

***TABLE.1**. Reagents Used for the test*

No. of Reagent	Reagent name	Quantity Used
RG_1	Standard of creatinine	3.0(mg/dl)
RG_2	Solution of Picric Acid	40(mmol/L)
RG_3	Alkaline solution NaOH	1.3(mol/L) approx.
RG_4	Acid -Trichloroacetic acid (TCA)	1.3(mol/L) approx.

The mixture of the solution RG_2+RG_3 is taken as the working solution. According to the requirement, solution preparation was done by adding the equal concentration of the solution from RG_2 and RG_3. In order to maintain the stability condition for the working solution, it was maintained at the temperature 20-250 C for 6 hours and stored in a dark bottle.

2.2 Specimen Preparation and Stability

Before the test is conducted, sample preparation is a crucial step and the process of specimen preparation can be divided into two steps which are as follows,

2.2.1 Serum or Plasma Specimen Separation

The subject need not prepare for the test and sample collection. When maintained at 420 C at least for seven days the sample remains stable while frozen.

2.2.2 Saliva specimen preparation

The subjects under analysis were asked to avoid drinking or eating before an hour for the sample collection and in order to avoid any kind of contamination their mouth was rinsed thoroughly. Around 2 mL of the saliva was collected using a spitting method and was stored in a sterile container. And nearly for15 min the saliva sample was centrifuged at 900g and the supernatant formed was removed from the mixture and was stored at the temperature of 890 C before final processing. For final processing, the steps below were followed.

- Manual setup preparation
- Wavelength was set to 500-560 nm
- The cuvette was placed 1 cm from the light source and detector
- The temperature was maintained at 20-270 C
- Against the blank reagent zero adjustments were made.
- Prepare the specimen of serum and saliva

Protein must be separated from the serum, add around 2 mL of serum with 2 ml of saliva and 1mL of TCA separately. After 30 minutes and at 280 C the mixture was mixed well and the absorbance of the specimen was measured and the standard solution against the blank was calibrated.

2.3 Estimation of Creatinine concentration and clearance level

The formula used to estimate the amount of the creatinine is given in the equation (2) below,

$$\text{Concentration of Creatinine} = \frac{\text{Specimen Absorbance rate}}{\text{Standard Absorbance rate}} \times \text{Standard Absorbance} \quad (2)$$

In order to prepare the saliva and serum samples, the result obtained is multiplied with the dilution factor within 24 hours from the collection in liter. Therefore, the creatinine clearance is estimated using equation (3) which is given below.

$$\text{Clearance rate of creatinine (ml/sec)} = ((\text{Creatinine Urine level}) * (\text{volume of urine})) / (\text{Creatinine of serum} * 1442) \quad (3)$$

3 HARDWARE IMPLEMENTATION

The proposed project consists of four major categories as shown in Fig. 1 and they are,

- Power Supply Unit
- Sensor unit
- Controller unit
- Display unit.

***Fig.1**. Final Product Prototype*

3.1 POWER SUPPLY

This unit provides the necessary supply for microcontroller and sensor unit. The functioning voltage required for the controller is (3.3-5) V and the sensor needs around 5V. The safety switching circuit is enabled in this unit. As directly the supply can't be used for driving the battery. It needs some mediator, so this safety circuit prevents any mishap and even makes the battery life for longer.

3.2 SENSOR UNIT

It consists of two sections namely

•**Transmitter:** It consists of LED (of 510 nm wavelength); this constantly passes the light rays in the fixed path and direction where the solutions need to be kept. And on the other end is the receiver.

•**Receiver:** It consists of a photodiode. Once the light rays pass through the solution. The amount of absorption rate

depends on the creatinine concentration in serum and saliva. So, the remaining light rays fall on the photodiode which produces current. And the output of the sensor is fed to the controller with the help of op-amp.

The transmitter and receiver section hold photodetector on one side and LED on the other side. In between the two, the sample solution is placed as shown in Fig. 2.

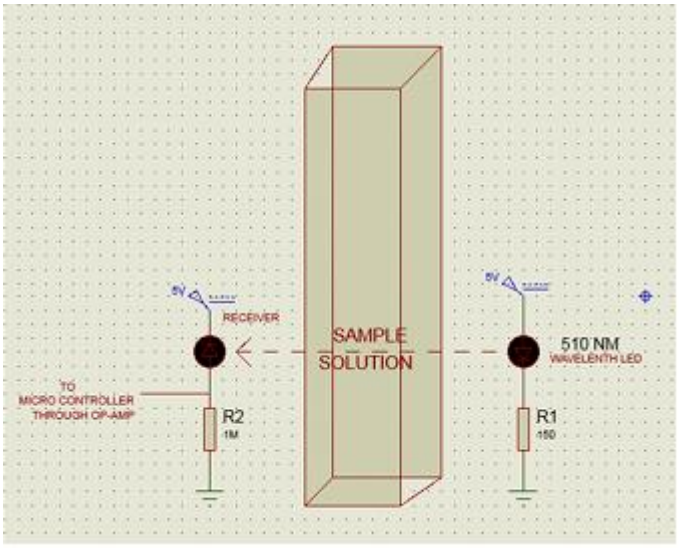

***Fig.2**. Transmitter and Receiver Section*

3.3 Controller Unit

It consists of the controller atmega328p and keys. The calculations done manually are all pre-programmed and hence on the proper signal they must get executed. For that, keys are of utmost use. Keys send the proper signal to the controller for doing a particular task.

3.4 Display Unit

This unit consists of an LCD display. Here, once the amount of creatinine present in the saliva is determined by the controller. The values are further sent to the LCD display. LCD acts as an output device which finally makes the task of the person handling the instrument to jot down the creatinine content.

4 RESULT AND DISCUSSION

The proper function of the kidney is analyzed with the help of Glomerular Filtration Rate (GFR) value which determines the quantity of blood passing through the glomeruli every minute. Depending upon the GFR rate of the participants, different stages of the CKD can be determined. The following Table 2 describes the stages with its GFR values.

***Table.2**. GFR values of different stages*

Stages	GFR (ml/ min/1.73m^2)
First	91
Second	61-90
Third	31-60
Fourth	16-30
Fifth	< 16

The data obtained from the subject was added to the chart and finally tabulated. The parameters like frequency of occurrence, mean value, Standard Deviation (SD) from the mean, median value, minimum and maximum values of the creatinine level were estimated and tabulated. For a different group of subjects like age, gender, etc., tests like Mann Whitney U Test were conducted and the creatinine values were compared among the groups. Finally, Spearman's Correlation Test was conducted and the correlation factor between serum and salivary creatinine level were estimated and compared. The first three stages of CKD are the most prevalent stage and hence the subject was around 50 but whereas the fourth and fifth stage subjects were around 40. Altogether the total number of the subjects under observation was around 230. The female subject creatinine level was more around 66.95% whereas the male subject creatinine level was about 37.39 % which is shown in table 3.

***Table.3**. Frequency Distribution of subjects with different stages of CKD*

Gender & Age	First Stage	Second Stage	Third Stage	Fourth Stage	Fifth Stage	Total (Percent)
Male subject	15	12	20	17	12	86 (37.39 %)
Female subject	35	38	30	23	28	154 (66.95 %)
All	50	50	50	40	40	230 (100%)
Age range Mid Age Mid age Range	18-55 32.0 (21.25-39.75)	18-70 39 (31.22-48.65)	18-70 38.5 (32.0-51.0)	20-80 41.5 (32.45-48.77)	22-65 46.5 (41.55-53.44)	
Subjects with comorbidities	34	45	43	35	42	199
	High BP and diabetes (1) High BP (20) Diabetes (8) Others (2)	High BP and diabetes (5) High BP (30) Diabetes (5) Others (5)	High BP and diabetes (3) High BP (25) Diabetes (10) Others (2)	High BP and diabetes (4) High BP (23) Diabetes (6) Others (2)	High BP and diabetes (6) High BP (28) Diabetes (6) Others (2)	

A subject's creatinine level of different stages is expressed in Table 4. It describes the range of CKD of stages first to last, and even the subjects with comorbidity combined. The creatinine levels in the subject showed some common features further it was observed that the creatinine level in the serum was greater than that present in the saliva.

***Table.4**. Salivary Creatinine and Serum Creatinine (µmol/L) of the subjects in all stages*

	First Stage	Second Stage	Third Stage	Fourth Stage	Fifth Stage	Total Subjects
Serum Creatinine Level	49-95	65-135	105-230	169-465	315-1551	49-1551
Salivary Creatinine Level	4-20	4-19	5-65	6-230	75-445	4-445

Nearly 120 subjects from the total 230 subjects around the age of 55 years were observed out of which 64 were males and the remaining 56 were females. The difference in the creatinine level observed among the category of subjects with different ages and gender was minimum and the factor was less than 0.05. when the creatinine level in the serum and saliva was compared it was observed that the serum creatinine level was greater than that present in the saliva which is as shown in Table 5.

***Table.5**. Creatinine Level in Saliva and Serum in subjects with CKD*

Groups of subjects	Level of Creatinine (µmol/L)				Mann Whitney U test results	Correlation factor q
	Creatinine present in Serum		Creatinine present in Saliva			
	Mean Value ±SD	Median Value	Mean Value ±SD	Median Value		
CKD subjects (n=30)	227.12±45.6	166	145±93	75	224	q=0.000 (<0.0009) high difference
Diabetes (n=30)	177±45	67	131±18	69	466	q=0.000 (<0.0009) high difference
High BP (n=30)	156±19	70	129±23	60	467	q=0.000 (<0.0009) high difference
High BP and Diabetes (n=30)	99±20	45	109±3	52	465	q=0.000 (<0.0009) high difference

The creatinine level present in the serum, as well as saliva samples during analysis, is updated to the cloud. A code using notepad++ and Xampp was developed which is useful in sending the data to the cloud. Now a separate web page is created in the server and a new domain name was given. The user has to register in the link www.awardspace.com which is one of the important websites to create a new domain. The webpage opens a new window in which the name, user id, and password of the domain are chosen. The Xampp code written in PHP for table creation is done which helps in creating the contents of the table which involves Patient ID, serum creatinine level, salivary creatinine level, time, and date. This technique of table creation on the web page also includes domain "name" and table "name". Once the procedures are correctly followed the final view of the phpMyAdmin window looks like Fig. 3 which depicts the creatinine value present in serum and a salivary sample of Stage 2 and Stage 3 subjects.

Patient ID	Serum Creatinine Level (µmol/L)	Salivary Creatinine Level (µmol/L)	Time_Date
1299570091	92	108	10:55_12-08-19
1299570092	118	92	11:22_12-08-19
1299570093	200	123	12:34_12-08-19
1299570094	122	120	13:09_12-08-19
1299570095	109	130	14:30_12-08-19
1299570096	89	110	15:45_12-08-19
1299570097	156	187	16:25_12-08-19

***Fig. 3**. Creatinine level present in the serum and saliva sample updated to the cloud*

5 CONCLUSION

Embedded system-based design can accurately estimate the salivary creatinine and serum creatinine values as simple as possible and requires very little time for analysis. The salivary creatinine level can be considered as one of the important indicators for the creatinine level as it is non-invasive in nature and easily collected without much pre-processing. But in the case of blood serum, the process involves the risk of contamination as well as infection and expensive too. Salivary assay and serum creatinine level provides a strong indication of the creatine level and easy classification of different stages of chronic kidney disease. Thus, salivary serum estimation is a stepping stone ahead and has the capability to revolutionize the era of medical diagnostics and provide more accurate results in the future.

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