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Designing A Non-Invasive Testing Device for Infant Diabetes using Saliva

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***Abstract*—Neonatal diabetes is estimated to affect 1 in every 95,000 to 1 in every 400,000 live births. Male and female infants are equally affected by the disease, which has been recorded in all ethnic groups. Neonatal diabetes mellitus is a type of diabetes that develops within the first six months of life. Insulin is a hormone that aids in the production of energy in our cells. This condition causes infants to produce insufficient insulin, resulting in elevated blood glucose levels. Therefore, it is very important to design a testing device that can monitor premature babies indicating symptoms of diabetes. Recognizing the signs of diabetes in an infant might be difficult because diabetes in babies is not common. Frequent wet diapers, a high appetite, dehydration, and weight loss are all signs of neonatal diabetes. Once you have diabetes, you're used to having to take tests to monitor your condition. Conventionally invasive methods are used to check neonatal diabetes which is a painful procedure for infants as it requires prinking to draw blood several times per day. Therefore, this paper aims to design a non-invasive testing design for neonatal diabetes that is not painful to babies. There are several non-invasive ways to test blood glucose levels; urine, sweat, and saliva. Urine tests can be used to detect glucose levels in urine and check for the presence of ketones. The presence of ketone above the threshold indicates a high blood glucose level of over 300ml/dL. Sweat is also used to test diabetes. Hyperhidrosis (excessive sweating) is caused by high blood sugar levels, and it indicates that blood sugar control has to be tightened. Saliva is a new medium to measure blood glucose levels and research is going on its use to measure diabetes. Previous research has found a strong relationship between blood glucose levels and saliva glucose levels. In this paper, saliva is used as a non-invasive method to measure neonatal diabetes. Saliva containing glucose is collected and treated with glucose oxidase. H2O2 produced during this reaction is further treated and an optical sensor along with a microcontroller is used to measure the concentration of blood glucose. The expected results based on the study indicate the amount of glucose in the saliva increases in proportion to the amount of glucose in the blood and confirms the relationship between glucose concentrations in the blood and saliva, implying that the proposed design can take the place of the existing devices. However, more tests should be carried out to improve the efficacy of the proposed design for detecting glucose in saliva.**

***Index Terms*: - Neonatal Diabetes, Invasive Testing, Non-Invasive Testing**

I. INTRODUCTION

In neonates, Neonatal Diabetes Mellitus (NDM) is a rare genetic disease, affecting one out of every 90,000 live births [1]. It is characterized by the occurrence of extreme hyperglycemia with inadequate or even no circulating insulin, which usually occurs before the age of six months and hardly between the ages of six months and one year[1]. The blood glucose concentration of most newborns is in the range of 63 mg/dL to 90 mg/dL. Regardless of gestational or postnatal age, neonatal hyperglycemia is defined as blood glucose levels of more than 125 mg/dL and levels of plasma glucose of more than 150 mg/dL. Continuous glucose monitoring reveals that hyperglycemia is more frequent during the first week following delivery, but it could be detected lasting up to 10 days or more after birth. In most neonates, severe hyperglycemia resolves in 2 to 3 days. The reasons of neonatal hyperglycemia are many and difficult to pinpoint. Hyperglycemia is defined as a combination of the infant's excessive glucose generation and diminished ability for glucose use. When glucose concentrations are abruptly elevated or dropped, no visible medical symptoms of neonatal hyperglycaemia and no deviations in neonatal medical status are observed. Preterm newborns, for example, exhibit glucosuria but not osmotic diuresis with higher flow rates of urine during experimental hyperglycaemia. As a result, hyperglycaemia remains a biochemical condition that cannot be identified without measuring glucose levels in the blood or plasma [2].

Currently used methods for measuring blood glucose levels encompass pricking the finger to get blood samples. This approach is painful, and multiple blood samples must be taken quite a few times daily. As a result, numerous researchers have

looked at developing non-invasive glucose monitoring devices that don't require a blood sample and instead rely on bodily fluids like saliva and sweat [3][4][5].

A. Sweat Testing

During the first several weeks, babies, especially preterm neonates, do not sweat. It's tough to make them sweat, even with the help of a thermally regulated incubator. This is because the sweat glands aren't fully functional yet. As a result, sweat testing patches for diabetes would be ineffective in neonates [6].

B. Urine Testing

Neonates with NDM have frequent urination, and urine may include extra glucose. Ketoacidosis is linked to severe NDM. To maintain normal blood glucose levels, most newborns who are not fed are given 4-6 mg/kg/min intravenous glucose. In these infants, blood glucose levels are checked every 4-6 hours. In newborns with hyperglycemia, testing glucose in each urine sample allows for fewer blood tests [2].

There are currently available ideas using a smart diaper with a QR code or a simple diaper with dipsticks. The urine sample is collected when it penetrates the diaper and reaches the reagent pads on the inside surface through capillary flow. Depending on which biomarker is detected, each reagent pad changes color as it comes in touch with the urine sample providing information on the nitrate and glucose levels present. When the diaper is removed from the baby, the color shift is evident through the translucent bottom layer. The reagent pad, like a dipstick, has a reference color chart to which the colors are compared or a QR code is scanned in the case of a smart diaper that displays the color chart for a variety of abnormalities which can be used to analyze the change [7][8].

However, this design is 3 to 4 times the cost of ordinary diapers and you must change 3-4 of these diapers to monitor blood glucose concentration at a regular interval in a day to comprehend exams, a smartphone app or a reference chart is required. It may not be very accurate, and the possibility of false positives is there when using a reference chart.

C. Saliva Testing

Saliva is a special liquid that is essential for the proper working of the oral cavity. Diabetes diagnosis by blood is difficult in infants, older individuals, and hemophilic patients, thus diagnosis by saliva analysis might be beneficial because saliva collection is non-invasive, simpler, and insensitive, unlike blood collection and newborns produce a lot of salivae so in our paper we used saliva as testing fluid. Also, previous research has found an important relationship between blood glucose concentrations and saliva glucose concentrations [9].

D. L-O-C (Lab-on-Chip) Design

Our design is based on the LOC idea proposed by D. G. Jung et al. [10] for finding the blood glucose concentration using saliva. We have modified the saliva collection, mixing, and measurement part of the LOC design. A pre-treatment part to produce H_2O_2 from glucose present in saliva, a mixing part to combine saliva with the chemicals together with the measurement section to assess the concentration of glucose in the saliva using a measurement technique comprises the LOC design to detect levels of glucose as shown in Fig. 1 and 2. Firstly, a micro-channel structure has been used in LOC to mix the chemicals. Secondly, glucose in saliva was optically analyzed using a colorimetric approach. Thirdly, using a LED and a photodiode, a technique for determining the colored sample's absorbance was suggested [10].

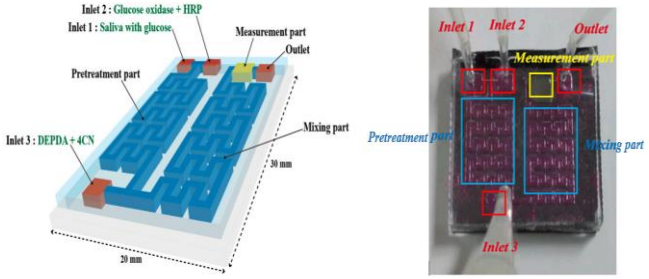

FIGURE 1. The design of LOC to detect glucose levels in saliva[10]

II. METHODOLOGY

A. Glucose Detection Principle

Our body needs energy in the form of ATP to perform functions. Glucose produced in the body undergoes glucose oxidation to convert glucose into gluconic acid, H_2O_2, and ATP. The H_2O_2 produced is directly proportional to the concentration of glucose. The principle of glucose detection is taken from D. G. Jung et al. on LOC [10] as shown in Fig. 2. The glucose in saliva undergoes glucose oxidation reaction, colorimetrical reaction, and glucose concentration measurement method [11]. The glucose in saliva reacts with the glucose oxidase enzyme to produce gluconic acid and H_2O_2. The H_2O_2 produced undergoes a colorimetric reaction with a combination of DEPDA (N, N'-diethyl-p-phenylenediamine), 4CN (4-chloro-1-naphthol), and HRP (horseradish peroxidase) enzyme. HRP gives color to the saliva-based on the concentration of H_2O_2 produced. HRP act as a catalyst in the breakdown of hydrogen peroxide into H_2O which changes the color of the saliva to the blue[10]. LED combined with the photodiode circuit measures the absorbance of the blue saliva and displays the concentration of glucose on an LCD screen.

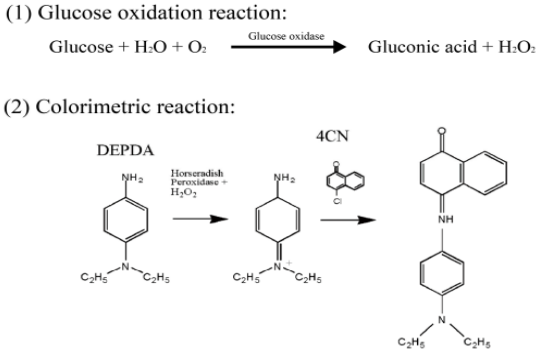

FIGURE 2. Glucose oxidation and a colorimetric reaction[10]

III. Design and Development

To improve the usefulness of the LOC idea for detecting glucose levels in saliva, we proposed a modified design. The design consists of saliva collection, pre-treatment, mixing, and measurement parts.

A. Saliva Collection

A pacifier is used to collect saliva in newborns who do not have any difficulties latching on (nipple/pacifier) or sucking. You're not only collecting saliva; preterm newborns may benefit from pacifier use as well; one study found that providing premature babies pacifiers resulted in faster sucking success and oral feeding transition [12]. A dropper or syringe without a needle might be used to help newborns who have difficulty latching on (nipple/pacifier) or sucking.

We devised a pacifier with a manual suction design that may be used by any baby as shown in Fig. 3. The doctor can put the pacifier in the patient's mouth and suck out the saliva if needed (syringe attached to the pacifier tube). A hole in the pacifier tip leads to a tube where the saliva will be collected. The collecting tube has measurements mentioned in the milliliters. A total of 2ml of saliva is collected for the pre-treatment part which is approximately equal to less than half of one teaspoon. Newborn babies are likely to produce more saliva. To collect pooled saliva, place the sucking end of the pacifier on the resting side of the infant's head between the cheek and bottom gum. Premature newborns, those in the NICU, and those who are unable to keep their heads up will have saliva collecting around their cheek and lower gumline rather than beneath their tongue [13].

The collecting part is not connected with the rest of the design because of the electrical and chemical hazards; any leakage or backflow of fluids and problems related to electronic circuitry may risk the baby's life. Therefore, the collecting part is separated from the rest of the device.

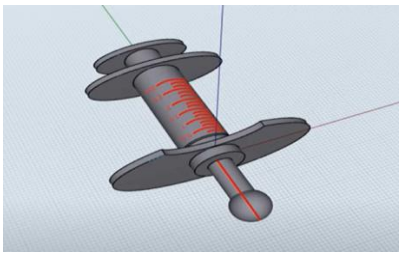

FIGURE 3. Pacifier with Manual Suction design

B. Pretreatment and Mixing Part

The pretreatment part is used to generate H_2O_2. Glucose in saliva and glucose oxidase reacts chemically to produce H_2O_2. The collecting tube is detachable with the pacifier. Once the saliva is collected, the collecting tube is attached with inlet-1 that opens in a container. The container has a valve that prevents the backflow of saliva. There are two more containers with inlet-2 and inlet-3 opening respectively. These containers are filled with glucose oxidase and a mixture of DEPDA, 4CN, and HRP enzyme; whenever the containers are emptied, the device sends a message to the user on the LCD screen to fill the containers first to measure the glucose concentration. The valves in front of these containers are controlled by a microcontroller to send the exact volume of all these reagents at an increased speed. 100 mg/mL glucose oxidase and 20 g/mL HRP in an equal ratio, and a colorizing agent containing 1 M 4CN and 1 M DEPDA in a 2:1 is released from both the containers in the pretreatment and the mixing part [10]. The release from the containers is made automatically for accurate mixing and time-saving. This part is manually done in the LOC device by inserting tubes inside the device and filling the inlets with the reagents every time you start the time-consuming testing.

When the saliva and glucose oxidase are released from the containers, they are mixed by passing through a series of micro-channel structures with obstacles to increasing the mixing efficiency by generating turbulent flow. The container in the mixing part releases a known volume of a mixture of DEPDA, 4CN, and HRP. The mixing part results in the change of saliva color to blue. The pretreatment and the mixing part are shown in Fig. 4 and Fig. 5.

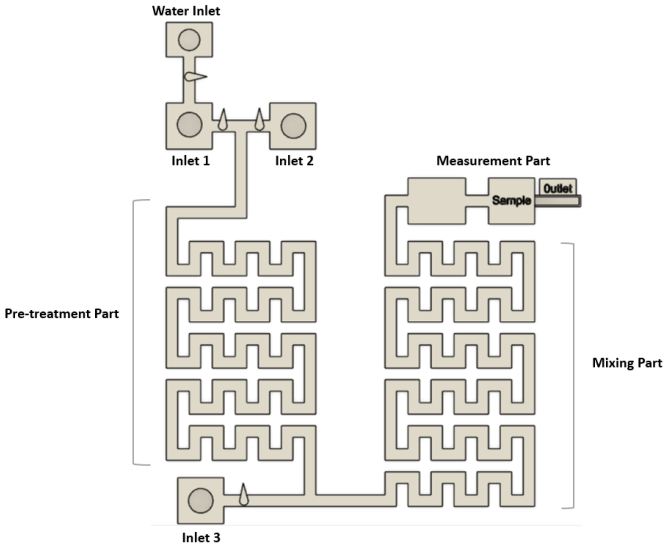

FIGURE 4. Pretreatment and Mixing part

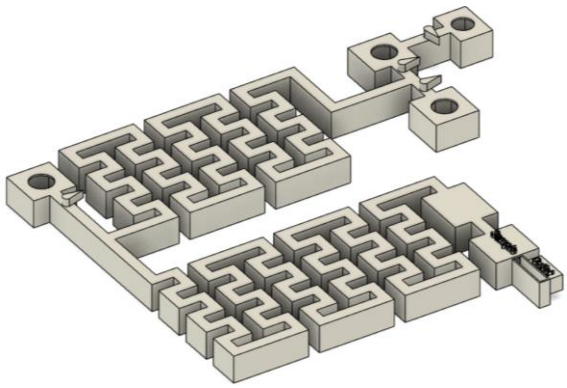

FIGURE 5. Overall view of Pretreatment and Mixing part

C. *Measurement Part*

In the measurement section, photo and light-emitting diodes are used to determine the levels of glucose in the colorized saliva. The photodiode measures the concentration of salivary glucose by finding the intensity of light that has been transmitted. As glucose concentration rises, the intensity of the transmitted light falls as it goes through the colorized sample. The maximum absorbance in LOC design was calculated at a wavelength of 630 nm [12]. Therefore, by using their work, we employed a 630 nm red LED and a 450–1050 nm photodiode in our design. The light from the LED travels through the colorized saliva and is converted to current by the photodiode.

Using a conversion formula based on linear approximation, an Arduino nano is used to convert the current values into glucose concentration The glucose measurements are then displayed on the LCD screen so that the user may see what their blood glucose levels are. A visual representation of the measurement part is made in fritzing software as shown in Fig. 6.

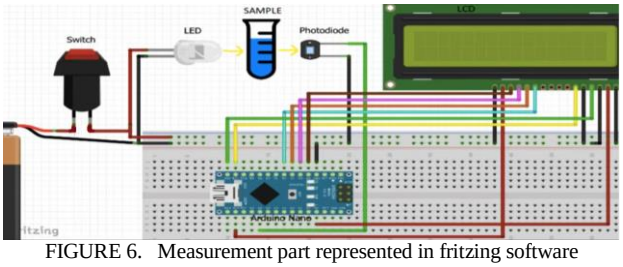

FIGURE 6. Measurement part represented in fritzing software

The final design of the glucose testing device is shown in Fig. 7.

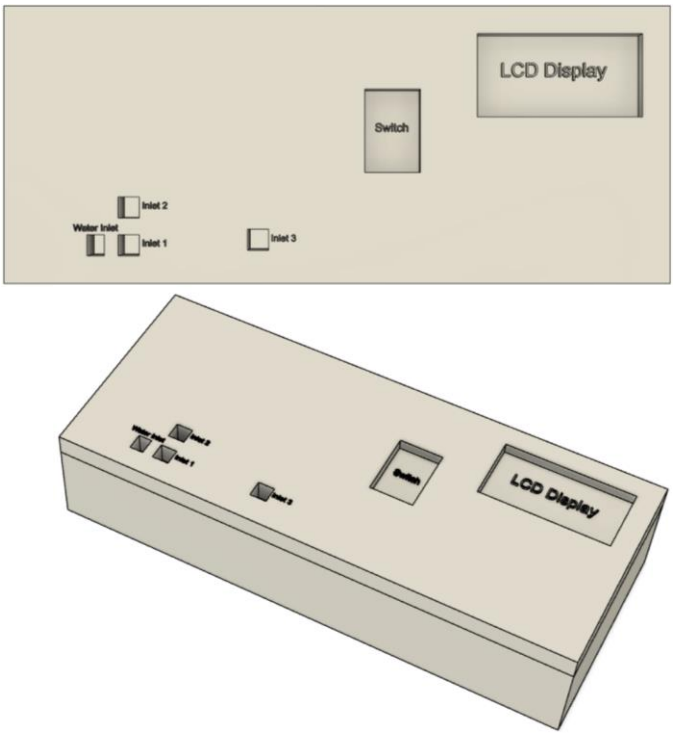

FIGURE. 7. The front and the side view of the final glucose testing device

IV. Result and Discussion

We designed a modified non-invasive glucose testing device based on the LOC design proposed by D. G. Jung et al. [10]. For that, we studied the correlation between the saliva glucose level and the blood glucose level through various studies. According to Zolotukhin et al. [14], salivary glands act as blood glucose filters, and hormones and neuromodulation can impact blood glucose levels. According to Abikshyeet et al. [15], diabetes patients had more glucose leakage from their salivary gland duct cells, which led to higher salivary glucose levels. This is due to basement membrane alterations in Diabetic patients and microvascular anomalies in blood vessels. Advanced glycation end products (AGEs) are produced more often when hyperglycemia is present. The AGEs damage the basement membrane and cause endothelial dysfunction, among other things, increasing their permeability and explaining the increased glucose entry from the blood into saliva in DM patients [16]. Unstimulated parotid saliva (UPS), according to Y. Cui et al. [17] had the highest correlation with blood sugar and served as a reference for the diagnosis of diabetes mellitus. Hence, the collecting method is a very important factor in affecting the saliva glucose concentration. Therefore, we will place the pacifier's sucking end close to the left parotid duct to implement effective glucose monitoring as the maximum correlation between blood glucose level and saliva glucose level is seen in the UPS.

The pre-treatment and the mixing part of the studied LOC design were modified by using valves and sensors. A predefined volume of reagents was released from each container. The containers were refilled after 6-7 uses. The device had an additional water inlet to clean the microchannels and an outlet port for removing the sample and water.

The process of saliva collection and glucose measurement takes about approximately 4 minutes to complete. The time taken to complete the test can be reduced with more accurate results by further working on the design fabrication. Our work is based on the literature we have studied, and the paper published by D. G. Jung et.al. This paper represents the AutoCAD drawing of our work and further work will be done to make a prototype of our design and evaluate the efficacy of our device using samples.

After the sample collection, Artificial Intelligence (AI) can be utilized to estimate the glucose level. AI has successfully solved several Biomedical problems in the past such as cancer diagnosis [18]–[20], disease prediction [21], [22], brain-computer interfaces [23]–[25], and assistive technologies [26], [27]. In the future, we aim to collect the data using our designed glucose testing device and then estimate the blood glucose levels using AI.

V. Conclusion

In this paper, different non-invasive methods were studied to design a non-invasive glucose testing device for neonates.

Sweat and urine testing for measuring blood glucose levels were rejected in our study and we chose saliva as a medium for testing blood glucose levels as infants produce a large amount of saliva and from prior studies, we know that diabetic patients have a glucose concentration of 170-180 mg/dL in blood and a saliva glucose level of 10-11 mg/dL. As a result, a saliva-based glucose sensor can be used to measure glucose concentrations as low as a few mg of fluid. Therefore, in this paper, we have designed a non-invasive testing device for infant diabetes based on the LOC-based optical sensor. We have designed our device with some modifications to the already proposed LOC design. After all the designing and modifications, we confirm that the suggested device can detect glucose concentrations and is appropriate as a device that can be easily carried out for continual glucose observation.

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The authors received no specific funding for this study.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest to report regarding the present study.

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