Assessment of Protein Aggregation in Aging Retina as an Early Retinal Biomarker

Author

Sameen Mehtab

Regn Number

00000277089

Supervisor

Dr. Saima Zafar

DEPARTMENT OF BIOMEDICAL ENGINEERING AND SCIENCES

SCHOOL OF MECHANICAL & MANUFACTURING ENGINEERING

NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY

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Sameen Mehtab

MS-BMS 00000277089

A thesis submitted in partial fulfillment of the requirements for the degree of MS Biomedical Sciences

Thesis Supervisor:

Dr. Saima Zafar

Thesis Supervisor's Signature:

_____

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1. Name: Dr. Syed Omer Gillani Signature: _____

2. Name: Dr. Asim Waris Signature: _____

3. Name: Dr. Nosheen Fatima Signature: _____

Supervisor's name: Dr. Saima Zafar Signature: _____

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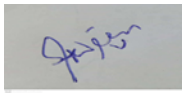

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Dedication

This work is dedicated to Allah Almighty, who is the epitome of all the knowledge that has ever existed, all the knowledge that exists now and all the knowledge that will ever exist in years to come.

My parents, Mr. & Mrs. Mehtab Ahmed, whose devotion, prayers and investment in me has made me reach where I am today.

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Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of amyloid beta (a-beta) protein and hyperphosphorylation of tau protein in brain and its peripheral regions. It is the leading cause of dementia worldwide which only worsens with time. An extensive body of research suggests that the disease onset is triggered at least 15 to 20 years prior to the appearance of any initial symptoms. There is no cure so far and the treatment lines only deal with slowing down the process of neurodegeneration. Owing to these facts, research paradigms are shifting towards early diagnosis of AD to prevent the irreversible neurodegenerative damage. A growing body of researchers is working on the use of ocular organelles for the early assessment of AD. Retina shares its embryonic origin with brain and research suggests that protein accumulation in brain as well as retina runs parallel in the patients. Lens has also been reported to be associated with providing a window towards any change related to neurodegenerative diseases. The contraction and dilation of iris are also being investigated as biomarkers for AD. The existing techniques have incorporated the use of MRIs, PET scans, OCT and other brain imaging techniques which are expensive and detect the disease indirectly. The techniques are although sensitive but do not specifically target the disease or disease stage. To rule out these factors, a robust, cheaper and direct technique is required which turns out to be specific, selective and sensitive at the same time. IR light has penetration power up to retina and it is safely being used in biometric identification using retinal scanning. Protein accumulation in retina has been confirmed by literature and it is hypothesized that more is the accumulation of protein in retina, more is the absorption of light and vice versa. A difference in incident and reflected light can precisely detect disease and disease stage. This study focuses on the use of infrared (IR) light for the investigation of a-beta stacks in patients when they are in pre-clinical stage of the disease.

Key Words*:* *Alzheimer's disease, amyloid beta, hyper phosphorylated tau protein, retinal biomarker, IR light*

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CHAPTER 1: INTRODUCTION

1.1: Background, Scope and Motivation

Alzheimer's disease (AD) is a neurodegenerative disease that gradually effects the cognitive functioning of brain. It is progressive in nature and if progression is not intervened by medication, the cognitive decline becomes irreversible. It is the leading cause of dementia worldwide. The term AD was first coined by a German physician Alois Alzheimer who published the first ever finding about presenile dementia in 1906. AD is distinguished by certain pathological characteristics which include the formation of plaques by amyloid beta protein, primarily in the cortical region of brain, which is the harbor of memory. These plaques are produced as a result of breakdown of amyloid precursor protein (APP). Another change in pathophysiology includes the formation of neurofibrillary tangles (NFT). These NFTs comprise of hyperphosphorylated tau protein, which is the protein associated with microtubules. Both amyloid beta plaques and tau NFTs are formed in extracellular region. In 2011, the National Institute of Aging (NIA), classified AD in three stages, namely preclinical AD, Mild Cognitive Impairment (MCI) and Dementia. Since then, gross working is done on halting the disease at an early stage in order to limit the cognitive decline. Another classification based on biomarkers was also needed so Jack et al. devised a new framework for AD classification in 2018. This new classification is based on ATN. ATN is the latest classification which is based on biomarkers of the disease, where A denotes to amyloid biomarkers, T denotes to tau biomarkers and N denotes to neurodegeneration based biomarkers. (Pais et al., 2020) Since the addition of this new classification system, the research paradigms have shifted towards biomarkers-based investigation of disease and disease stages.

As of now, the early diagnosis of AD is prime focus of every research because literature suggests that so far, diagnosis is made at a much later stage when most of the irreversible cognitive loss has already occurred. Another issue that arises is the machinery and diagnostic techniques which are not only expensive and not available to every individual in general public, but they are also time consuming and indirectly diagnose AD. The current techniques being used to culminate the effects of AD include magnetic resonance imaging (MRI), optical coherence tomography (OCT) scan, positron emission tomography (PET) scan, blue light autofluorescence (BAF) and scanning laser ophthalmoscopy (SLO).

The need of hour is to develop a robust and user-friendly technique that specifically targets AD in its pre-clinical stages and directly measures the level of cognitive decline. A growing body of research supports the fact that since cortical region of brain and retina share a common embryonic origin, the retina can be used as biomarker of AD. Literature also suggests that physiological changes such as protein accumulation and tangle formation occur in patients safely fifteen to twenty years before the appearance of initial symptoms. It is also reported that protein formation in retina and brain are symmetric in nature, hence any changes is retina can give a direct clue to what is happening inside the brain.(Alber et al., 2020)

1.2: Research Problem

The pathological findings in AD include extracellular protein accumulation in cortex region of brain. They are currently being diagnosed by analysis of cerebrospinal fluid (CSF), brain imaging techniques or by post-mortem. Although these approaches are effective, yet they pose certain problems mainly due to high costs and difficulty in gathering sample. Research conducted by J. Lim et al. reported multiple retinal biomarkers such as thickness of optic nerve as biomarker, retinal nerve fiber layer as biomarker, retinal vasculature as biomarker, retinal blood flow as biomarker and choroidal thickness as biomarker for the probable early diagnosis of AD. (Lim et al., 2016)

There is clearly a need of such technology which is sensitive and selective at the same time and is not overpriced as well. Research conducted by Kerbage et al. have proposed a new model for diagnosis of AD. This model is based on SAPPHIRE system which uses fluorescent ligand and laser scanning device for the early detection of AD. The ligand used is compound 11 and the laser scanning device measures its emission spectra. They performed experiments in three regions of eye and found out that the substantia nigra region which is deeply located inside, showed maximum bounding of ligand with amyloid beta stacks. (Kerbage et al., 2013)

Extensive research is still underway to validate ocular biomarkers as the new model for diagnosis of AD in its pre-symptomatic stage. This study has focused on the use of retina for the investigation of amyloid beta stacks. The inspiration for this study came from retinal scanning which uses IR light to access the deeply located retina in the eye. IR light has penetration power up to retina and

in AD, protein accumulation is also reported in retina before any accumulation in the cortical region of brain. Any obstacle placed in the path of light causes the light to reflect, so in this study, it has been hypothesized that protein accumulation in retina can serve as blockage in the path of incident IR light. According to Beer-Lambert law, there is a linear relationship between absorbance and concentration. Hence it is assumed here that more is the accumulation of protein in retina, higher is the absorbance and lower is the reflectance.

CHAPTER 2: LITERATURE REVIEW

PubMed, Science Hub and Google Scholar were used for literature review of this study. Keywords used included AD, amyloid beta accumulation, early diagnosis, ocular biomarkers, retina, IR light, sensor and hyperphosphorylated tau protein. A total of 25 articles were initially downloaded, based on most recent to past 10 years. After thorough reading, 6 of the articles were excluded because they appeared to be beyond the scope of this study. 3 of the articles were not included because they were older than past 10 years. A total of 16 articles out of 25 were finally shortlisted for the literature review.

The findings from review of literature from the above-mentioned articles are mentioned below.

2.1: AD Detection through Deep Learning (DL)

A recent study conducted by Castro et al. was aimed at diagnosing AD through MRIs of the sagittal plane which is not usually used. The purpose was to detect AD automatically without using any further extensive procedures. Transfer learning (TL) technology was employed to improve the accuracy of results. Another reason for using TL was the fact that MRI data sets are very small sized. (Puente-Castro et al., 2020)

The neural network used in this study was ResNet. After extraction of feature vectors, the data sets were divided into two classes namely train set and test set. The train set was used in the development of model and test set measured behavior of data that was not previously seen.

Their study demonstrated that both the data sets measured AD at an early stage when usually the disease is hidden. It is pertinent to mention that diagnosis of disease at such an early stage is hardest because of absence of any clinical manifestations. It can be inferred that targeting AD through sagittal plane can be a promising avenue but requires further testing.

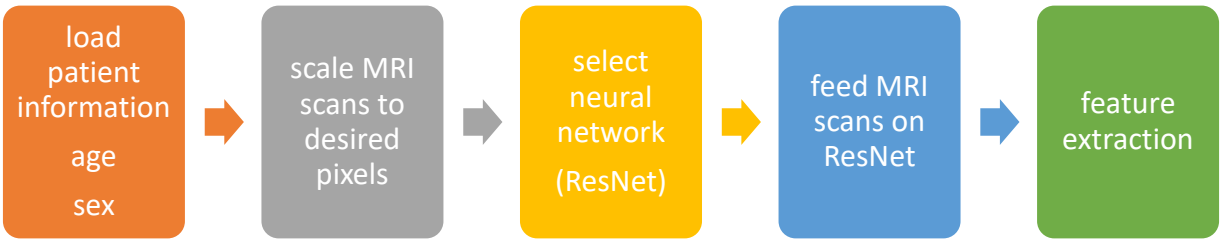

Figure 1: Basic Scheme of AD detection by DL method

2.2: AD Detection through Hyperspectral Imaging

A growing body of research suggests that retinal changes that are experienced by human patients of AD or rodents, co-relate with the changes that occur in brain. It has also been reported that amyloid beta accumulation in retina runs parallel with its accumulation in cortex region of brain. Hadoux et al. conducted first ever study on human subjects to find out the effect of hyperspectral imaging.

The study concluded that retina could serve as non-invasive, free of ligand biomarker for early detection of AD because the hyperspectral imaging successfully demonstrated protein accumulation in patients that had undergone PET scan and that had not undergone PET scan. However, it was found that reflection of light varies not only from patient to patient but differences in reflection were also reported in different regions of retina in the same patient. Considering this finding, it was realized that raw spectra of reflection did not reveal much information or did not discriminate controls from patients. To eliminate the error caused due to raw data, removal of main axes from within group spectral difference was done. This slight change demonstrated noticeable variations in reflection spectra of patients and controls. It was observed that greater differences appeared at shorter wavelengths.

Transgenic mice compared with controls also replicated the same findings hence it was reported that results of hyperspectral imaging of retina may predict accumulation of protein in brain as well.(Hadoux et al., 2019)

2.3: Effect of Light in AD

Pupillary light response (PLR) is thought to be potential biomarker for AD primarily because protein accumulation in AD affects the sympathetic as well as parasympathetic arms of the PLR. Neurodegenerative burden in AD causes changes in locus coeruleus (LC) and Edinger Westphal nucleus (EWN) of the eye. LC is located in pons, and it sympathetically controls size of pupil and PLR. EWN on other hand is involved in control of pupil by parasympathetic means. Keeping these facts in consideration, pupillometry becomes an easy and low-cost biomarker for AD since it ensures detection of PLR not only in AD but in other neurological diseases as well.

PLR is dependent on many variables such as age, alertness, rate of respiration and emotional stress. Due to aging and neurodegeneration PLR is generally reduced. PLR in AD is based on three features namely baseline pupil dilation, constriction phase and re-dilation phase. The effect of AD on pupil diameter has shown varying results and is not consistent in all the studies. This happens due to differences in measuring environments and sample size. Talking about the constriction phase, pupil constriction velocity is the first to change with respect to time followed by the change in acceleration. Studies suggest that this happens due to parasympathetic deficiency in AD. Pupil re-dilation phase has also shown varying results because of multiple factors such as flash offset, re-dilation time and average velocity during dilation.

These studies suggest that PLR alone may not be helpful in timely diagnosis of AD however it could be employed in conjunction with other diagnostic techniques to amplify the outcome.(Chougule et al., 2019)

2.4: Scope of Near Infrared Spectroscopy in AD Diagnosis

A study conducted by Panseiri et al. has reported the intrinsic optical properties of amyloid fibers. It is reported that the amyloid fibers intrinsically display ultraviolet (UV), visible and near infrared (NIR) properties.(Pansieri et al., 2019)

Investigating the formation of amyloid fiber is of prime importance to decipher the cascade of events that result in disease formation at molecular level. Till date, fluorescent markers have been used to observe the process of fiber formation. It is noteworthy that NIR signal is observed only when amyloid fibers are formed. This fact makes the NIR signal a crucial tool in finding the disease and disease stage. It has also been observed that NIR signal is proportional to the concentration of protein. The study concluded that NIR signal can be observed both in-vitro and in-vivo, leading to the direct detection of amyloid fibers without the need of being labelled. It is suggested by authors that in-vivo detection of protein should be further subjected to trials among human subjects. (Pansieri et al., 2019)

Cognitive neuroscience aims at understanding brain and its working and link it to everyday life. With the advent of cognitive diseases in the past few years, novel approaches to deal with the diseases have also emerged. One of the emerging technologies that is progressing at a rapid pace is the use functional near infrared (fNIR). It is a non-invasive neuroimaging technique that measures change in concentration of oxygenated and deoxygenated blood in the brain tissue. In the process, fNIR is shone on patient who wears a specific cap, and the technique takes advantage of transparency of tissues which absorb this light. fNIRs have been reported to indirectly measure cognitive decline in patients with AD and MCI. (Pinti et al., 2018)

Another study conducted by Li et al. confirmed the use of fNIRs based on hemodynamic response in patients with varying levels of cognitive decline. The fronal and bilateral parietal cortices were focused for investigating the disease. The study included digit verbal span task (DVST). The experiment was set up such that patients were seated and relaxed for 3 minutes in order to measure values of baseline fNIRs. This step was followed by DVST which comprised of 30 blocks. Every block initiated with an encoding task and while task, number sequence of varying digits was displayed on individual monitors. The subjects were asked to cram the sequence. Followed by encoding phase, resting phase began in which all participants were allowed to rest after which the

subjects were asked to recall the sequence they had crammed earlier. That is how their cognitive performance was examined.

This study was first ever study to effectively distinguish hemodynamic response patterns in diversified brain regions. The study inferred that significant changes in hemoglobin concentration were observed in all subject groups, hence making their differentiation possible. It was further suggested that although sample size and testing procedure needs to improve yet, this study does indicate positive findings in employing FNIRs as the treatment line for the prediction of disease as well as disease stage. (Li et al., 2018)

2.5: Retinal Imaging in Diagnosis of AD

A recent study conducted by Koronyo et al. attempted to analyse the possibility of detecting AD in its early stage through retinas of live patients. They used flourescent compound, curcumin, to non-invasively assess AD. The study was first of its kind to use live patients as subjects. The results turned out to be encouraging such that they demonstrated a rise in RAI scores in AD patients compared to control group. Furthermore, the histopathological exam confirmed that AD patients had amyloid deposits in various layers of their retinas. The results also showed similarity with previous studies related to amyloid deposition in retina. It was also observed that neuronal loss in retina was also linked to the plaques formation in the region. The most significant highlight of this study was the fact that in AD patients, retinal amyloid deposits were in higher concentration as compared to the control group.(Koronyo et al., 2017)

Researchers	Year	Tests employed	Findings
Koronyo et al.	2017	• Electron microscopy • Congo red staining	Verification of existence of: • retinal amyloid fibrils • proto fibrils Potential existence of: • Amyloid beta oligomers

Table 1: Research findings of Koronyo et al.

2.6: Structure of Eye

Light travels in straight line and any object placed in path of light, causes the light to deflect. In humans and in animals, retina is the last structure to exist in eye, henceforth, all the light ultimately reaches retina. Retina senses the photo signals and transmits them to brain through the optic nerve which converts photo signals to neural signals. This whole cascade of events leads to image formation and enables vision.(Downie et al., 2021)

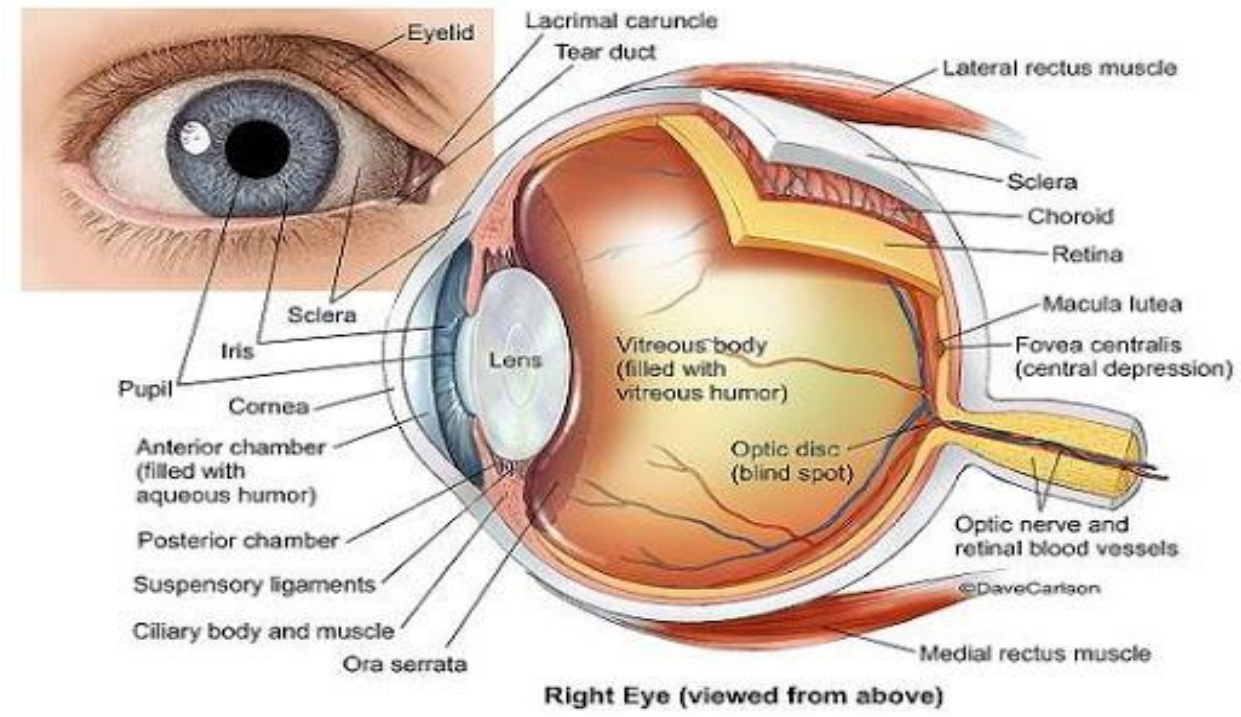

Figure 2: Anatomy of eye

Following table depicts the brief description of internal structure of eye and the respective function of each organelle.

Organelle	**Function**
Cornea	Transparent part that receives light
Aqueous Humor	Watery part that causes refraction of light due to change in medium (medium changes from air to water when light enters eye)
Iris	The part with colored pigments. It absorbs light and the light it reflects is its original color. It is also called eyeball
Pupil	Small aperture that controls the amount of incoming light. In excess of light, it contracts to allow minimum light to enter interior of eye and dilates in limited light to allow maximum light to enter interior of eye to ensure vision.
Ciliary Muscles	It controls the contraction and dilation of iris for near and far vision
Lens	Transparent structure that focuses light on retina.
Vitreous Humor	Jelly like transparent structure to support the structure of eye and keep it intact.
Retina	Light sensitive part containing rods and cones where image is formed.

Optic Nerve	Converts image into electric impulse and sends message to the brain.

Table 2: Functions of internal organelles of eye

2.7: Safety Limit for IR

In considerably higher concentrations, the ultraviolet (UV), infrared (IR) or visible light can cause damage to human eye. Till date, no such data is found that confirms any damage to consumers using light emitting diodes (LEDs). An important feature to consider here is the fact that damage depends on power and time of exposure. To ensure the safety, the International Electrotechnical Commission (IEC) has formulated certain standards. Strictly adhering to these standards poses no threat while using LEDs because all safety requirements are met. (Exposure, 2016)

According to the schematic of human eye, the parts that directly fall in the path of eye might be damaged if the exposure limit exceeds. To control the amount of light reaching retina, the pupil adjusts itself in very bright and very low light conditions. Its diameter varies from approximately 2mm to 7mm depending upon the conditions of light exposure. Literature suggests that human eye is prone to danger by the exposure of UV light and visible blue light of short wavelength but over exposure of NIR may also damage eye, specifically cornea and retina. It is noteworthy that most of the consumer NIR LEDs have not reported to cause any damage to human eye, but certain conditions and mode of operation may cause exposure limits set by IEC to exceed. (Exposure, 2016)

The International Commission on Non-Ionizing Radiation Protection (ICNIRP) has provided duration of exposure limit, that poses no harm. The duration is 0.25 seconds to not more than 10 seconds. (14)

CHAPTER 3: MATERIALS AND METHODS

3.1: Design and Fabrication of Prototype

The prototype for this study is simple in its design and application. The intended purpose is to incident IR light on individual eyes and record the amount of reflected light on sensor. Following list of materials was used to design the prototype:

- IR LED 5*5 mm (940 nm)
- TSL 1401 camera (used as sensor)
- Resistors 220 ohm
- Arduino Nano (made in Italy)
- Battery
- 5V charging module
- Connecting wires (male to male, male to female, female to female)
- Soldering iron
- Glue gun

The prototype consists of two parts, the eye piece and the circuitry. The mechanical design of eye piece as well as circuitry were 3D printed using polylactic acid (PLA). The PLA used was green in color, so the internal side of eye piece was colored black to block the effects of any irrelevant external light. The circuit was designed on fritzing software and the mechanical design of eye piece and circuitry were designed on solidworks software.

3.2: Stepwise Procedure

3.2.1: Circuit Diagram

On fritzing software, schematic was drawn. All the parts were assembled and connected using wires of varying colors. The positive arm of LED was connected with resistor which was connected with digital pin D8. The negative arm was connected to ground. VCC pin of sensor was connected to 5V pin of Arduino, CLK and SI pins were connected to D2 and D3 pins respectively, A0 was connected to A0 and GND was connected with ground of Arduino board. 3.7 V battery was connected for power supply and reset button was connected for obtaining data values.

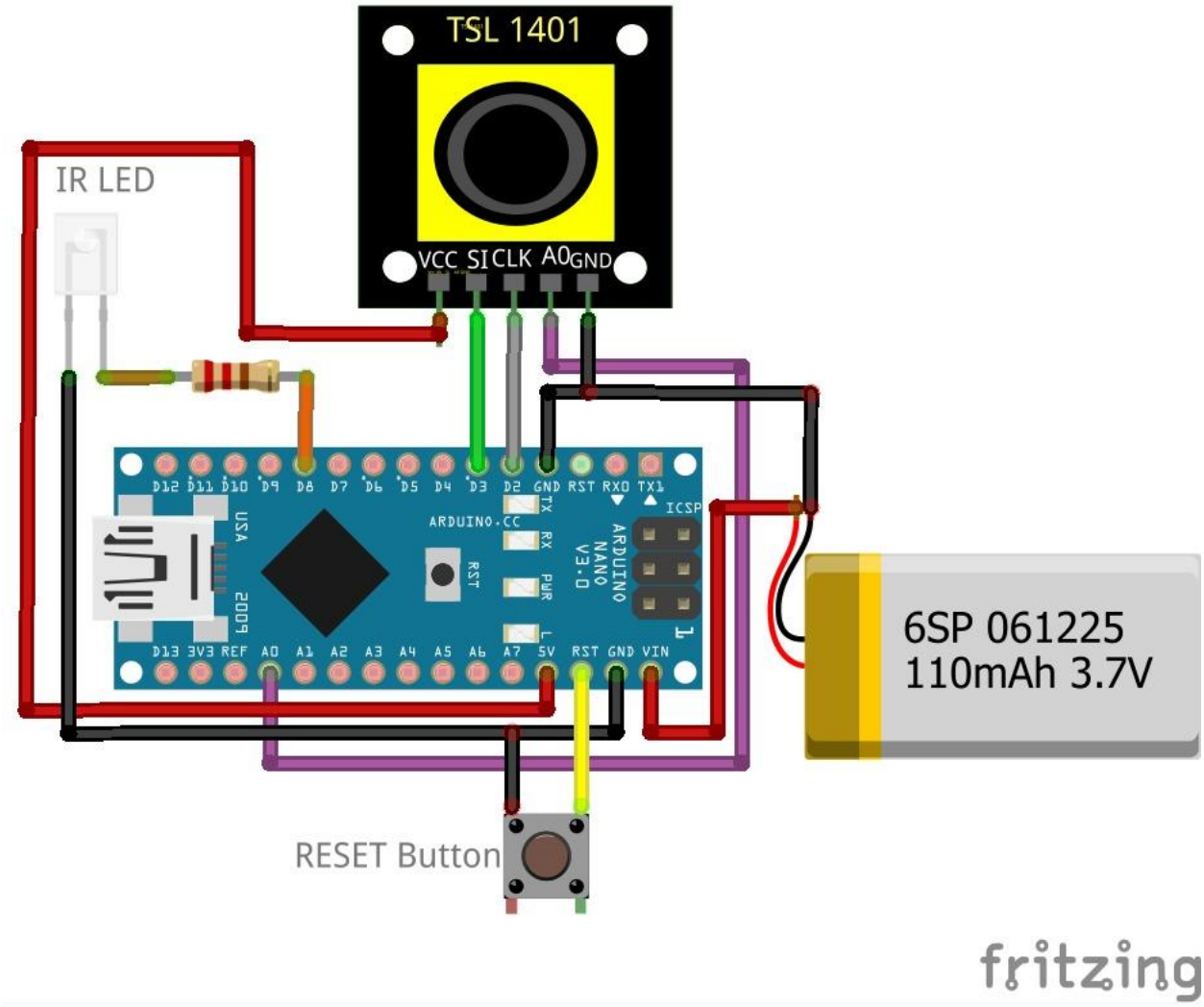

Figure 3: Circuit diagram

3.2.2: Hardware Design

As per schematic, TSL 1401, IR LED and resistor were soldered to Arduino board. Wires were tapped to secure connections.

3.2.3: Hardware Enclosure

The hardware enclosure was prepared on solidworks software, and the diameter of eye piece aperture was in accordance with the diameter of eye, i.e., 24 mm

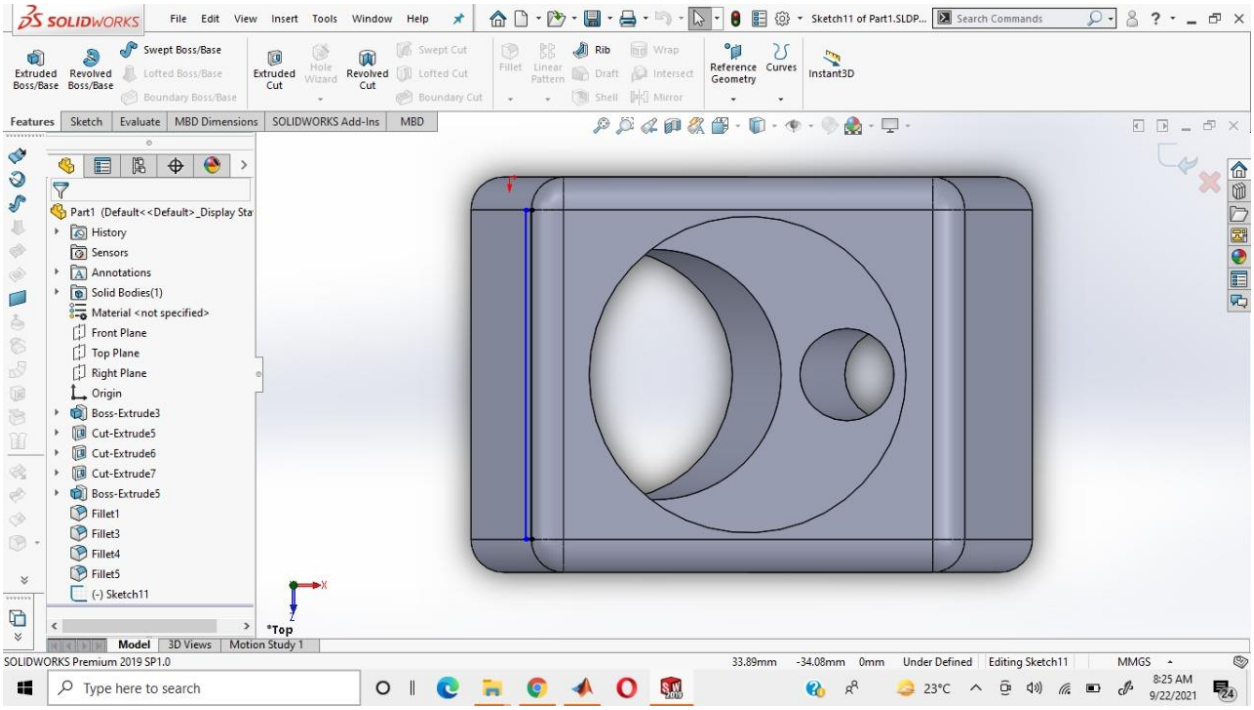

Figure 4: Top view of enclosure

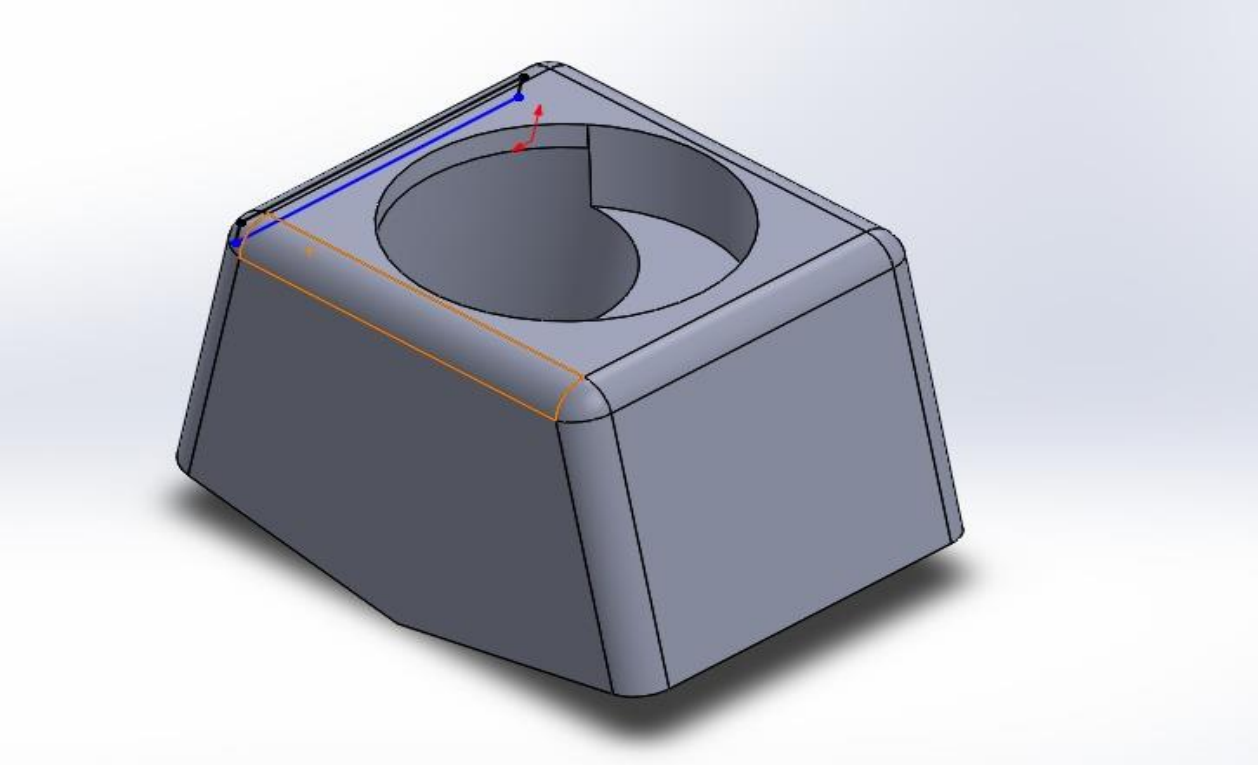

Figure 5: Side view of enclosure

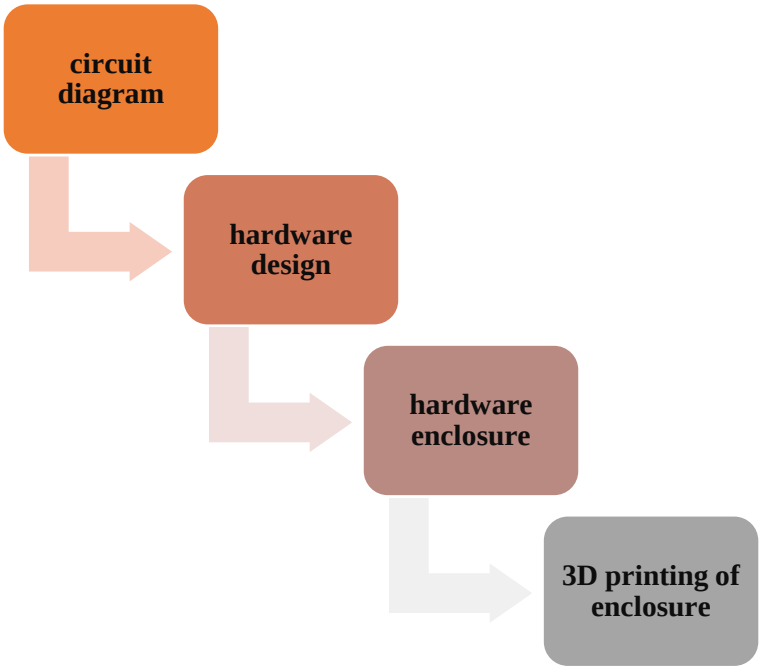

Figure 6: Schematic of procedure

3.3: Calibration of Prototype

The prototype was calibrated using already calibrated 3.2 mm UV silicon photo pin diode whose spectral range was 200-1100 nm. The IR LED was connected with the diode and different objects were first placed in path of LED. The readings were recorded. After reflection from objects was recorded, human subject was asked to hold LED close to eye and readings were recorded. Another IR LED was connected with TSL 1401 camera, and the same procedure was repeated to record readings. Then the same procedure was again repeated with human subject. The difference in values was corrected by adjusting the lens of camera. Lens adjustment was carried out until both sensor readings were in accordance with each other.

3.4: Experiment Methodology

3.4.1: Sampling

Participants were recruited based on convenience sampling. All the participants were briefed about experiment and its outcome. Queries of all sorts were addressed and an inform written consent was signed prior to the initiation of experiment.

3.4.2: Participants Details

10 human subjects of varying age groups were shortlisted for this study based on their consent. Among these 10, 6 were females and 4 were males. None of the subject showed any symptom of cognitive decline.

3.4.3: MMSE

MMSE is an acronym for mini mental state exam. MMSE is a questionnaire-based test which assesses the cognitive abilities of patient. It covers questions from language, calculation, repetition and identification. Its evaluation range spans from 0 to 30. A score below 20 is considered as an indication of cognitive decline or initial problems with memory. (Celik et al., 2021)

3.4.4: Experiment

For the experiment, firstly MMSE was taken from each subject and their scores were calculated and saved. The experiment was arranged in dark room to avoid the effects of any irrelevant external light source. The Arduino was connected to laptop and eye piece was free to be held by the subject. To compute total incident light, the shiny surface of a self-reflecting mirror was placed in front of LED in dark room, and reading was recorded. Then each subject was taken to experiment room,

seated and made comfortable. All sorts of queries were answered and once the subject was fully ready, he was asked to hold eye piece as close to eye and was directed to not close eyes. The reading was recorded when light source and eye were in line, using the Arduino button. Same procedure was adopted for both eyes. It took less than a second to record reading from each eye. It is noteworthy that each value was recorded thrice to ensure reliability and repeatability of results.

CHAPTER 4: RESULTS

4.1: Statistical Analysis

Reflectance is the optical property which predicts the amount of light that reflects from surface when placed in path of light. It ranges between 0 to 1. 0 reflectance value indicates that none of the light is reflected and 1 indicates all the light is reflected by the surface. For the ease of understanding, reflectance is taken as percent reflectance, which is obtained by multiplying reflectance value with 100.

It is calculated by following formula

p(y)= Gr(y)/Gi(y)

where:

p= reflectance

y= wavelength

Gr= reflected radiation

Gi= incident radiation

4.2: Computation of Results

For the computation of results, above mentioned formula of reflectance is used to predict the differences between incident and reflected light among eyes of subjects.

The subjects were divided into three categories based on age groups.

- Group 1: 50+ years
- Group 2: 25+ years
- Group 3: 15+ years

The percent reflectance values that were recorded from 20 eyes ranged from 10.8 to 19.9 %

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Sr. No.	Incident radiation (Gi)	Reflected radiation (Gr)		Reflectance (p) = Gr/Gi		% p = p(100)		MMSE score	Age	Gender
		left eye	right eye	left eye	right eye	left eye	right eye			
1	980	122	195	0.124	0.199	12.4	19.9	25	60	M
2		123	120	0.126	0.122	12.6	12.2	25	56	F
3		120	126	0.122	0.129	12.2	12.9	27	22	M
4		115	121	0.117	0.123	11.7	12.3	29	18	F
5		115	113	0.117	0.115	11.7	11.5	29	27	F
6		106	119	0.108	0.121	10.8	12.1	29	31	M
7		117	121	0.119	0.123	11.9	12.3	25	37	F
8		115	121	0.117	0.123	11.7	12.3	26	63	F
9		120	119	0.122	0.121	12.2	12.1	26	66	M
10		112	116	0.114	0.118	11.4	11.8	24	24	F

Table 3: Values of reflected light

Sr. No	Age Group	Reflectance Range	
		Minimum	Maximum
1	50+ years	11.7 %	19.9 %
2	25+ years	10.8 %	12.3 %
3	15+ years	11.4 %	12. 9 %

Table 4: Comparison of Age Group with Reflectance Values

Sr. No	Age Group	Reflectance Range		MMSE scores
		Minimum	Maximum	
1	50+ years	11.7 %	19.9 %	25-26
2	25+ years	10.8 %	12.3 %	25-29
3	15+ years	11.4 %	12. 9 %	24-29

Table 5: Comparison of age group and reflectance with MMSE scores

Sr. No	Gender	MMSE scores
1	M	25-29
2	F	24-29

Table 6: Comparison of Gender with MMSE scores

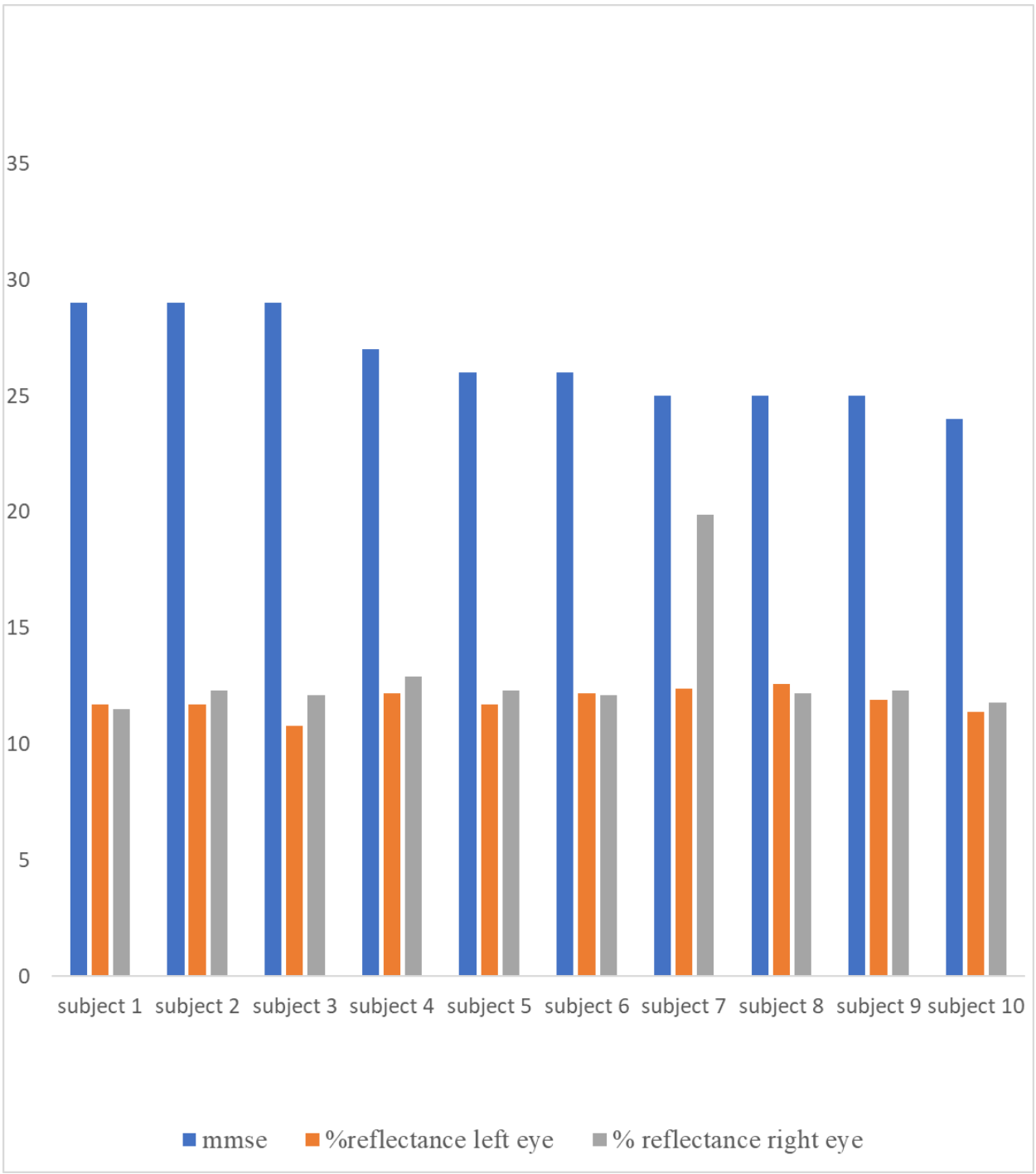


Figure 7: Comparison of MMSE scores with % reflectance. Blue bars indicate MMSE scores of each subject. Orange bars indicate values of % reflectance from left eye of subjects and grey bars indicate values of % reflectance from right eye of subjects. The recorded values do not signify any specific trend, suggesting that the MMSE scores are independent of reflectance of light.

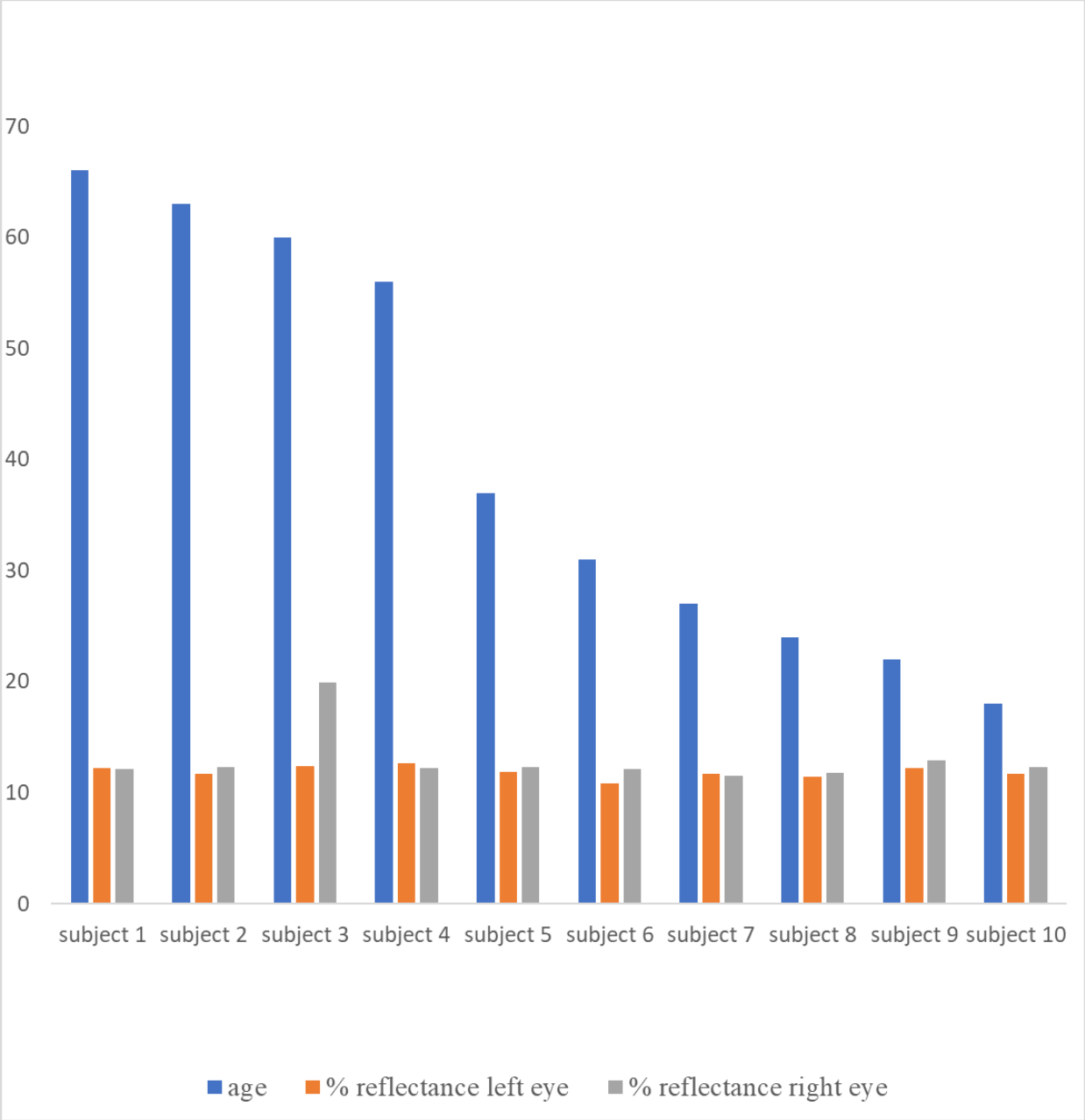


Figure 8: Comparison of Age with % reflectance. Blue bars indicate age of each subject. Orange bars indicate values of % reflectance from left eye of subjects and grey bars indicate values of % reflectance from right eye of subjects. The trend suggests that reflectance tends to remain stable in younger subjects and it varies among subjects of older age group.

CHAPTER 5: DISCUSSION

In the findings of experiment, it was observed that the lowest recorded value, i.e., 10.8 was observed in subject whose MMSE score was exceptional among all others (29/30), yet his percent reflectance value suggested maximum light was being absorbed at the retina.

Contrarily, the highest recorded value, i.e., 19.9 was observed in subject whose MMSE score also did not suggest any cognitive decline. To explain the reason behind this maximum reflection, his eyes were closely monitored, and it was observed that early cataract was being formed in his lens. Since rest of the eyes had no such issue, normal reflection of light was observed in their cases but in this particular case, the layer formation at lens did not allow light to penetrate till retina properly.

It was also observed that the values recorded using prototype did not correspond with MMSE scores and the readings were also independent of age or gender. Observing the trend, it was inferred that reflectance tends to remain stable in young subjects and its range varies in older subjects. Varying range in older subjects could be due to multiple reasons such as the age-related changes that occur at older age, which effect absorption of light. The age-related changes that effect light absorption include cataract, diabetes and cardio-vascular diseases. Thinning of retinal blood vessels is the major reason that effects absorption of light at the retina.

It is noteworthy that this experiment was first of its kind on human subjects and it was conducted to investigate whether low power IR light penetrates up to retina or not. A difference in incident and reflected light could reveal information about the level of penetration. It was observed that if the incident light did not face any obstacle when directed to eye, the penetration was deep till retina. If due to any phenotypic anomaly such as cataract, the reflection of light was obstructed. As per the schematic of experiment, we had light source and sensor both at same ends and data was collected based on reflectance values because there is no such mechanism that can place sensor at the back of alive subject.

However, literature suggests that post-mortem analysis of human eyes can place light source at one end and sensor at the other end to calculate the transmittance of light. Whenever light source encounters any object, there are three possibilities. Some of the light is reflected, some gets absorbed and some of the light gets transmitted. In case of human eye, diffused reflection is observed whenever light hits. Some of the light gets back scattered as well.

In case of our recorded data, the percent reflectance ranges from 10.8 % to 19.9 %. For the ease of understanding, if we round off these values to 11 % to 20 %, the values suggest that the subject who showed lowest reflectance i.e., 11% had maximum absorption at the retina i.e., 91%. On the other hand, the subject with highest reflectance i.e., 20% had minimum absorption at the retina i.e., 80%.

With these stats we can roughly assume that percent absorption values range from 80% to 91%. According to the hypothesis of this study, higher the absorption of light, higher is the a-beta protein accumulation at retina of eye and lower the absorption, lower is the protein accumulation. The subject with 80% absorption had initial signs of cataract so it can be presumed that the light did not penetrate retina deeply. In case of subject with maximum absorption i.e., 91%, his MMSE score was 29 out of 30 which is exceptional and he lied in middle range of age group i.e., 31 years. Although the subject did not show any symptom of cognitive decline and his MMSE score was also good, yet his percent absorption value represents maximum protein accumulation in his retina.

If we take average of the 20 values of percent reflectance, it is observed that 12.4 % is the average reflectance value. 12.4 % reflectance suggests an absorption of 87.6 % in the retina. Rounding off this value to 88%, it is inferred that ranging from 80% to 91%, the average percent absorption among all subjects is 88%. It is presumed that on average, 88% of the light is absorbed by retina among the subjects recruited. The value drops whenever there is obstruction in path of light and the high values could be due to higher protein accumulation.

Three out of ten subjects had eyesight issues as well however neither far sightedness nor short sightedness had any impact on the level of penetration of light. The reflectance values were also observed to be independent of age and gender. The MMSE scores also did not correspond with reflectance values. Reflectance was observed to be affected only by the obstacles in path of light from its source till retina.

It is pertinent to mention that this study was limited to 10 subjects which is not enough to validate the results. The obtained values also do not clearly specify that reflection is due to retinal amyloid proteins. It is also noteworthy that the obtained values of reflectance suggest whether there is accumulation or not. They do not indicate the number of proteins involved in accumulation.

CHAPTER 6: CONCLUSION & RECOMMENDATIONS

AD has become the leading cause of dementia world-wide and if its propagation is not intervened by timely diagnosis or treatment, there can be an irreversible cognitive and neuronal loss. To mitigate the worse effects, researchers around the globe are working on targeting the disease in earliest stages when even the symptoms are not reported.

Our pilot study was also aimed at targeting AD in its pre-clinical stage. The challenge was to select the right light source which not only penetrates dip till retina, but also does not harm eye in any way. Our results are encouraging in a way that they do answer our primary research question, but number of participants is not enough to predict the reliability and accuracy of prototype.

It is suggested that the recruitment of maximum number of participants can assess the potential and reliability of prototype. It is also suggested that with certain design modifications, the prototype can be replicated to evaluate the reflection of light in synthetic or extracted amyloid plaques. To validate the results, mice model can also be used in conjugation.

For the confirmation of reflectance values as correct, the prototype can be re-designed with slight modifications such that it measures reflectance values of synthetic a-beta plaques first and then those values can be matched with the reflectance values of a-beta in subjects.

Appendix

AD: Alzheimer's Disease

APP: Amyloid Precursor Protein

NFT: Neuro Fibrillary Tangles

NIA: National Institute of Aging

MCI: Mild Cognitive Impairment

CSF: Cerebro Spinal Fluid

MRI: Magnetic Resonance Imaging

OCT: Optical Coherence Tomography

PET: Positron Emission Tomography

BAF: Blue Light Autofluorescence

SLO: Scanning Laser Ophthalmoscopy

LC: Locus Coeruleus

EWN: Edinger Westphal Nucleus

PLR: Pupillary Light Reflex

fNIR: functional Near Infrared

NIR: Near Infrared

IR: Infrared

UV: Ultraviolet

LED: Light Emitting Diode

IEC: International Electrotechnical Commission

ICNIRP: The International Commission on Non-Ionizing Radiation Protection

Informed Consent Form for Experimental Participants

Please read the following information carefully. You can also request a copy for further reference.

Experiment

Assessment of amyloid beta stacks as retinal biomarkers for the early detection of Alzheimer's disease

Investigator

Sameen Mehtab

Supervisor

Dr. Saima Zafar

Affiliation

Department of Biomedical Engineering and Sciences, SMME-NUST

Introduction

You are being invited to take part in a research study. Before you decide, it is important for you to understand that why the research is being done and what it will involve. Please take time to read the information carefully and you are encouraged to raise any question you may have in your mind.

Purpose of study

The purpose of this study is to determine whether the exposure of unperceived beam of IR light on eyes, gives same reflectivity values or different. If the values are different, the difference is based on the concentration of certain proteins in retina of eye. Our aim is to target those proteins and verify that the light reaches retina or not.

Time involvement

Your participation will take approximately half hour. You will be asked some questions and the investigator will fill the form on your behalf. This will take 15-20 minutes. Further, you will be asked to hold device near eye with some instructions and the investigator will record readings. This will take less than a minute.

Risks of Participation

The device has been prepared by complying all the international safety standards. It has also been tested on investigator and no current or future risk has been reported. It will only take your 30 minutes.

Benefits of Participation

Subjects will be informed about their risk of developing AD at any later stage of life. Any underlying issue will also be communicated if observed.

Confidentiality

We plan to publish the results, but we will not include any information that would identify you. To keep your information safe, we will

- Assign unique study codes to all study participants that will be used on the notes and documents.
- Not make your data available to any other researcher for other studies following completion of this research study.

Voluntary Withdrawal

Participation in this study is voluntary. It is up to you to decide whether to take part in this study or not. If you decide to take part, kindly sign the consent form. You are free to withdraw your consent and discontinue participation in the study at any time throughout the study without negative consequences to your relationship with the research staff in anyway.

Participant Signatures

I have read this informed consent form carefully and my signature below indicates that I have been instructed accurately about the experiment procedure both verbally and in written form and that, I have read and understood the information and completely answered all questions in this document. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study.

Participant's Signature: _____ Date: **_____**

Signature of Investigator: _____ Subject code: _____

Mini mental state exam (MMSE)

National University of Sciences and Technology, H-12 Islamabad, Pakistan

Subject Particulars:

Serial #: _____

Name: _____

Gender: _____

Age: _____

Contact #: _____

1. ORIENTATION:

Year: _____

Month: _____

Day: _____

Date: _____

Season: _____

Country: _____

City: _____

Area/District: _____

Hospital/ Department: _____

Ward/Floor: _____

2. REGISTRATION:

Repeat these words: bell, jar, fan

*keep in mind

3. ATTENTION AND CALCULATION:

Count backwards from 100 with subtracting 7 each time.

93, 86, 79, 72, 65

4. RECALL:

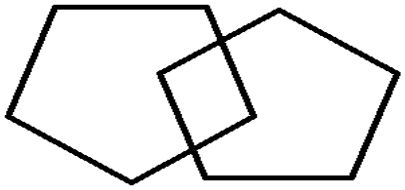
Repeat the words previously mentioned

5. LANGUAGE:

a) Show wristwatch, ask what it is called.
b) Show pencil, ask for correct identification.
c) Ask subject to repeat “no ifs, ands or buts” in correct sequence
d) Give paper with “close your eyes” written on it. Does subject follow instructions or read it out loud.
e) 3 stage command. Ask subject if he is right/left-handed. Give him a piece of paper, ask him to take it in non-dominant hand, fold it in half and place on ground.
f) Ask subject to write a complete sentence on a piece of paper. It should have subject, verb and object in it.

6. COPYING:

Ask subject to copy the design.



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