Determination of 650nm wavelength for rapid detection of sickle cell anemia

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Research

Keywords: Biosensor, Photo-diagnosis, Sickle Cell Anemia

Posted Date: March 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17409/v1

Abstract

Background Sickle cell anemia (SCA), is a type of inherited hemoglobin diseases, It is resulting from point mutation in the β-hemoglobin gene, that resulted from replacement of adenine with thymine, producing a phenotype of sickle hemoglobin (HbS). This paper represents a first try of hemo-photodiagnosis for such disorders using single wavelength, that absorbed by normal blood, so that any change in absorbance value interprets as pathological disorders of hemoglobin.

Methods The proposed photobiosensor was designed according to Beer-Lamberts law. The system was involved diode laser of 650nm wavelength, with 100 mW output power, where the laser beam passed through a cuvette of blood sample, and then transmitted light emite to an optical lens to focus transmitted laser beam in to 2mm in diameter of laser spot size on optical sensor window of laser power meter detector that measure the amount of transmitted light in volts.

Results The readings that displayed on LCD of laser power meter, had been analyzed using compare means values and ANOVA table of SPSS program.

Conclusion The main conclusion that photobiosensor design was able to distinguish between experimental blood groups with respect to hemoglobin pathological conditions.

Introduction

Sickle cell anemia (SCA) is congenital chronic hemolytic disease, characterized by several cellular abnormalities like ; crescent-shape or sickle-shape erythrocyte, excessive blood destruction and active blood formation, are seen in samples of blood smear [1]. There are multiple systemic complications associated with sickle cell disease- which result from reduction in tissue perfections or infarction of small vessels and capillaries – like; vaso-occlusive pain crises, hyposthenuria, acute chest syndrome, central nervous system disease, that could lead to early death [2].

There are several protocols of laboratory techniques, that has been described by many investigations, recently and commonly used as diagnostic method for sickle cell anemia carrier individuals, each one has its advantages and limitations. The most common method, is moist stasis method [3]. According to this method, the sickling course observed by microscope of blood smear taken after reduction of oxygen and carbon dioxide supply, by occluding patients finger for five minutes. Itano and Pauling in 1949 proposed a direct method of diagnosis by reducing time consuming for sickling smear process by adding reducing agent to the blood sample before microscopic examination [1].

Other types of screening techniques that are currently used, as the first-line technique or confirmatory tests, based on many different chemical mechanisms to differentiate sickle hemoglobin, which include; sickle solubility assay that only detects the presence or absence of sickle hemoglobin without differentiate between sub-types of sickle hemoglobin (HbS, sickle cell trait SCT, fetal hemoglobin HbF) [4].

Also, Hemoglobin electrophoresis, isoelectric focusing (IEF), and high-performance liquid chromatography (HPLC), in spite of their highly accuracy, but they lack standardization for further hemoglobin discrimination[5].

Recent trail is aimed to design proposed laser photobiosensor for standardize, qualify and accurate assay of SCD, and differentiate it from normal hemoglobin and iron deficiency anemia, according to the optical absorption characteristics of hemoglobin types (HbS, SCT, HbF) and blood group type.

Materials And Methods

Blood Sample: Thalassemia Center (TC), Blood Bank in Kut, AL-Karama Teaching Hospital /Wasit Health Department/ KUT/Wasit , are the sources of SCD, blood groups, iron deficiency blood samples respectively. The total number of patients in Wasit province - according to SCD statistical documents of patients - are 8, but only 5 agreed to give blood samples for research purpose.

Sample Replicates Groups: the collected blood samples were divided into 11 groups labeled as following;

A+NO: Normal blood group A+.

A+ SC: Sickle cell erythrocyte after blood transfusion group A+

A+ ANI: Iron deficiency anemia group A+

B+ NO: Normal blood group B+

B+SC1:Sickle cell erythrocyte after blood transfusion for paitient no1, group B+.

B+ SC2: Sickle cell erythrocyte after blood transfusion for paitient no2, group B+.

B+ SC3: Sickle cell erythrocyte after blood transfusion for paitient no3, group B+.

B+ ANI: Iron deficiency anemia group B+.

O+NO: Normal blood group O+

O+SC: Sickle cell erythrocyte after blood transfusion group O+.

O+ANI: Iron deficiency anemia group O+.

The range of recipient age was between 16 -35years.Each blood samples were diluted in distilled water (1:100) with 5 replicates, before testing .

Photobiosensor design: The proposed biosensor consist of :diode laser (JD-R303,HUONJE TM/ China), with 650nm wavelength, 100mW max output power, convex lens, laser power meter LP1(SANWA),which set at 40mW, the readings that represent value of output power of transmitted laser irradiation from

diluted blood sample, the set-up design is analogous to the set up explained by [6], with omitting polarizer and adding confocal lens (focal length 6.5cm) before photodiode probe (figure 1).

Results And Discussion

In this research, SPSS statistical analysis program was used to analyze the data readings (output transmitted power) that appear on LCD display screen of detector. One-way ANOVA table was applied for mean transmittance value comparisons, with confidence value 95%. Indeed, it was needed to demonstrate the optical discriminatory ability (power of optical resolution) of proposed biosensor to distinguish between SCA blood samples of different blood group types and different cases of anemia.

ANOVA tables showed that the mean comparisons of data readings between groups of SCA has statistical significance, but the transmittance value of B+SC2 and B+SC3 has no significant difference (sig. =.279 0.05), that it means the proposed biosensor cannot distinguish significantly between B+SC2 and B+SC3, in figure2 the plot show clearly the result of convergence in mean value of B+SC2 and B+SC3. At the same time, the biosensor differentiates significantly between B+SC1 and B+SC2 , B+SC3. Honestly, the sample B+SC1left at room temperature for 12h and hemolysis occur, for the purpose of determination of biosensor ability to distinguish between blood groups of haemolized and intact RBC. As a results, the mean values of transmitted voltage from haemolized B+SC1 samples, that measured at power meter, was the least.

To give logical explanation of why wide reading intervals among B+SC1, B+SC2 and B+SC3 groups and between B+SC2 and B+SC3, there were several scientific interpretations that cause such fluctuations in readings of power meter, beside the blood hemolysis leads to release iron out of RBC, which increase the absorbance of B+SC1. On the other side, for comparisons between B+SC2 and B+SC3, it depends on whether B+SC heterozygotes AS hemoglobin or homozygotes SS resulting in sickle cell trait SCT (50% normal Hb and 50% Sickle Hb) and SCA phenotype respectively. The documental archive of patients did not contain such information. Generally, it was known that the absorption coefficient of blood hemoglobin is constant at certain laser wavelength and chemical structure of hemoglobin [7] ;therefore instability of sickle hemoglobin (HbS) of certain type of SCD, leads to precipitate of iron groups on membrane of erythrocyte in the form of hemichromes [8], that cause decomposition of phospholipid bilayer of red blood corpuscles of B+SC1 homozygote-as we assumed. Also there was a trail concluded that heme binding to a primary binding site of plasma membrane resulting in a noticeable large bathochromic shift, up to 38 nm[9], that quit enough to shift absorption coefficient in form of decrease transmittance power displayed by biosensor.

At that approach, we examine the ability of biosensor to distinguish between normal Hb, Hbs and iron-deficiency anemia sample, the resultant readings shows a significant deference between variables at the same blood groups (figures 3 a , b and c).

Figure3 proves that there is no fixed rule in determining the range of readings of normal blood groups compared with other pathological groups. The hypothesis is that the amount of transmitted 650nm

wavelength depends on dissociated iron concentrations in membrane of erythrocyte (fig 3 a and b) [8,9].

However, fig3c represent the inverse relationship between the transmitted light and amount of dissociated hem group residues.

From the overview of fig. 3 it is found that the emitted power of the laser beam at wavelength of 650nm for iron deficiency anemia, are the less readings than normal blood samples, meaning iron deficiency anemia absorbs 650 nm more than normal blood, and this result makes sense to emphasize that there are a reciprocal relationship between absorbance and hem-group concentration of blood -regardless of blood type- according to research samples.

On the other hand, the emitted power values of sickling samples seem fluctuated compared with normal and iron deficiency anemia samples (fig. 3), where transmittance values range from the highest value for O type (fig. 3b), and the least than NO and above than ANI for A and B blood types, with exception of B+SC1 case. This finding improve that the a mount of absorbed irradiation with certain wavelength affected with presence or absence of A,B antigen -for B and A blood type (fig. 3a and c), and O blood type (fig. 3b) respectively- as well as severity of sickling disease (heterozygote SCT or homozygote HbS) which confirmed in fig. 2.

Conclusion

Dissociation of heme group from globin and rearrange with protein plasma membrane of erythrocyte, lead to decrease absorption of 650nm wavelength, with each involved blood group were read.

Recommendation

It is needing to study the relation between blood group type (ABO, Rh system) with different content and types of hemoglobin, with sickle cell, iron deficiency anemia and the normal hemoglobin.

Abbreviations

SCA
Sickle cell anemia.
HbS
Sickle hemoglobin.
LCD
Liquid crystal display.
ANOVA
Analysis of variance.
SPSS
Statistical package for the social sciences.
SCT

Sickle cell trait.
HbF
Fetal hemoglobin.
HPLC
High-performance liquid chromatography.
TC
Thalassemia Center.

Declarations

Ethics approval and consent to participate: This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication: the manuscript does not contain individual information of donors.

Availability of data and material: The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Competing Interests: The authors declare that they have no competing interests

Authors' contributions: The two authors contributed to the study conception and design. Material preparation, data collection and analysis, as well as first draft of the manuscript was written by correspondence author. Second author read and approved the final manuscript.

Role of funding source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement: We would like to extend our sincere thanks to the health personnel in Thalassemia Center, Blood Bank in Kut and AL-Karama Teaching Hospital , in Kut city, for their cooperation in providind blood samples for healthy and sick people for the purpose of carrying out this research.

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Figures

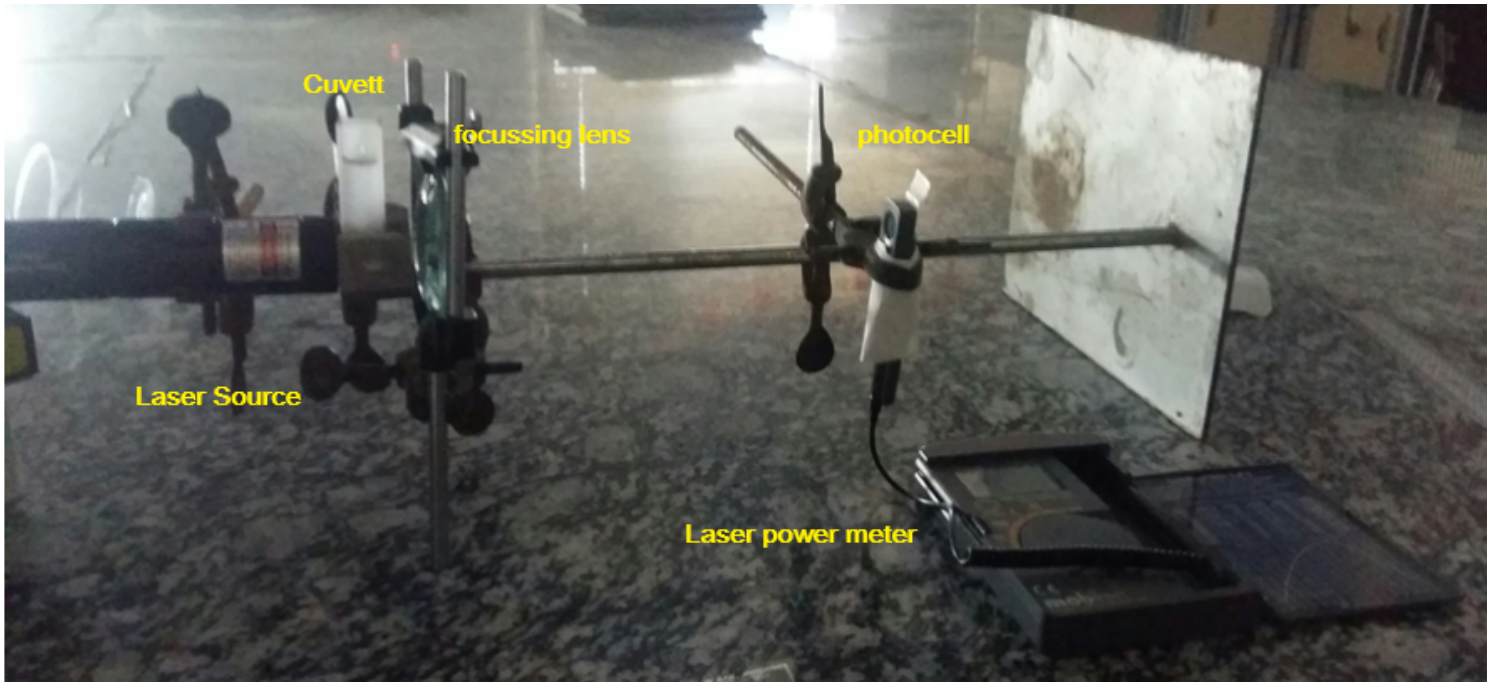

Figure 1

Photobiosensor arrangement

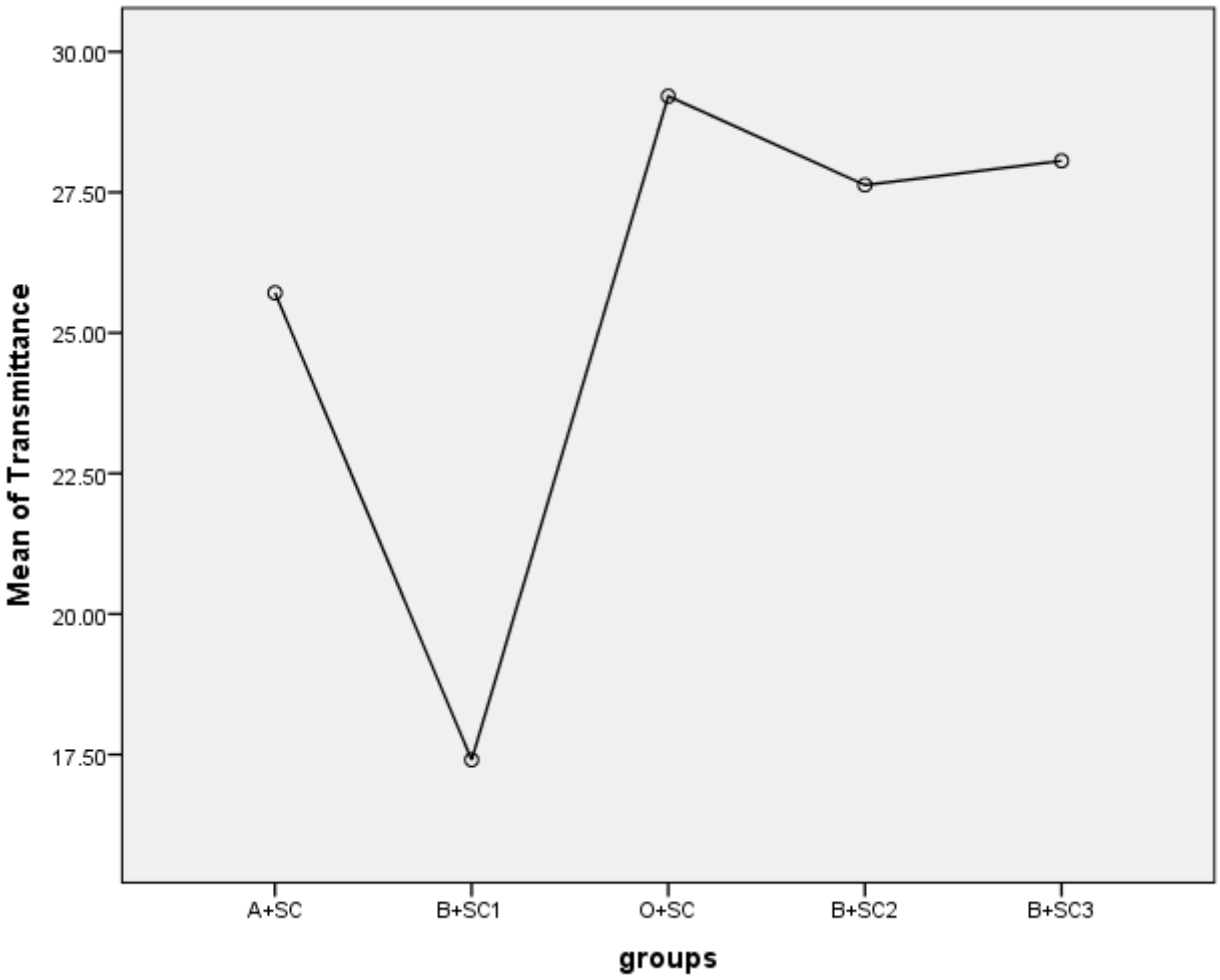

Figure 2

A comparison between transmittance mean values (in mW power) of SCD blood samples

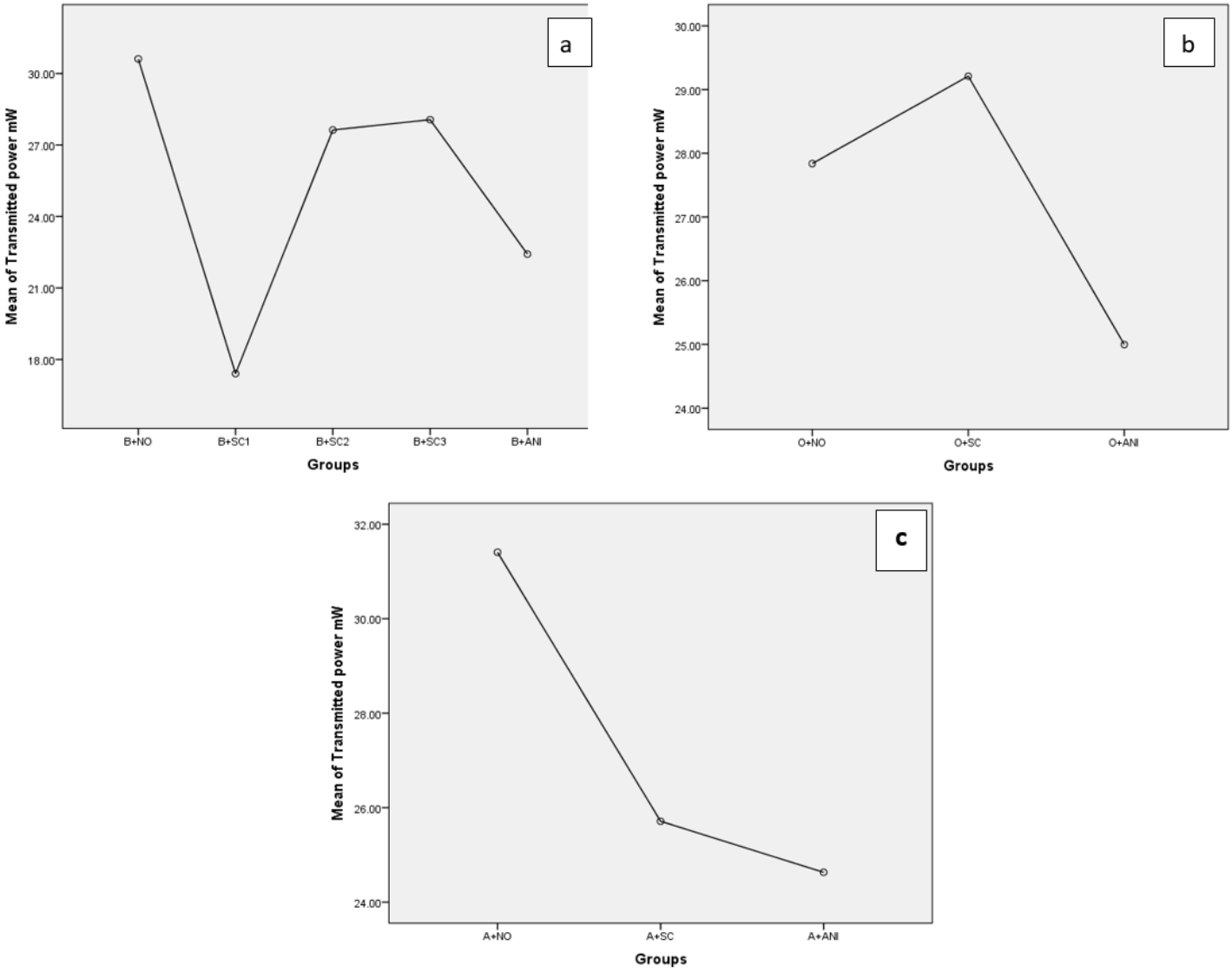

Figure 3

a. comparison plot between transmitted power(mW) for B+ groups, b. comparison plot between transmitted power (mW) for O+ groups, c. comparison plot between transmitted power (mW) for A+ groups.