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Original Article
Endothelial Dysfunction Profile in Patients of Sickle Cell Anemia

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ABSTRACT

Sickle cell disease (SCD) is monogenic disorder of haemoglobin due to substitution single nucleotide change (GAG → GTG) in the 6th codon of exon 1 of the B-globin chain leads to replacement of valine for glutamic acid at 6th position of the beta globin chain of hemoglobin causes the formation of Sickle hemoglobin (HbS). Primary pathophysiology is b polymerization of deoxy HbS with formation of long fiber like structure called tactoid[1] within the RBCs causing a distorted sickle shape which eventually leads to increased haemolysis and vaso-occlusion of sickled red cells[2,3]. Acute exacerbations of sickling called vaso-occlusive crises (VOC) leads to adverse events such as acute pain, acute chest syndrome, multi-organ dysfunction, stroke, renal dysfunction & pulmonary dysfunctions[4].Factors that may trigger vaso-occlusion are red cell dehydration, abnormal adhesion of RBCs to the vascular endothelium, inflammatory events5. Endothelial dysfunction & blood hyper-viscosity impairs the local blood flow by vasoconstriction, which leads to decrease in local perfusion and increase in transit time of sickle RBCs[6,7,8].

1. Introduction

Deoxygenated hemoglobin could remain longer in the microcirculation where the hemoglobin is more likely to polymerize hence enhancing the risk for VOC. Role of Endothelial Dysfunction and noxious microenvironment due to pro-inflammatory and pro-oxidative[9] factors in pathophysiology of VOC is not well addressed which is the major cause of morbidity & mortality [10] in these cases. This study will provide deep insight about VOC and will help to predict and prevent painful crisis in patients of SCD. If VOC can be predicted in such patients, proper clinical management can be introduced, leading to reduced risk of VOC. The purpose of this study is to establish the role of endothelial dysfunction in pathophysiology of vaso-occlusive crises (VOC) with induced vasoconstriction which may play role in triggering crisises as well as the mechanism of endothelial dysfunction in SCD. The role of microvascular changes and the noxious environment in the pathophysiology have been acknowledged, but mainly the factors such as vascular spasms and the coagulation system have been substantiated with any backing research for their involvement in vaso-occlusive crises [11]. The use of Photoplethismography(PPG)[12] is still relatively rare in this scenario, as it is one of the new techniques. PPG is quite a sensitive measure, as it depends only on the effects seen in the microvasculature, which might reflect even small disturbances. This technique has the potential to detect Sickle cell anemia in a non-invasive[16,17] manner and can help in predicting the course of vaso-occlusive crises in patients, thus making their treatment more streamlined and improving their quality of life.

METHODOLOGY:

To accomplish this aim the specific objectives are:

1. To assess Endothelial Function by reactive hyperemia in adult patients of sickle cell anemia.

2. To quantify & compare the vascular reactivity to reactive hyperemia by using parameters of PPG in healthy subjects & SCA patients.

This was a Cross sectional study with sample size (n) 30.15 Young Adult patients of age group 18 to 40 yrs. diagnosed with SCD from Sickle cell OPD of AIIMS Raipur and 15 healthy age-matched controls were recruited after taking their informed consent between perodod of Nov 2018 to October 2019. Patients having History of Seizures & other Psychiatric problems, Bed ridden Patients and History of Diabetes & Hypertension or any other cardiovascular disorder were excluded from study.

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Experimental Protocol:

On experimental day, each patient was familiarized with the experimental design and procedure of Reactive hyperemia to assess endothelial function by Photoplethysmography (PPG). The subjects were asked to restrain from tea & caffeine intake for 12 h before the experimental sessions & were asked to come to the laboratory after 2 h of light meal. All experiments were conducted at an ambient room temperature of 25° to 27°C with minimal external disturbances at Autonomic & Vascular testing clinic, AIIMS Raipur.

Experimental Measurements:

Each subject's Heart rate was continuously monitored using a lead II electrocardiogram (ECG). ECG and PPG from right middle finger were recorded on 8-channel digital physiograph (Labchart, ADI, Australia) at Autonomic & Vascular Clinic AIIMS Raipur.

Photoplethysmography (PPG) [12] is a simple and low-cost optical technique that can be used to detect blood volume changes in the microvascular bed of tissue. The PPG waveform comprises a pulsatile ('AC') physiological waveform attributed to cardiac synchronous changes in the blood volume with each heart beat, and is superimposed on a slowly varying ('DC') baseline with various lower frequency components attributed to respiration, sympathetic nervous system activity and thermoregulation.

Parameter extraction of PPG:

a) PP interval

b) Amplitude

c) Pulse transit time

PPG from right middle finger were acquired under supine resting condition for 5 min as a baseline measurement followed by procedure of reactive hyperemia.

Reactive Hyperaemia [15], for assessment Endothelial Function, BP cuff was tied on right forearm. After baseline recording of PPG for 5 mins, pressure in the cuff was raised to 200mm Hg (suprasystolic pressure) & kept for 3 minutes (or till patients tolerate). After that pressure was released and recovery recording was done for further 5 minutes.

Calculation of RH:

1. Pulse Transit Time
2. Amplitude.

Data analysis:

From the baseline (resting) recordings we analyzed 5min steady state records as control data. PPG and ECG were used to obtain beat-to-beat changes of systolic peak amplitude, height of the systolic peak point from the foot; slope, maximum of the first derivative of upstroke PPG pulse; systolic peak to peak interval (PPI), the time difference between the successive systolic peak instances of PPG. In addition, the beat-to-beat changes of PTT were computed as the time delay between R wave peak of ECG and onset of corresponding PPG pulse. After determining the distribution of data appropriate statistical analysis were done by using SPSS 20 software.

Table 1: Demographic Profile and Basal parameters of SCD Patients & Healthy Controls

Baseline Parameters	Patients(n=15)	Controls (n=15)	p value
Age (yrs)	29.4 ± 6.9	27.8 ± 6.01	NS
Height (cm)	162.6 ± 7.8	164.9 ± 8.05	NS
Weight (Kg)	53.3 ± 9.05	60.9 ± 11.51	NS
BMI	20.19 ± 3.2	22.42 ± 4.3	NS
Systolic blood pressure (mmHg)	122 ± 10.3	115 ± 5.2	NS
Diastolic blood pressure (mmHg	77 ± 9.7	76 ± 5.2	NS
Heart rate (per minute)	80.6 ± 18.2	70.4 ± 17.5	NS

Values shown are mean ± S.D., * = p<0.05

There was no significant demographic parameter difference between Sickle Cell patient group and healthy controls.

Table 2: Basal analysis of PPG parameters of SCD Patients & Healthy Controls

Parameters	Patients (n = 15)	Controls (n = 15)	p value
Amplitude (V)	0.12 (0.07-0.2)	0.15 (0.09-0.19)	N.S.
Pulse Transit Time(ms)	0.24 (0.23-0.29)	0.27 (0.23-0.37)	N.S.
UpstrokeSlope (V/s)	1.2 (0.78-3.0)	1.1 (0.68-2.26)	N.S.
Pulse Timing(ms)	66.8 (62.2-72.5)	66.9 (59.2-108)	N.S.

[Values are expressed as median (1 quartile – 3 quartile, * = p<0.05). Abbreviations: ms: milliseconds; V: volts; V/s: Volts/second]

There was no significant parameter difference between Sickle Cell patient group and healthy controls.

Table 3: Comparison of PPG values of Patients before and after RH

Patient Parameters (n=15)	Pre RH	Post RH	p value
Peak Amplitude (V)	0.05 ± 0.02	0.04 ± 0.01	0.08
Pulse Transit Time (ms	0.48 ± 0.05	0.39 ±0.03	0.51
UpstrokeSlope (V/s)	0.98 ± 0.39	0.84 ±0.19	0.15
Peak to peak interva (ms)	0.78 ± 0.09	0.39 ± 0.03	0.7

Values shown are mean ± S.D., * = p<0.05

[Abbreviations: ms: milliseconds; V: volts; V/s: Volts/second]

There was no significant parameter difference between Sickle Cell patient PPG parameters before and after RH

Table 4: Comparison of PPG parameters of a Healthy Control before and after RH

Control Parameters(n=15)	Pre RH	Post RH	p value
Peak Amplitude (V)	0.06 ± 0.01	0.09 ± 0.02	0.005*
Pulse Transit Time (ms	0.69 ± 0.08	0.82 ± 0.06	0.007*
UpstrokeSlope (V/s)	0.98 ±0.38	0.64 ± 0.03	0.001*
Peak to peak interva (ms)	0.38 ±0.05	0.79 ± 0.02	0.001*

Values shown are mean ± S.D., * = p<0.05

[Abbreviations: ms: milliseconds; V: volts; V/s: Volts/second]

There was a significant change in parameters of PPG in the control group before and after RH.

Discussion

Reactive Hyperemia (RH) is the commonly used non-invasive technique, which is used to study the physiological function of Endothelial function (EF).The transient increase in the blood flow and the resultant increase in the shear stress on the vascular endothelium, followed by a period of ischemia, due to arterial occlusion (AO), cause endothelium dependent vasodilation. During the period of occlusion, tissue hypoxia and vasodilatory metabolites dilate the arterioles and decrease vascular resistance. When the perfusion pressure is restored, flow is elevated because of the reduced vascular resistance.

In the present study, there is no significant difference in the baseline, demographic parameters in the Control and Patient Groups. Their baseline PPG parameters are comparable, suggestive of normal resting functions of cardiovascular system. For understanding how the vascular functions respond in both the groups, the variability of PPG for the monitoring of vasodilation during RH has been attempted in the present study. The maximal vasodilation and reduced vascular resistance, produced by the accumulation of metabolites post-arterial occlusion, was manifested by the significant increase in the amplitude in the control group, than that in the patient group, suggestive of an effective metabolic response. Similarly, arterial stiffness depends on structural elements within the arterial wall, distending pressure and vascular smooth muscle tone. Endothelial NO causes the relaxation of vascular smooth muscle and the resultant decrease in vascular tone during RH. Pulse transit time(PTT) is inversely proportional to the tone of vasculature, loss of tone during AO was evident by the significantly longer PTT observed controls, as compared to patients immediately after the release of occlusion, suggestive of intact endothelial function due to release of Endothelial NOS. The present study is in accord with Itzhaki et al. [16], Maltz and Budinger [17] that peripheral arterial tone measured by PTT can also be considered as a noninvasive method of assessing Endothelial Function. Endothelial function and can be used clinically to assess the risk of vaso-occlusive crisis happening in sickle cell patients, thus drastically improving their quality of life.

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