Vascular endothelial dysfunction in sickle cell disease by brachial artery flow mediated dilatation

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ABSTRACT

Objective: The present study was conducted aiming to assess endothelial function in sickle cell disease (SS), sickle cell trait (SA) and compare to endothelial dysfunction between sickle cell anemia (SS), sickle cell trait (SA) cases and control (AA) patients to evaluate correlation of endothelial dysfunction. Methods: The study population comprised of, total 25 cases having sickle cell disease and sickle cell trait and 25 age and sex matched normal control. Endothelial dysfunction as assessed by brachial artery flow mediated dilatation by colour Doppler (non-invasive method) by using Siemens Sonoline 500. Statistical analysis was performed using Software Statistical Package for Social Sciences (SPSS) version 20, and P value of less than 0.05 was considered as statistically significant at 95% confidence intervals. Results: Significant difference were observed in FMD (flow mediated vasodilatation) in case and control group (p<0.05), also significant difference was demonstrated between AS and SS group. Conclusion: The percentage of flow mediated dilatation of vessel is a marker of endothelial function was significantly lower in cases as compared to controls and was also lower in AS & SS when compared to control group & significantly lower in SS group than AS group.

Key words: Sickle cell disease, Sickle cell trait, Flow Mediated Vasodilatation

INTRODUCTION

Endothelium once believed to be an inert edge between artery and blood, is now recognized as organ per se. Vascular endothelium performs an array of homeostatic functions within normal blood vessels and endothelium cells plays a critical role through endothelium derived relaxing factor (EDRF), which was identified as nitric oxide (NO) by Flavahan NA (1992). Vaso-occlusive crisis is a characteristic manifestation of sickle cell disease (SCD). According to Lonsdorfer J (1983), the interactions between sickle red blood cells (SSRBCs) and the endothelium may contribute to the pathogenesis of vaso-oclusive crisis. Sickle erythrocytes may occlude microvessels directly by adhering or indirectly by altering endothelial functions such as endothelium-dependent vasodilation. Thus, enhanced interactions between SSRBCs and the vascular endothelium on one hand and abnormal vasomotor tone regulation on the other may contribute synergistically to the occurrence of vessel in sickle cell disease. In vivo studies have identified specific hemodynamic conditions in the microcirculation of patients with SCD. During the intervals between crises, there is an increase in peripheral blood flow with a periodic microcirculatory flow pattern, which may compensate for the alterations in red blood cell rheology. Arteriolar diameter is increased during vaso-occlusive crisis as compared with steady state phases of the disease. Thus, the relationship between endothelial regulation of vascular tone and red blood cell rheology seems to play a key role in SCD. Sickle cell disease is a autosomal recessive disorder of structural haemoglobinopathy involving substitution of thiamine instead of glutamine in the 6th position of β-chain. Average incidence of sickle cell disease is approximately 4.3% in India (Kar BC et al 1987). Endothelial dysfunction in sickle cell disease has been demonstrated by various workers by biochemical and physiological methods.

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MATERIALS AND METHODS

The present study was conducted in department of medicine and radio diagnosis L N Medical College and Research Center Bhopal Madhya Pradesh India. The study population comprised of, total 25 cases having sickle cell disease and sickle cell trait and 25 age and sex matched normal control.

Inclusion criteria
For cases
Cases included sickle cell disease (HbSS) and sickle cell trait (HbAS) both during crisis and steady phase. Twenty-five and sex matched controls were also included accordingly in sickle cell anaemia and trait groups. The consent taken before study from all participants.

For control
Controls included subjects those who have no evidence of any disease clinically and belonging to the same age and sex group as the patients.

Exclusion criteria
For case includes the history of blood transfusion during last 3 months, History of treatment with vasodilators, hydroxyurea, History of smoking, History of Diabetes Mellitus, History of hypertension, Congestive cardiac failure, Renal insufficiency, Ischemic heart disease, Dyslipidemia, treatment with vasodilators, anti-inflammatory drugs, or hydroxyurea; and concomitant systemic disease.

Flow mediated vasodilatation (FMD)
Endothelial dysfunction as assessed by brachial artery flow mediated dilatation by colour Doppler (non-invasive method) by using Siemens Sonoline 500. The two reading were opted as follows, First – After the rest of 10 minutes, Second – After the forearm compression by pneumatic tourniquet at 200 mmHg for five(5) minutes. The diameter of brachial artery were measured one and half minutes after the release of pressure. Scanned area was marked to measure the same segment of brachial artery repeatedly i.e. 1st at rest, 2nd after reactive hyperemia.\(^\text{1-3}\)

Calculation of FMD% (flow mediated dilatation)
Brachial Artery diameter after hyperaemia – Brachial Artery diameter at rest \times100.

Table 1: Age & Sex distribution of the study group

<table>
<thead>
<tr>
<th>Age Group (year)</th>
<th>Sickle cell trait (AS)</th>
<th>Sickle cell disease (SS)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (%)</td>
<td>F (%)</td>
<td>T (%)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>1 (14.3)</td>
<td>2 (40)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>20-29</td>
<td>5 (71.4)</td>
<td>1 (20)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>30-39</td>
<td>1 (14.3)</td>
<td>2 (40)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>24.00±7.32</td>
<td>23.80±8.25</td>
<td>23.92±7.35</td>
</tr>
</tbody>
</table>

Statistical analysis

Statistical analysis was performed using Software Statistical Package for Social Sciences (SPSS) version 20, T test, chi-square test, and Chi-square trend for linear association were performed. P value of less than 0.05 was considered as statistically significant at 95% confidence intervals.

RESULTS

In control group majority of males (82.67%) and females (75%) were found in age < 30 yrs. The mean age of male and female were 24.41 (± 6.59) yrs and 25.00 (± 7.56) yrs respectively (Table 1). The difference in mean age was statistically insignificant (p > 0.05) (Table 3). In sickle cell trait group majority of males (85.7%) and females (60%) were found in age < 30 yrs. The mean age of males and females were 24.00 (± 7.32) yrs and 23.80 (± 8.25) yrs respectively (Table 1). The difference in mean age was statistically insignificant (p > 0.05). In sickle cell disease group majority of males (75%) and females (80%) were found in age < 30 yrs (Table 3). The mean age of male and female were 23.63 (± 6.47) yrs and 21.80 (± 6.87) yrs respectively (Table 1). The difference in mean age was statistically insignificant (p > 0.05) (Table 3). In AS group majority of males (85.7%) and females (60%) were found in age < 30 yrs. The mean age of males and females were 24.00 (± 7.32) yrs and 23.80 (± 8.25) yrs respectively (Table 1). The difference in mean age was statistically insignificant (p > 0.05). In SS group majority of males (75%) and females (80%) were found in age < 30 yrs. The mean age of male and female were 23.63 (± 6.47) yrs and 21.80 (± 6.87) yrs respectively. The difference in mean age was statistically insignificant (p > 0.05) (Table 3).

As compared to control group significant difference was observed in mean FMD (%) in both trait & disease group. (p<0.05), also significant difference was demonstrated between AS and SS group. In all age groups significant difference was observed in cases as compared to control group (p<0.05) (Table 2). Significance difference was observed between male in case and control groups (p<0.0001). Similar findings were observed between females (p<0.0001 as the male by FMD in both case and control group (Table 4).
Endothelial cell dysfunction was significantly more in sickle cell anemia as compared to controls (p < 0.05). They found that FMD% was significantly lower in cases as compared to controls. This suggests a potential mechanism underlying reduction in EDRF/NO activity.

**DISCUSSION**

Study included total 50 subjects equally divided between cases and controls. Cases included sickle cell disease (SS) and sickle cell trait (AS). Twenty-five age and sex matched controls were also included among those who had no evidence of any disease clinically and belonging to the same age and sex group as the patients. Twenty-five sickle cell anaemia cases were studied and divided into 2 groups – sickle cell disease (HbSS) & sickle cell trait (HbAS) which included 13 and 12 patients respectively. In the present mean flow mediated vasodilatation (FMD)% that is mean % increased in the luminal diameter of brachial artery after stress (i.e., reactive hyperaemia), was significantly reduced in sickle cell anaemia cases (8.08±2.73) as compared to control (15.39±3.04), p<0.001. Compared to control group (15.39 ± 3.04) significant difference was observed in mean FMD% in both sickle cell trait (9.72 ± 1.97) and sickle cell homozygous (SS) group (6.56 ± 2.50) p < 0.05. FMD% was significantly lower in patients with steady phase as compared to control group (8.42±0.99 v/s 15.39±3.04 in SS group and 11.42±1.48 v/s 15.39±3.04 in AS group. These findings corroborate with the works of Zawar SD et al (2002-03). They found that FMD% was significantly lower in cases as compared to controls (p < 0.05). Results of the present study consistent with the work of Zawar SD et al. They found that endothelial dysfunction was significantly more in sickle cell anemia as compared to sickle cell trait cases. In all age groups significant reduction in mean FMD% were observed in cases as compared to control group, according to Blum (2005). Flow mediated dilation (FMD)% was 4.57+/- 4.11 at steady state, compared with the control group FMD of 11.64+/- 7.69% (p<0.001), compared with control group FID of 24.17+/- 11.87% (p<0.001) & findings shoed reduction in FMD. A Aessopos (2007) & Rambaran & B Jhang (2007) significant reduction in FMD in disease compare to control. Significant reduction in mean FMD% was observed between male in case and control. When compared to male and female in both case and control group no significant difference were observed. The conclusion drawn from the present study are as follows the percentage of flow mediated dilatation of vessel is a marker of endothelial function was significantly lower in cases as compared to controls and was also lower in AS & SS when compared to control group & significantly lower in SS group than AS group.

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**REFERENCES**