Ocular manifestations of sickle cell disease and genetic susceptibility for refractive errors

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Abstract:
PURPOSE: Sickle cell disease (SCD) is the most common and serious form of an inherited blood disorder that lead to higher risk of early mortality. SCD patients are at high risk for developing multiorgan acute and chronic complications linked with significant morbidity and mortality. Some of the ophthalmological complications of SCD include retinal changes, refractive errors, vitreous hemorrhage, and abnormalities of the cornea.

MATERIALS AND METHODS: The present study includes 96 SCD patients. A dilated comprehensive eye examination was performed to know the status of retinopathy. Refractive errors were measured in all patients. In patients with >10 years of age, cycloplegia was not performed before autorefractometry. A subset of fifty patients’ genotyping was done for NOS3 27-base pair (bp) variable number of tandem repeat (VNTR) and IL4 intron-3 VNTR polymorphisms using polymerase chain reaction-electrophoresis. Chi-square test was performed to test the association between the polymorphisms and refractive errors.

RESULTS: The results of the present study revealed that 63.5% of patients have myopia followed by 19.8% hyperopia. NOS3 27-bp VNTR genotypes significantly deviated from Hardy–Weinberg equilibrium (P < 0.0001). Although IL4 70-bp VNTR increased the risk of developing refractive errors, it is not statistically significant. However, NOS3 27-bp VNTR significantly reduced the risk of development of myopia.

CONCLUSION: In summary, our study documents the prevalence of refractive errors along with some retinal changes in Indian SCD patients. Further, this study demonstrates that the NOS3 VNTR contributes to the susceptibility to development of myopia in SCD cases.

Keywords: IL4, NOS3, refractive errors, retinopathy, sickle cell disease

Introduction

Sickle cell disease (SCD) is the most common and serious form of an inherited blood disorder that lead to higher risk of early mortality.¹ The average life expectancy for people with SCD is estimated to be between 42 and 48 years of age,² among which ~85% survives for at least 20 years of age. Sickle cell retinopathy is one of the complications of sickle cell anemia that occurs due to occlusion of retinal vessels, especially in temporal periphery.³ Vitreous hemorrhages caused by vaso-oclusion in SCD patients may cause transient visual impairment or retinal detachment with permanent blindness.⁴,⁵ Further, majority of SCD patients showed varying degree of refractive errors.⁶ Furthermore, patients with SCD showed more structural abnormalities of the cornea when compared to healthy volunteers.⁷ Ophthalmologic characteristics of among pediatric and teenage patients from Northeastern Brazil demonstrated that retinal changes have early onset in patients with SCD disease.⁸
Clinical manifestations of SCD are also related to processes and complex metabolic pathways that include endothelial activation, inflammation, nitric oxide (NO) bioavailability, oxidative stress, and regulation of the adhesiveness of several types of blood cells. Adherence of sickled cells to vascular endothelium triggers an inflammatory process by releasing inflammatory agents. High levels of reactive oxygen and/or nitrogen species and decreased levels of NO contribute to increased production of proinflammatory and anti-inflammatory cytokines. Studies in animal models showed some evidence for the changes in the expression of NO synthase isoforms in the form-deprived chickens. NO synthase inhibitors block the development of form-deprivation and lens-induced myopia. Interleukin-10 (IL-10), IL-8, and monocyte chemotactic protein-1 (MCP-1) were much higher in patients with myopic choroidal neovascularization strongly suggests an involvement of inflammatory processes. Further, screening of inflammatory cytokines in the aqueous humor of high myopic cataract patients showed decreased expression of IL-1ra and increased expression of MCP-1. The relationship between endothelial NO synthase (eNOS) and several devastating complications of SCD led to the hypothesis that it could be a modifier gene in SCD. IL-4 is an anti-inflammatory cytokine produced by CD4+ Th2 cells, basophils, and mast cells. IL-4 promotes differentiation of Th2 cells and simultaneously inhibits differentiation of Th1 cells. A 70-base pair (bp) variable number of tandem repeat (VNTR) in the intron-3 of IL4 gene is known to alter IL4 production. In the present study, we investigated the possible association between the NOS3 27-bp and IL4 70-bp VNTR polymorphisms and refractive disorders in SCD patients.

Materials and Methods

The present study included 96 homozygous SS disease (SCD-SS) patients. The study volunteers are mainly from the outpatient clinic of the Sickle Cell Institute Chhattisgarh. Institutional Ethics Committee of Sickle Cell Institute Chhattisgarh, Raipur, has approved the study protocol. Informed written consent was obtained from study participants. The refractive error was measured without cycloplegia. In case of children <10 years of age, cycloplegic autorefraction was performed 20 min after the use of 0.8% tropicamide and 5% phenylephrine combination (Tropicacyl Plus; Sunways India Pvt. Ltd., India). The refractive error was measured with autorefractometers (Canon R30 Autorefractor) for all eyes. First, the instrument can be set so that the user has to depress the trigger button when the user believes that the instrument is properly focused and secondly the instrument can automatically trigger itself when properly focused. The instrument was refocused after each individual measurement. A dilated comprehensive eye examination was performed to know the status of retinopathy. All measurements were done by the same ophthalmologist (Santosh Patel). Myopia and hyperopia were defined as a sphere power of -0.50 D or worse and +0.50 D or greater, respectively, in either or both eyes. A negative cylinder notation was chosen and astigmatism was defined as a cylinder error ≥0.50 D.

SCD patients suffering from severe anemia or patients who have received a blood transfusion were excluded from the DNA analysis. A volume of 3 ml of peripheral blood samples was collected from fifty participants, and DNA was extracted using the following the standard protocol. Polymerase chain reaction (PCR)-based methods were adopted to delineate the genotypes of NOS3 27-bp VNTR and IL4 intron-3 VNTR. Allele frequencies were determined by direct counting of alleles at each locus. The genotype distribution of NOS3 27-bp and IL4 70-bp VNTR genotypes was analyzed using Hardy–Weinberg’s equilibrium (HWE) using goodness-of-fit Chi-square test. The association between the polymorphisms and refractive and cylindrical groups was analyzed using Chi-square test. Odds ratios (OR) and 95% confidence interval (CI) were calculated. All statistical analyses were performed using SPSS statistical software version 17.0 (SPSS Inc., Chicago, Illinois, USA) for Windows.

Results

The present study includes 96 SCD patients from the outpatient department of Sickle Cell Institute Chhattisgarh, Raipur. Patient demographics and ocular parameters are summarized in Table 1. Age distribution of study participants was depicted in Figure 1. The mean age of the SCD patients was 18.16 ± 8.28 years. Overall the patients were dominated by male participants and comprised 56.3% of total participants [Table 1]. Hemoglobin in the study participants is 9.27 ± 2.1 mg/dl. The mean fetal hemoglobin of the patients is 19.58%. Myopia is the major refractive error (51%) followed by hyperopia (5.2%). Astigmatism was found in about 53% of SCD patients. Further, retinal changes were observed in 14 (14.6%) patients [Table 1]. Among the retinal changes observed, tortuous blood vessels is the major followed by disc edema and increased cup-disc ratios (>0.7) with temporal pallor [Table 2].

Genotyping of NOS3 27-bp VNTR and IL4 70-bp VNTR was performed for only fifty participants.
NOS3 27-bp VNTR genotypes significantly deviated from HWE \( (P < 0.0001) \). NOS3 27-bp VNTR distribution among hyperopia, myopia, and normal groups is not significantly different (Fisher exact test \( P = 0.187 \)). Similarly, distribution of NOS3 VNTR variants among participants with or without astigmatism is not significant (Fisher exact test \( P = 0.318 \)). Risk analysis revealed that the NOS3 27-bp VNTR genotypes significantly decreased risk for myopia in dominant model (OR: 0.23; 95% CI: 0.04–1.11; \( P = 0.036 \)). However, decrease risk noted for NOS3 27-bp VNTR genotypes is not statistically significant in all models for hyperopia and astigmatism [Tables 3 and 4].

IL4 70-bp VNTR variants were found to be in HWE \( (P = 0.157) \). IL4 70-bp VNTR distribution among hyperopia, myopia, and normal groups are not significantly different (Fisher exact test \( P = 0.369 \)). Similarly, distribution of IL4 70-bp VNTR variants among participants with or without astigmatism is not significant (Fisher’s exact test \( P = 0.579 \)). Analysis of risk caused by IL4 70-bp VNTR variant genotypes for hyperopia and myopia showed that IL4 70-bp VNTR variant genotype increased the risk for hyperopia, myopia, and astigmatism, but in all conditions, it is not statistically significant [Tables 3 and 4].

Discussion

Analysis of 96 SCD patients revealed that 51% of patients have myopia followed by 5.2% hyperopia. Screening of fifty SCD patients for NOS3 27-bp VNTR and IL4 70-bp VNTR revealed that both markers are polymorphic in the study population. Although IL4 70-bp VNTR increased the risk of developing refractive errors, it is not statistically significant. However, NOS3 27-bp VNTR significantly reduced the risk of developing myopia. SCD patients are known to manifest different types of ocular problems such as refractive errors, nonproliferative retinopathy (PR), and PR.[7] The prevalence of refractive errors varies greatly from population to population and cannot be directly compared. The prevalence of myopia and hyperopia in the younger population (<15 years age) of India was 3.3% and 62.6%, respectively, while in participants >15 years of age, the prevalence of myopia and hyperopia was 19.45% and 8.38%, respectively.[23] In the present study, 56.2% patients had refractive errors which are higher than the (10.78%) previous report in SCD patient from Maharashtra, India.[14] Complete ophthalmic examination of 46 SCD Arab children revealed visual acuity decrease in 93.7% of patients without any sickle cell retinopathy.[24] Retinal changes were consistently more common in Jamaican children with SCD-SS disease.[25] The vaso-occlusive phenomena of the ocular microvasculature trigger ocular manifestations in the retina leading to visual impairment. Tortuous blood vessels and temporal pallor observed in the present study support microvascular damage and optic neuropathy, respectively, in the retina.[26] Optic disc edema noted in this study might be the results of increased intracranial pressure caused due to abnormal retinal vessel formation.[11]

Several lines of evidence demonstrated that the NO released from the endothelial cells plays a major role in...
regulating the local hemodynamics and systematic blood pressure.[23] In normal tissues, both eNOS and neuronal NOS are activated to produce NO for physiological functions. A 27 bp VNTR in the intron-4 of NOS3 gene is known to alter eNOS expression. Changes in production or actions of NO could contribute to several eye diseases such as uveitis, retinitis, glaucoma, and retinal degeneration. Changes in eNOS messenger RNA expression profiles and oxidative stress in the eye tissue microenvironment may have important roles in ocular neovascularization and permeability in PR. Immunological NOS (iNOS) induced during pathological conditions by inflammation will produce large amounts of NO for long periods of time. [25] It has been documented that, restoring functional hyperemia by iNOS inhibition limited the progression of retinopathy in diabetic patients. Suppression of iNOS-derived NO production lowers the intraocular pressure; hence, a precise regulation of NO may lead to a new therapeutic option for a wide range of ocular diseases.[27]

Estimation of IL4 levels in steady state SCD patients and controls showed elevated levels of IL4 in SCD patients compared to normal healthy individuals.[28,29] In contrast to this, low levels of IL4 is documented in SCD patients.[30] Further, IL4 levels differed in SCD patients belongs to various races.[31] Although inflammation plays a pivotal role of in SCD and the ocular changes produced by inflammation, SCD direct measurement of IL4 levels in SCD patients is controversial. No work has been done on the allergic reactants in ocular cells of SCD in relations to retinopathy. The treatment for SCD retinopathy is still controversial. However, the ocular changes in SCD patients are the result of a complex pathophysiological process affecting the eye. With our current knowledge, most of these devastating ocular complications could be prevented through the use of drugs that focus on the physiopathology of the SCD.

Conclusion
The present study has some limitations; inflammatory and ischemic biomarkers levels were not determined to correlate with the SCD retinopathy, as well as gene variants. However, our study documents the prevalence of refractive errors in Indian SCD patients. This study further demonstrated that the NOS3 VNTR contributes to the susceptibility of myopia in SCD cases. Large-scale association studies may provide a powerful tool for identifying alleles associated with complex phenotypes such as refractive errors and retinopathy in SCD.

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Conflicts of interest
The authors have no any conflicts of interest to declare.

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Table 3: Association between refractive errors and variable number of tandem repeat polymorphisms of NOS3 and IL4 genes

| Genotype   | Normal | Myopia | Hyperopia | Normal versus myopia | Normal versus hyperopia |
|------------|--------|--------|-----------|----------------------|------------------------|
|            | OR (95% CI) | P     | OR (95% CI) | P                    |
| NOS3 27-bp VNTR |        |        |           |                      |                        |
| 4bb        | 11     | 24     | 3         | Reference            | Reference              |
| 4ab        | 4      | 3      | 0         | 0.34 (0.04-2.48)     | 0.192                  |
| 4aa        | 4      | 1      | 0         | 0.11 (0.00-1.41)     | 0.056                  |
| 4ab + 4aa  | 8      | 4      | 0         | 0.23 (0.04-1.11)     | 0.036                  |
| IL4 70-bp VNTR |        |        |           |                      |                        |
| 3R3R       | 14     | 15     | 1         | Reference            | Reference              |
| 2R3R       | 4      | 9      | 2         | 2.10 (0.44-11.35)    | 0.289                  |
| 2R2R       | 1      | 4      | 0         | 3.73 (0.31-196.7)    | 0.250                  |
| 2R3R + 2R2R | 5     | 13     | 2         | 2.43 (0.59-10.87)    | 0.163                  |

Table 4: Association between astigmatism and variable number of tandem repeat polymorphisms of NOS3 and IL4 genes

| Genotype   | Normal | Astigmatism | OR (95% CI) | P     |
|------------|--------|-------------|-------------|-------|
| NOS3 27-bp VNTR |        |             |             |       |
| 4bb        | 17     | 21          | Reference   |       |
| 4ab        | 4      | 3           | 0.61 (0.08-4.18) | 0.421 |
| 4aa        | 4      | 1           | 0.20 (0.00-2.38) | 0.156 |
| 4ab + 4aa  | 8      | 4           | 0.40 (0.08-1.86) | 0.185 |
| IL4 70-bp VNTR |        |             |             |       |
| 3R3R       | 17     | 13          | Reference   |       |
| 2R3R       | 6      | 9           | 1.96 (0.47-8.38) | 0.291 |
| 2R2R       | 2      | 3           | 1.96 (0.19-26.23) | 0.415 |
| 2R3R + 2R2R | 8     | 12          | 1.96 (0.54-7.28) | 0.248 |

VNTR = Variable number of tandem repeat, OR = Odds ratio, CI = Confidence interval
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