Molecular and Pathophysiological Basis of Vaso-Occlusive Crisis in Sickle Cell Disease

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4th Global Congress Sickle Cell Disease

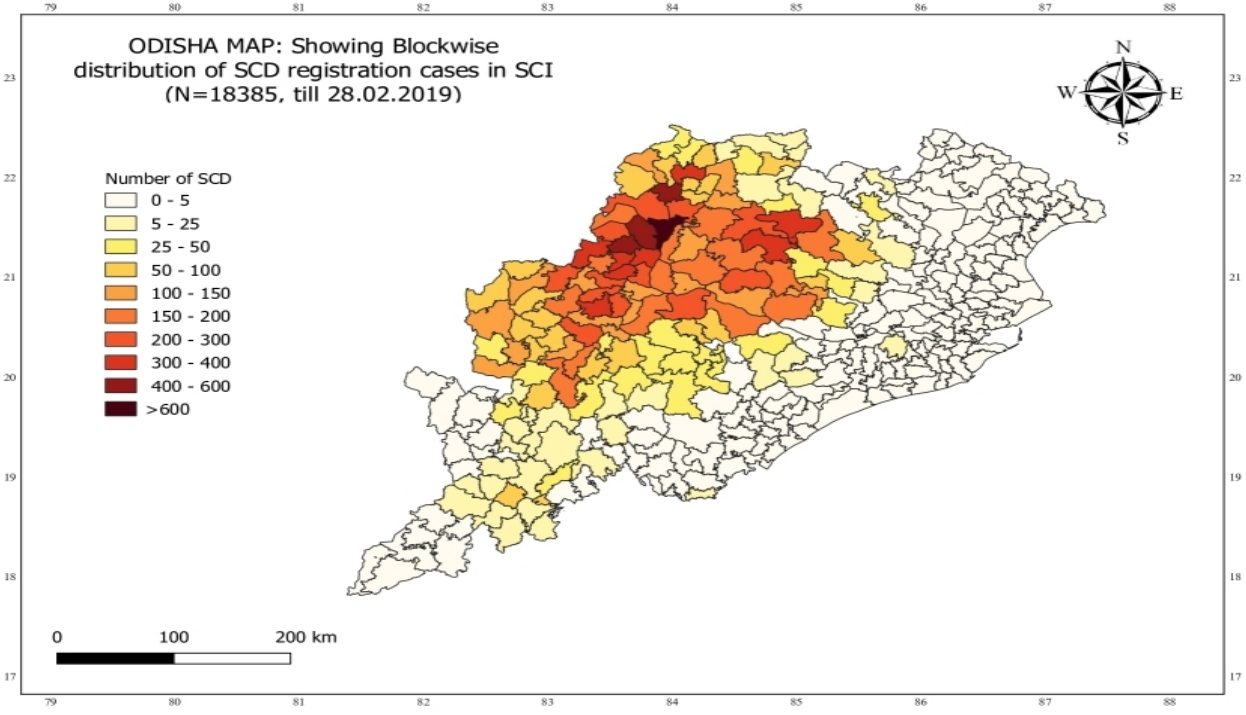
In-person and on-line Congress

JUNE 16-18 2022

Paris, Novotel Tour Eiffel

Late Prof. B.C.Kar, Mrs Beryl Serjeant, Prof. G.R.Serjeant, and Prof. B.P.Dash 1997, Burla, Odisha, India.

Distribution of Sickle Cell Patients in Odisha, India



Features of SCD in Odisha

* Higher foetal haemoglobin, total haemoglobin and interaction of alpha thalassaemia
* Lower reticulocyte count
* Spleen enlargement in 68% cases
* 95% cases were between 1-30 years of age
* Age of 1st manifestation of symptoms before 5 yrs in 50% and 25% before 10 yrs of age
* About 7% of cases were asymptomatic
* Bone & joint pain in >80% of cases
* Splenic sequestration in 8% of cases
* Hip bone necrosis 6%, leg ulcer 2.5%
* Priapism very rare (0.16%)

Vaso Occlusive Crisis (VOC)

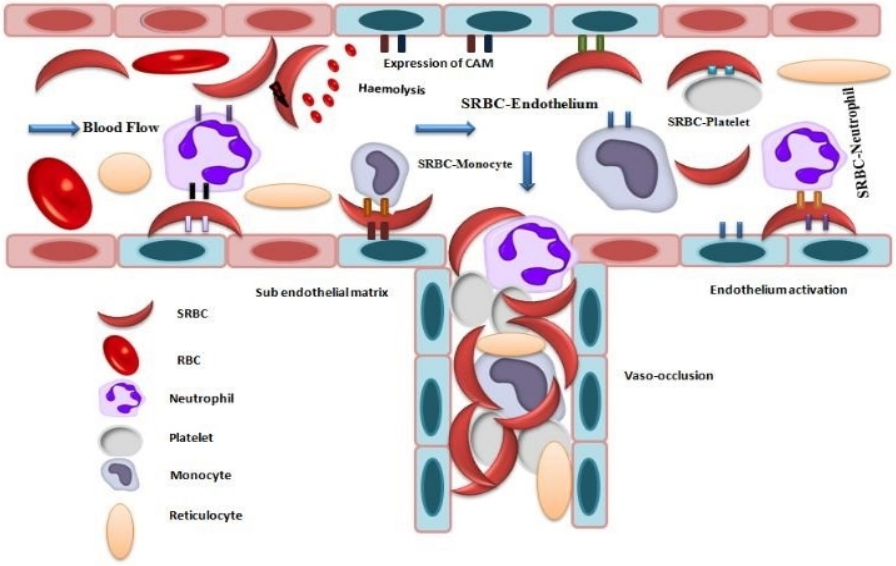
VOC may be defined as an acute painful events that lasted for at least 4 hours and required oral/injectable analgesics when no other cause could explain the symptoms. (Charache S, et al. 1995).

Pathophysiology

- Reduced Oxygen Affinity of HbS
- Polymerization of HbS in erythrocytes
- Adhesion of sickle erythrocyte
- Involvement of different cells

Factors influencing VOC in SCD

- Beta-globin gene haplotype
- Higher Fetal Hemoglobin level
- Alpha-thalassemia association



Cell activation and interaction in vasculature leading to VOC (Manwani and Frenette, 2013).

Introduction

- HOMOCYSTEINE, SULFUR-CONTAINING AA FORMED AS AN INTERMEDIARY PRODUCT DURING METHIONINE METABOLISM (KUMAR ET AL. 2017).
- HYPERHOMOCYSTEINEMIA IS AN INDEPENDENT RISK FACTOR OF THE VASCULAR DISEASE, LEADING TO ISCHEMIC COMPLICATIONS AND ATHEROSCLEROSIS.
- THE C677T GENE POLYMORPHISM (RS1801133) IN THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE. THE C677T GENE POLYMORPHISM IS ALSO INVOLVED IN HCY METABOLISM.
- HAPTOGLOBIN (HP) IS AN ACUTE PHASE GLYCOPROTEIN SYNTHESIZED MOSTLY FROM THE HEPATOCYTE.
- THE HP GENE IS ENCODED IN CHR-16Q22.
- IT HAS TWO GENE ALLELES: HP1 AND HP2.
- HP-1 ALLELE -1757BP WHEREAS
- HP-2 ~1700BP+ ~1700= 3481BP.
- THREE TYPES OF PHENOTYPES PROTEIN PRODUCTS WERE FOUND SUCH AS : HP1-1, HP1-2, AND HP2-2.
- HAPTOGLOBIN FORMS BONDS WITH THE FREE HB IN 1:1 COMPLEX AND RELEASED FROM THE CIRCULATION BY HB SCAVENGER RECEPTOR “CD163” THAT PRESENT ON THE MACROPHAGE AND MONOCYTES.

Introduction –cont.

- Nitric oxide (NO) is produced by the enzyme nitric oxide syntheses (NOS) during the enzymatic conversion of L-arginine to citrulline.
- The NO vasodilate endothelium vessels and inhibits its activation and platelet aggregation.
- At promoter position -786 a substitution mutation of T>C.
- In Intron four, the 4b/4a, a 27bp variable number of tandem repeat (VNTR) exists.

Objectives of the study

- To study the incidence Vaso Occlusive Crisis in SCD in Odisha.
- To study the bio-chemical and hematological markers in relation to Vaso Occlusive episodes in patients with Sickle Cell Disorder.
- To study the association of molecular markers (MTHFR, Haptoglobin, eNOS) with Vaso Occlusive Crisis in patients with Sickle Cell Disorder.

METHODS AND MATERIALS

Patients and Controls recruitment from SCI, VIMSAR, Burla with proper consent and IEC

Exclusion criteria:
- Those who have taken blood transfusion within 3 months of investigation.
- Any infection on Malaria, Tuberculosis etc.
- Those who refuse to consent for the study

Inclusion criteria:
- Those who allow consent for the study
- Age: 15 to 65 years;
- Sex: Male and Female

Collection of Blood and Serum Sample from recruited subjects

Blood Sample Processing Confirming Sickling Status

CBC

Serum Sample Processing

DNA isolation for mutational Study

(1) Sickling Slide Test

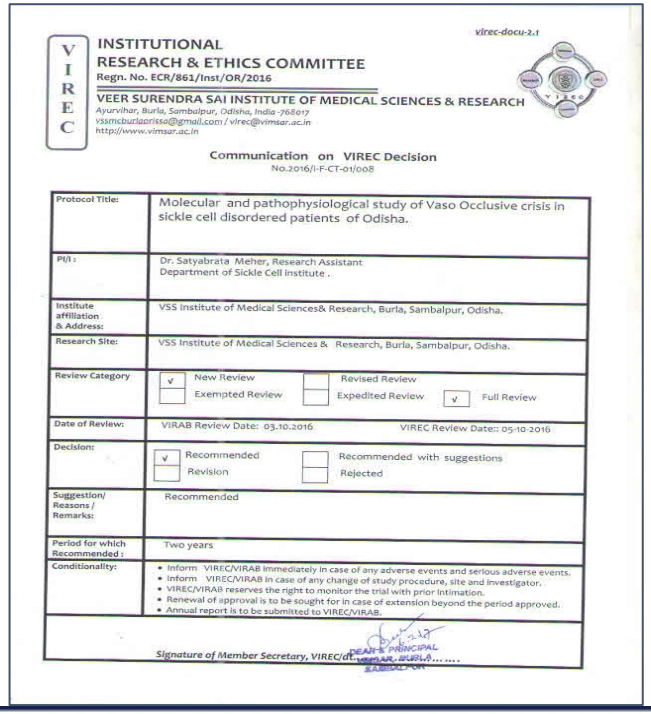
(2) Hb Electrophoresis (pH 8.6)

(3) CE-HPLC

(4) Confirmation of sickle mutation by ARMS-PCR

LFT, RFT, LDH, Homocysteine, Haptoglobin

Ethical Clearance Certificate



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VIREC

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
Regn. No. ECR/861/Inst/OR/2016

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Communication on VIREC Decision
No.2016/I-F-CT-01/008

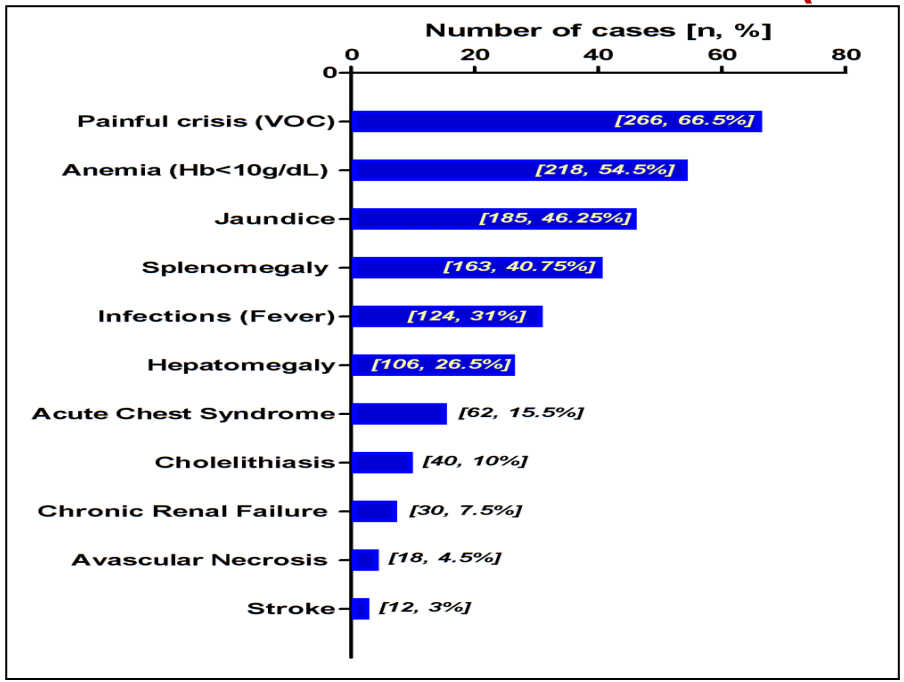
Protocol Title:	Molecular and pathophysiological study of Vaso Occlusive crisis in sickle cell disordered patients of Odisha.
PI/I :	Dr. Satyabrata Meher, Research Assistant Department of Sickle Cell Institute .
Institute affiliation & Address:	VSS Institute of Medical Sciences& Research, Burla, Sambalpur, Odisha.
Research Site:	VSS Institute of Medical Sciences & Research, Burla, Sambalpur, Odisha.
Review Category	[√] New Review [ ] Revised Review [ ] Exempted Review [ ] Expedited Review [√] Full Review
Date of Review:	VIRAB Review Date: 03.10.2016 VIREC Review Date:: 05-10-2016
Decision:	[√] Recommended [ ] Recommended with suggestions [ ] Revision [ ] Rejected
Suggestion/ Reasons / Remarks:	Recommended
Period for which Recommended :	Two years
Conditionality:	• Inform VIREC/VIRAB immediately in case of any adverse events and serious adverse events. • Inform VIREC/VIRAB in case of any change of study procedure, site and investigator. • VIREC/VIRAB reserves the right to monitor the trial with prior intimation. • Renewal of approval is to be sought for in case of extension beyond the period approved. • Annual report is to be submitted to VIREC/VIRAB.

Signature of Member Secretary, VIREC/at. VIMSAR, BURLA..... DEAN & PRINCIPAL

Age and Gender wise distribution of the Studied SCD cases and Controls

Age groups (in Years)	STUDY INDIVIDUAL S (N=615); [n, %]					
	Control (N=215)			SCD (N=400)		
	Male	Female	Total	Male	Female	Total
11-20	14 (6.6)	23 (10.7)	37(17.3)	77(19.3)	72 (18.0)	149(37.2)
21 - 30	45 (20.9)	53 (24.7)	98(45.7)	75(18.8)	78(19.5)	153(38.2)
31 - 40	32 (14.9)	17 (7.9)	49(22.7)	47(11.7)	28 (7.0)	75 (18.8)
41 - 50	15 (6.9)	9 (4.2)	24(11.2)	6 (1.5)	14 (3.5)	20 (5.0)
51 - 60	5 (2.3)	2 (0.9)	7 (3.1)	3 (0.7)	0 (0)	3 (0.8)
Total	111(51.6)	104(48.4)	215 (100)	208(52.0)	192(48.0)	400 (100)

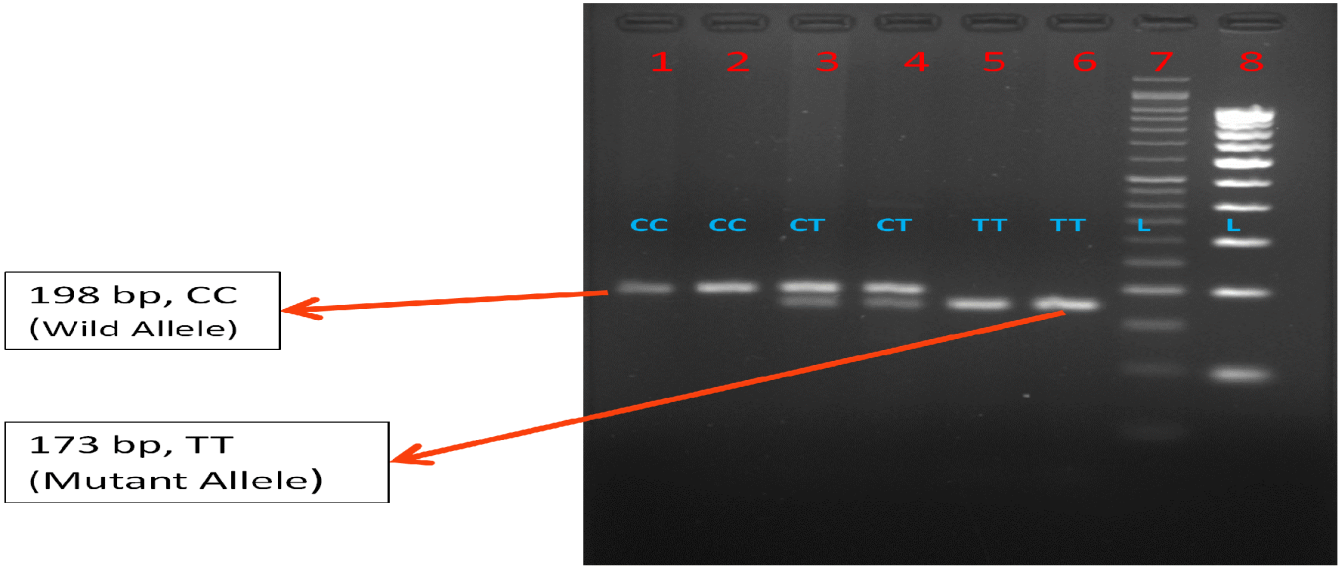
Incidence of various clinical phenotypes in SCD cases (N=400)



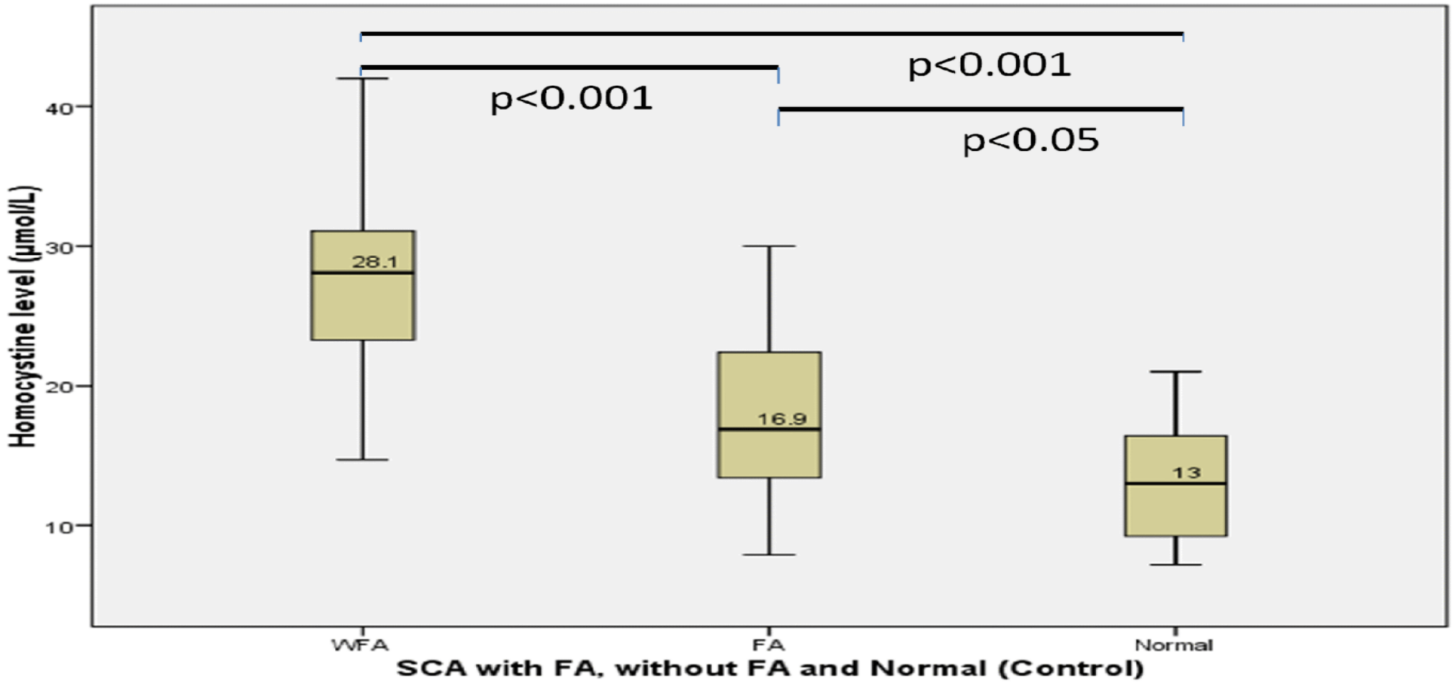
SCD with VOC (N=400)			
Number of VOC/Yr	Male	Female	Total (%)
No VOC (steady state)	64 (16.0)	70 (17.5)	134 (33.5)
1-2	39 (9.8)	26 (6.5)	65 (16.2)
3-4	51 (12.8)	34 (8.5)	85 (21.3)
5-6	26 (6.5)	25 (6.3)	51 (12.8)
7-8	23 (5.7)	16 (4.0)	39 (9.7)
9-10	12 (3.0)	5 (1.3)	17 (4.3)
>10	6 (1.5)	3 (0.7)	9 (2.2)
Total (%)	221 (55.3)	179 (44.8)	400 (100)

MTHFR 677C>T mutational analysis

Gene	Restriction Site	Primer 5' → 3'	Product size
MTHFR	RE: Hinf I (MTHFR C677T)	TGAAGGAGAAGGTGTCTGCGGGA AGGACGGTGCGGTGAGAGTG	198 bp



Homocysteine level in SCA with, without folic acid supplement and normal healthy control



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ORIGINAL ARTICLE

Association of plasma homocysteine level with vaso-occlusive crisis in sickle cell anemia patients of Odisha, India

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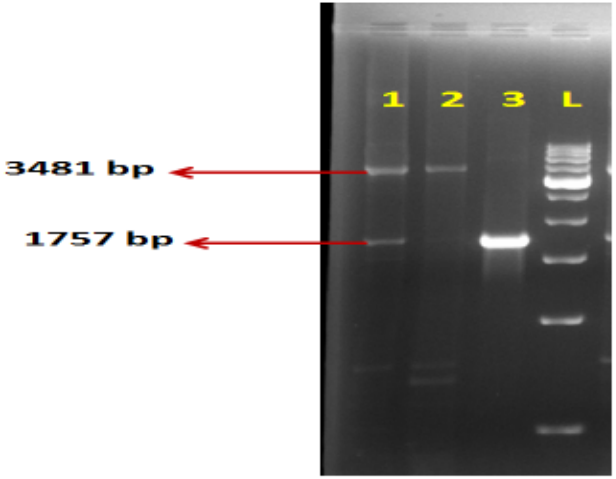
Abstract

Vascular complications of sickle cell anemia (SCA) are influenced by many factors. Elevated plasma homocysteine (Hcy) is supposed to be an independent risk factor and is either genetic or nutritional origin. The present study evaluated the plasma Hcy level, MTHFR C677T gene polymorphism, effect of folic acid (FA) supplementation, and hemato-biochemical parameters in SCA and their effect on the vaso-occlusive crisis (VOC) in SCA patients of an Asian-Indian haplotype population. One hundred twenty cases of SCA (HbSS) and 50 controls with normal hemoglobin(HbAA) were studied. It was found that the plasma Hcy level is significantly higher ($p < 0.0001$) in patients with SCA (22.41 ± 7.8 μmol/L) compared to controls (13.2 ± 4.4 μmol/L). Moreover, patients without FA supplementation had a significantly ($p < 0.001$) higher Hcy level (27 ± 7 μmol/L) compared to those with supplementation (17.75 ± 5.7 μmol/L). Turkey-Kramer multiple comparison tests show that there is a significant difference ($p < 0.05$) in HbF percent, hemoglobin (Hb), platelet count, serum bilirubin (direct:Bil-D and total:Bil-T), aspartate transaminase (AST), lactate dehydrogenase (LDH), and plasma Hcy levels between mild and severe VOC. Between moderate VOC and severe VOC, there was a significant difference ($p < 0.05$) in HbF%, Bil-D, AST, Hcy. Pearson correlation revealed that plasma Hcy had a significantly ($p < 0.05$) positive correlation with AST, serum bilirubin (indirect and total), LDH, jaundice, stroke, VOC per year, and hospitalization per year whereas it was inversely correlated with HbF percentage, Hb level, and FA treatment. In the study population, increased plasma Hcy level, hemolysis, and platelet activation were found to influence VOC in SCA.

Haptoglobin genotype analysis

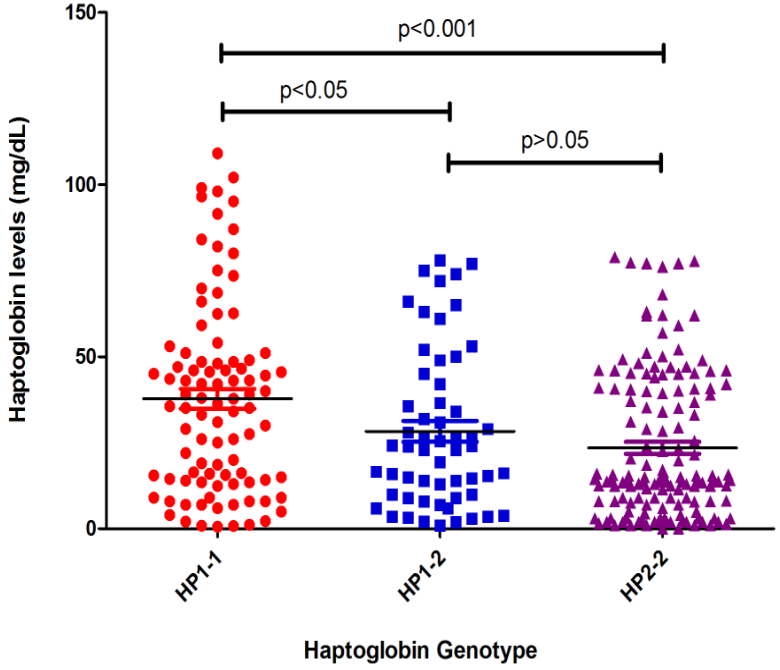
PCR method using a pair of primers to identified HP 1 and 2 (Koch et al. 2002):

HP-A (forward primer) 5'-GAGGGGAGCTTGCCTTTCCATTG-3'
and
HP-B (reverse primer) 5'-GAGATTTTTGAGCCCTGGCTGGT-3'



Lane 1(Sample 1)= HP 1-2 (Heterozygote)
Lane 2(Sample 2)= HP 2-2 (Homozygote)
Lane 3(Sample 3)= HP 1-1 (Homozygote)
Lane 4(Ladder)= 500 bp

Haptoglobin level with different Haptoglobin genotypes in SCA



HEMOGLOBIN
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ORIGINAL ARTICLE

Haptoglobin Genotypes Associated with Vaso-Occlusive Crisis in Sickle Cell Anemia Patients of Eastern India

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ABSTRACT

Sickle cell anemia is hallmarked by hemolysis, which releases hemoglobin (Hb) into the plasma promoting vaso-occlusive crisis (VOC). Haptoglobin (Hp) clears free Hb and decreases Hb-related pathophysiology in sickle cell anemia. There are two alleles (HP1 and HP2) and three genotypes (HP1-1, HP1-2 and HP2-2) of Hp with different frequencies in different populations. This study involved Hp level and genotype among normal and sickle cell anemia patients with varying severity of VOC. A total of 297 sickle cell anemia patients and 98 healthy controls were selected for the study. The sickle cell anemia patients were categorized as 'mild-phenotype' with no pain episodes and 'severe-phenotype' as having three or more acute pain episodes in the preceding 12 months. The Hp level was significantly lower ($p < 0.001$) in sickle cell patients anemia than controls; HP1-1 genotype had a higher Hp level compared to HP1-2 and HP2-2 ($p < 0.05$). Turkey-Kramer multiple comparison tests showed that mild and severe phenotypes have significant differences ($p < 0.05$) in Hb F%, Hb, platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct-bilirubin (Bil-D), total-bilirubin (Bil-T), lactate dehydrogenase (LDH) and Hp level. Pearson correlation revealed that Hp level has a positive ($p < 0.05$) correlation with Hb F%, Hb, packed cell volume (PCV) and serum urea; in contrast its level is negatively correlated with AST, ALT, Bil-T and LDH. A significantly higher frequency of HP2 allele and HP2-2 genotypes was found in severe phenotypes. In the studied population, it was found that higher HP2 frequency, low Hp level and more hemolysis favors the onset of VOC in sickle cell anemia.

ARTICLE HISTORY
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KEYWORDS
Haptoglobin (Hp); sickle cell anemia; vaso-occlusive crisis (VOC)

SUMMARY

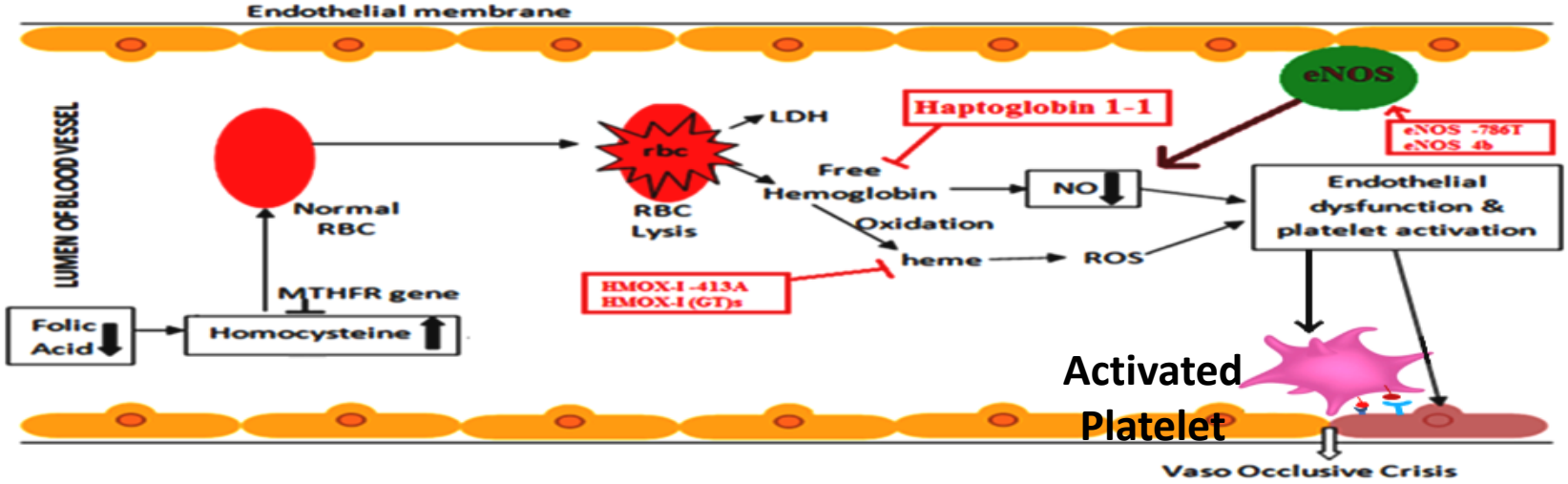
VOC CATEGORY	HOMOCYSTEINE LEVEL	MTHFR 677 T>C	HAPTOGLOBIN LEVEL	HP-GENOTYPE	eNOS -786 T>C	4b/4a
SEVERE VOC SCA	HIGH	NO EFFECT	LOW	HP2-2	CC	aa
MILD VOC SCA	LOW		HIGH	HP 1-1	TT	bb

Summary

- The mean haptoglobin level was almost below three and half below than normal level.
- Inverse relationship of haptoglobin with LDH level (r=-353, p=0.001)
- Hemoglobin level was positively linked to the haptoglobin level (r=0.637, p=0.035)
- HP 1-1 has a greater level of Hp as compared to the HP 1-2 and HP2-2.
- Healthy control obey Hardy-Weinberg equilibrium on -786 T>C polymorphism. But, SCA and severe SCA doesn't obey, mutant allele (C) of -786 T>C gene polymorphism is linked to the VOC.
- eNOS 4b/a 27bp VNTR analysis showed that the mutant allele of 'aa' is linked to VOC.

- Higher Hcy level ($p<0.0001$) in patients with SCA has been reported as compared to that in the normal controls.
- Higher Hcy level was recorded among the SCA patients those are not on FA therapy than among those on the therapy.
- Increase in both LDH and Hcy level were recorded, indicating a possible effect of Hcy on the hemolysis of RBC.
- LFT were elevated during severe VOC as compared to mild VOC.
- Hcy was negatively correlated with HbF%.

Over all Mechanism ?



Cartoon representing Vaso Occlusive Crisis: Increase homocysteine lead to hemolysis of RBC and release of free Hb which decreases nitric oxide and endothelial activation and disfunction. Free heme activate ROS then lead to endothelial disfunction.
MTHFR-Methyl tetrahydrofolate reductase, NO-nitric oxide, ROS-reactive oxygen species.

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- Organizers of 4th Global Congress on Sickle Cell Disease

THANK YOU ALL