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A study on C-reactive protein as an early marker of vasococclusive crisis in homozygous sickle cell disease (HbSS) and sickle cell-β thalassemia disease (Hb S-β thal)

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Abstract
Background: *Sickle cell disease (SCD) is associated with a chronic inflammatory state. SCD patients often seek care in the Emergency Department (ED) due to vaso-occlusive crisis (VOC), the most common complication of SCD. Currently, no diagnostic test can determine if a SCD patient is having an acute VOC. Methodology: Irrespective of the clinical diagnosis and type of sickle cell crises the confirmation of haemoglobin pattern in sickle cell disease patients and healthy subjects were done by sickling test, hemoglobin electrophoresis or high performance liquid chromatography.*
Results: *CRP status in Group I (Hb electrophoretically AA) healthy subjects were analyzed. It showed that irrespective of their age and sex 100% were CRP negative during their healthy state. Diarrhoea (26%), LRTI (16%), URTI (14%) and malaria (12%) were more prevalent infection in sickle cell patients in the present study. It was observed that out of 21 patients with CRP positivity in early phase 17 patients (80.9%) developed vaso-occlusive crisis during their follow up.*
Conclusion: *The present study demonstrated a strong association between the inflammatory biomarker hs-CRP and vasococclusive crisis in homozygous sickle cell disease (HbSS) and sickle cell-β thalassemia disease (Hb S-β thal). We believe these results highlight the clinical relevance of inflammation in microvessel occlusive complications, and provide a basis for the study of hs-CRP as a potential biomarker for predictive modelling of clinical outcomes in SCD.*
Keywords: *Sickle Cell Disorder, homozygous sickle cell disease (HbSS), sickle cell-β thalassemia disease, vaso-occlusive crisis, hemoglobin electrophoresis, biomarker, C-reactive protein.*

Introduction

Sickle Cell Disorder (SCD) is a congenital hemoglobinopathy. It is also known as drepanocytosis, is a quadrumvirate of anemia and its sequelae, pain syndromes, organ damage including infection, and comorbid conditions. [1-4]

It is a chronic blood disorder characterized by red blood cells that assume an abnormal, rigid, sickle shape. The striking deformity of the red blood cells led to the disorder being termed 'sickle cell anemia' and 'sickle cell disease',[5] names that reflected the subsequent acceptance that erythrocyte distortion was central to the development of its symptoms. In 1917, Emmel[6] noted that the degree of erythrocyte deformation varied with time demonstrated that this eponymous change was induced by deoxygenation. Microvessel occlusion, the main pathological process underlying the cardinal clinical manifestation of sickle cell disease (SCD), is frequently termed as vaso-occlusive crisis (VOC) or pain crisis (Stuart & Nagel, 2004)[7].

Patients with sickle cell disease have intermittent painful crisis caused by occlusion of microcirculation with sickled cells. Crisis varies considerably in severity ranging from mild episodes that respond at home to simple analgesics and oral rehydration to extensive tissue infarction requiring parental opiates and intravenous fluid in hospital.

V.S.S. Medical College is situated in Western Orissa and cater to a population of 6 – 8 millions of adjoining districts and also part of Chhattisgarh. Large number of patients of sickle cell haemoglobinopathy attends paediatric Outdoor and Indoor. Considering the magnitude of this disease in this area this study was undertaken with following aim and objectives to find out the value of measurement of acute phase reactants like C-reactive protein as an objective marker of tissue ischemia during steady state, as an early marker in the diagnosis of vaso-occlusive crisis in sickle cell disorder and on response to treatment.

Materials & Methods

This study was conducted in the Outdoor and Indoor patients of paediatric department and sickle cell clinic of V.S.S. Medical College, Burla. Study subjects in the age group of 6 month to 14 yrs were enrolled in the study. The study subjects were divided into 5 separate group as per case definition specified.

Inclusion Criteria

- **Group I:** It includes healthy subjects, cases of HbAA proved by electrophorectically or high performance liquid chromatographically where other chronic or acute inflammatory disease, infection or malignancy was excluded.
- **Group II:** It includes patients of electrophorectically or high performance liquid chromatographically proved cases of Homozygous sickle cell disease (HbSS) and sickle β thal disease (Hb S- β thal) who being in the steady state (as defined in group I) were not in any crisis but presenting with some other clinical problem like LRTI, URTI, UTI, diarrhoea, malaria, enteric fever, mumps, measles, pyoderma, primary complex etc.

Exclusion Criteria

Patients of acute rheumatic fever, collagen vascular disease, infective hepatitis, infective endocarditis, portal hypertension, recent typhoid vaccination and meningitis were excluded from the study group.

Methodology

Irrespective of the clinical diagnosis and type of sickle cell crises the confirmation of haemoglobin pattern in sickle cell disease patients and healthy subjects were done by sickling test, hemoglobin electrophoresis or high performance liquid chromatography. After careful selection of cases a detailed history and thorough clinical examination was done. In each case the following investigations were done as required for the study.

Hematological Investigations

A. Routine Hematological Investigations

a. Sickling Test: The sickling phenomenon was demonstrated by following methods. A drop of capillary blood was obtained by finger prick and. taken over a clean glass slide. A cover slip was put over it and carefully sealed with Vaseline. This preparation was kept at room temperature for 24 hrs and then examined under microscope to detect presence of sickled erythrocyte. An alternative quick method of detecting sickled RBC was done by adding a drop of freshly prepared 2% sodium-metabisulfite to the drop of blood taken over glass slide and covered with cover slip and sealed with wax. The slide was examined for sickle erythrocytes after half an hour to one hour.[8]

b. Haemoglobin Estimation (Hb%): This was done by Sahli's acid haematin method.

B. Special Hematological Investigations: Were done as and when necessary

a. Haemoglobin Electrophoresis[8, 9]

Although the sickling test and haemoglobin solubility test detect the presence of HbS, haemoglobin electrophoresis is mandatory for precise diagnosis of sickle haemoglobinopathies. The principle of Hb-electrophoresis at alkaline pH is based on the alteration of haemoglobin molecule which follows certain amino acid substitution. In practice the method requires a source current, a buffer system and a supporting medium. Of the medias most used, paper is cheapest but gives poor results, starch gel gives good results but is time consuming. Cellulose acetate gives excellent results but is expensive. At present cellulose acetate is the most widely used medium with Tris-EDTA borate buffer system at a pH of 8.4.

Haemoglobin electrophoresis on acid agar gel provides valuable confirmatory and additional evidence. It is done in a citrate buffer at pH 6.0 to 6.2. HbF migrates slightly towards cathode and HbS and HbC towards anode, whereas most other variants remain in position of HbA close to the origin. The use of both alkaline and acid agar gel electrophoresis serves to identity HbS and HbC separately and indicates presence of less common variants.

Procedure

Venous blood was collected in a clean vial containing anticoagulant (EDTA). A haemolysate of blood was prepared by the following method: 2 ml of anti-coagulated blood was taken in a test tube and was washed with normal saline (9 gm/dl) for 3 times. Each time the supernatant liquid was thrown out. After washing distilled water, double the volume of packed cell was added to test tube to lyse the cells. 0.5ml to 1 ml of carbon tetrachloride was added and the test tube was shaken well for 5 to 10 times. The clear haemolysate was present at the top layer in the test tube and carbon tetrachloride at bottom. Agarose and buffer solution was boiled to become liquid. The agarose gel was applied on a glass slide and was allowed to become solid. The hemolysate was applied on one end of the gel flooded slide with an applicator. The slide was placed in the electrophoresis tank with the applied end at the negative pole. Current was applied at a constant voltage of 200 mv for 25 to 30 mins. The different bands get separated. The slide was taken out dipped in the fixative for 3.5 minutes. After fixation the slide was dried in the air and then it was stained with Benzidine H2O2 solution or Amidoblack stain.

b. Automated High Performance Liquid Chromatography (HPLC)[9]

Automated cation-exchange HPLC is being used increasingly as the initial diagnostic method in hemoglobinopathy laboratories with a high workload. Both capital and consumable costs are higher than with hemoglobin electrophoresis but labour costs are less, overall costs may be similar. In comparison with haemoglobin electrophoresis, HPLC has four advantages: The analyzers are automated and thus utilize less staff time and permit processing of large batches. Very small samples (5µl) are sufficient for analysis; this is

especially useful in Paediatric work. Quantification of normal and variant haemoglobins is available on every sample. A provisional identification of a larger proportion of variant haemoglobins can be made.

Principle

HPLC depends on the interchange of charged groups on the ion exchange material with charged groups on the haemoglobin molecule. A typical column packing is 5 μm spherical silica gel. The surface of the support is modified by carboxyl groups to have a weakly cationic charge which allows the separation of haemoglobin molecules with different charges by ion exchange. When a haemolysate containing a mixture of haemoglobins is adsorbed onto the resin, the rate of elution of different haemoglobins is determined by the pH and ionic strength of any buffer applied to the column. With automated systems now in use elution of the charged molecules is achieved by a continually changing salt gradient; fractions are detected as they pass through an ultraviolet visible light detector and are recorded on an integrating computer system. Analysis of the area under these absorption peaks gives the percentage of the fraction detected. The rate of elution (retention time) of any normal or variant haemoglobin present is compared with that of known haemoglobins, providing quantification of both normal haemoglobins (A, F and A2) and many variants.

Method

Procedure was done by instrument "VARIANT β thalassemia short programme" supplied by Bio-Rad Laboratory.

Procedure

Latex gloves were weared before reagents preparation. Elusion buffers, wash solution and analytical cartridge were installed. HbA2 / F calibrator was reconstituted by adding 10ml HbA2 / F calibrator diluent per vial. Vial was swirled gently to dissolve and to ensure complete mixing. Calibrator was allowed to stand for 10 minutes at 15 - 30°C. A set of normal (HbF – 1-2%, HbA2 – 1.8 – 3.2%) and abnormal (HbF – 5 – 10%, HbA2 – 4.6%) controls supplied by manufacturer was runned at the beginning and end of each group of patients specimens. Haemoglobin primer was used at the beginning of each run to condition the cartridge for analysis. Haemoglobin primer was reconstituted by adding 1ml of deionized water to the vial. Vial was swirled gently to dissolve and to ensure complete mixing. Primer was allowed to stand for 10 min at 15 - 30°C. 2ml of venous blood was collected in a clean vial containing anticoagulant EDTA. 5 μl of collected blood sample was taken into a separate 1.5ml sample vial. 1ml of haemolysis reagent was added to sample vial containing collected blood sample. Sample vial was covered with paraffin and was mixed by inversion. Sample vial was then placed into VARIANT. Power of system was pressed. Start key was pressed to flush the system. The system performed a 5 minute warm – up programme after which the system was entered IDLE status. The RUN SET UP screen was then displayed. 250 μl of each of the above mentioned reconstituted reagents were transferred into sample vials. The reagents and prepared patients specimens were placed into the variant sample tray as indicated below:

WELL	REAGENT
STAT WELL	Haemoglobin primer
1	Deionized water
2	HbA2 / F calibrator
3	Normal Control
4	Abnormal control
5 to N*	Patient Haemolysates
N+1	Normal control
N+2	Abnormal control

*N referred to the last patients well number included in the run.

The run was initialized. The calibrator was analysed. The calibration response factors for haemoglobin A2 and F were automatically calculated. The calibration response factors were used in the calculation of area percentages for haemoglobin A2 and F for all subsequent analyses in the run. Test menu key was pressed followed by '1' to select the β thalassemia short programme. Enter was pressed to confirm the selection. At the completion of each run the system was

automatically initiated a 5 minutes WASH cycles. At the completion of wash cycle the results of the test was interpreted.

Interpretation and comments

Results are accurate and reproducible but as with every method of haemoglobin analysis, controls should be run with every batch. The system was used for the detection of haemoglobin variants, elution times compared with those of known controls. Actual times however are affected by the batch of buffer and column, the age of the column and the laboratory temperature. A better comparison was obtained by using the relative elution time which is calculated by dividing the elution time of the variant with that of the main HbA fraction. Hb A was separated into its component fractions of A0, A1 and the Al fraction frequently subdivides into several peaks. Analyte identification 'windows' was intended in the interpretation of normal and abnormal haemoglobins detected in the patient's samples. The 'windows' were established time ranges in which common variants had been observed.

Analyte Identification Window

Analyte Name	Retention time (mins)	Band (mins)	Window (mins)
F	1.15	0.15	1.00-1.30
P2	1.45	0.15	1.30-1.60
P3	1.75	0.15	1.60-1.90
A0	2.60	0.40	2.20-3.30
A_2	3.83	0.15	3.68-3.98
D-window	4.05	0.07	3.98-4.12
S-window	4.27	0.15	4.12-4.42
C-window	5.03	0.15	4.88-5.18

The retention time: The center of the window (was measured from the time of the sample injected to the maximum point of each peak).

The band: The half-width of the window. HPLC usually separates Hb A, A2, F, S, C, D Punjab and G Philadelphia from each other. However, both Hb E and Hb Lepore co-elute with haemoglobin A2 (as other haemoglobins co-elute with A, S and F). The retention time of glycosylated and other derivatives of HbS can be the same as those of Hb A0 and A2. For these reason and because there are more than 750 variants identified, HPLC can never definitively identify any haemoglobin. It is important to analyze variants found using second-line techniques, such as sickle solubility, alkaline and acid electrophoresis.

c. Estimation of foetal haemoglobin: (Batke method)

0.6 ml of prepared hemolysate was taken with 10 ml of Drabkins solution and was allowed to stand for 10 minutes. This mixture is called Cyanmeth haemoglobin (HICN). 5.6 ml of HICN was taken and 0.4 ml of 1.2 N sodium hydroxide was added to it. It was allowed to mix well for 2 minutes. Then 4 ml of saturated ammonium sulphate solution was added to it and mixed well. The mixture was filtered. The filtrate was compared with standard using green filter calorimeter with light of wave length 540 μ. Standard: 1.4 ml of HICN was taken in a test tube and 1.6 ml of distilled water was added to it. Then 2ml of saturated ammonium sulphate solution was added and mixed well. 0.5ml of the mixture was taken in to 4.5 ml of Drabkins solution and was compared in the calorimeter in green filter with the test. Calculation: (Reading of test x 5)/Reading of standard = % of HbF

d. Semiquantitative Estimation of C-reactive protein[10, 11]

Semiquantitative estimation of C-reactive protein estimation was done by diagnostic kit manufactured by Span Diagnostic Ltd. (Surat, India) by rapid latex slide technique.

Principle

This test based on the immunologic reaction between CRP as an antigen and latex particles have been coated with monospecific anti-human CRP and sensitized to detect levels greater than 6 μg/ml CRP. The latex slide test has the advantage of rapid performance in comparison to other tests for detection of CRP.

Sample

5-10 ml venous blood was collected into sterile tube/vial without anticoagulant. Sample was allowed to clot at room temperature for several hours. After complete formation of clot serum was separated and was stored at 2-4°C.

- **Reagents / Accessories (Supplied in the Kit)**

  Reagent 1: CRP Latex Reagent
  Reagent 2: Positive Control Serum
  Reagent 3: Negative Control Serum

Precautions

The CRP Latex Reagent vial should be properly closed to avoid drying & formation of flakes during storage at 2-8°C. It should never be frozen. It should not be left at room temperature for a long period. Specimen bottles or tubes and the test slide must be free from detergent. While performing the test the slide should be rocked very gently as vigorous rocking may disturb the agglutination pattern. Drying of the mixture at the periphery may lead to erroneous results. Plasma should not be used in place of serum of the test. Excessively lipemic and bacterial contaminated sera can cause false positive results.

Procedure

Qualitative Slide Test

All reagents as well as the sample were allowed to reach room temperature:

Using the disposable plastic dropper, one drop of undiluted test serum was placed within the circled area on the special slide provided in the kit. One drop of Latex CRP Reagent (the vial was shaken gently immediately before use) was added to the one drop of undiluted test serum and was mixed well with a disposable applicator stick and was spread out to the edge of the test area. The slide was rocked gently to and fro for 2 minutes and was examined for macroscopic agglutination under direct light source. Note: For positive and negative controls, the same procedure was followed as mentioned above by taking pre-diluted control serum from respective vials. Note: Controls were not diluted.

Semi – Quantitative Slide Test

A series of dilutions of the test serum were prepared in normal Saline i.e. 1:2, 1:4, 1:8 etc. One drop of each of these dilutions was tested with one drop of Latex CRP Reagent. Agglutination was observed for no longer than 2 minutes on glass slide provided in the kit. The highest dilution which showed agglutination was taken as CRP titre of the test serum.

Interpretation: Qualitative Slide Test:

Observation	Conclusion
i) Coarse agglutination	Strongly positive
ii) Finer agglutination	Weakly positive
iii) Smooth suspension without any noticeable change	Negative

Semi-Quantitative Slide Test

CRP level was calculated in μgm/ml by multiplying the highest dilution giving clear cut agglutination with a factor of 6 (Sensitivity of antigen is 6 μg/ml)

In **Group I** total 15 patients were selected and their C-reactive protein status were studied every 6 monthly during their regular health checkup. Initially 24 cases were enrolled in this group but we have to drop out 9 cases due to irregular follow up or appearance of any infective and inflammatory condition. Ultimately 15 cases could satisfy the inclusion criteria of minimum 2 healthy state CRP reports out of a maximum 4.

In **Group II** total 50 patients were selected and their C - reactive protein status was done on early (day 1 – 3) and middle (day 4–7) phase of clinical problems. Initially 56 cases were enrolled in this group but we have to drop out 6 cases due to irregular followup and non cooperation of the patients. All the entries of individual cases were entered into a master chart and observations were analyzed in details.

Results

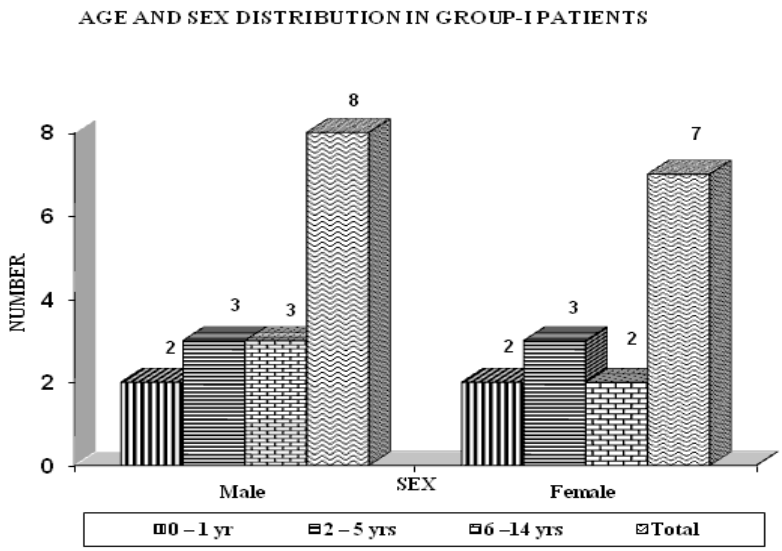

Figure 1: Age and sex distribution among group I subjects [healthy subjects]

There were 8 (53.3%) male and 7 (46.7%) female patients enrolled in Group I. The age and sex distribution of subjects in this group were comparable to those in the other study groups [Fig. 1].

Table 1: CRP status in Group I patients in healthy state

Age (yrs)	No. of pt.	CRP Status							
		Positive (> 6mg/l)				Negative (< 6mg/l)			
		M	F	T	%	M	F	T	%
0 – 1 yr	04	00	00	00	00	02	02	04	100
2 – 5 yrs	06	00	00	00	00	03	03	06	100
6 – 14 yrs	05	00	00	00	00	03	02	05	100
Total	15	00	00	00	00	08	07	15	100

CRP status in Group I (Hb electrophoretically AA) healthy subjects were analyzed. It showed that irrespective of their age and sex 100% were CRP negative during their healthy state. B. Shine, MB Pepys (1998)[66] showed that median value of CRP in 468 healthy children was 1.6 mg/l, 90% were less than 3 mg/l and 99% were less than 6 mg/l [Table 1].

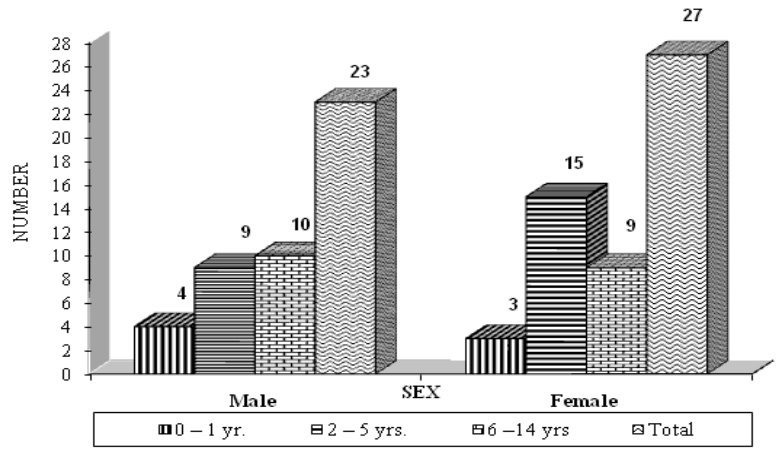

Figure 2: Age and sex distribution among group II subjects [homozygous sickle cell disease (HbSS) and sickle cell-β thalassemia disease (Hb S-β thal)]

There were 23 (46%) male and 27 (54%) female patients enrolled in Group II. Largest number of cases -24 (48%) were between 2 – 5 yrs of age. The female patients outnumbered the males by 4, ratio being 1.2:1 [Fig. 2].

Table 2: Clinical diagnosis in Group II patients

Clinical diagnosis	No of patients		Total	Percentage
	Male	Female		
URTI	04	03	07	14
LRTI	05	03	08	16
UTI	01	02	03	06
Malaria	02	04	06	12
Diarrhoea	06	07	13	26
Enteric fever	01	03	04	08
Mumps	01	01	02	04
Measles	00	01	01	02
Pyoderma	02	02	04	08
Primary complex	01	01	02	04
Total	20	30	50	100

Distribution of cases according to their clinical diagnosis in Group II patients was analyzed. Clinical diagnosis was confirmed after doing definitive and supportive laboratory investigation. Diarrhoea (26%), LRTI (16%), URTI (14%) and malaria (12%) were more prevalent infection in sickle cell patients in the present study [Table 2].

Table 3: CRP status in early phase of clinical problem in Group II patients

Clinical Diagnosis	No. of pt.	CRP Status							
		Positive (> 6 mg/l)				Negative (< 6 mg/l)			
		M	F	T	%	M	F	T	%
URTI	07	01	01	02	28.5	03	02	05	71.5
LRTI	08	02	02	04	50	02	02	04	50
UTI	03	00	00	00	00	01	02	03	100
Malaria	06	01	02	03	50	01	02	03	50
Diarrhoea	13	04	05	09	69.2	02	02	04	30.8
Enteric fever	04	01	00	01	25	01	02	03	75
Mumps	02	00	00	00	00	01	01	02	100
Measles	01	00	00	00	00	00	01	01	100
Pyoderma	04	01	01	02	50	01	01	02	50
Primary complex	02	00	00	00	00	01	01	02	100
Total	50	10	11	21	42	13	16	29	58

Irrespective of clinical problem, age and sex CRP status became positive in 21 (42%) cases and negative in 29 (58%) cases during their early phase of presentation [Table 3].

Table 4: Outcome in Group II patients with early phase CRP positivity [n=21]

Clinical Diagnosis	No. of pts.	Vaso-occlusive crisis						No Vaso-occlusive crisis					
		M	F	CRP Status				M	F	CRP Status			
				Positive (>6mg/l)		Negative (<6mg/l)				Positive (>6mg/l)		Negative (<6mg/l)	
				M	F	M	F			M	F	M	F
URTI	02	01	01	01	01	00	00	00	00	00	00	00	00
LRTI	04	02	02	02	02	00	00	00	00	00	00	00	00
UTI	00	00	00	00	00	00	00	00	00	00	00	00	00
Malaria	03	01	02	01	02	00	00	00	00	00	00	00	00
Diarrhoea	09	02	04	02	04	00	00	02	01	00	00	02	01
Enteric fever	01	01	00	01	00	00	00	00	00	00	00	00	00
Mumps	00	00	00	00	00	00	00	00	00	00	00	00	00
Measles	00	00	00	00	00	00	00	00	00	00	00	00	00
Pyoderma	02	01	00	01	00	00	00	00	01	00	00	00	01
Primary complex	00	00	00	00	00	00	00	00	00	00	00	00	00
Total	21	08	09	08	09	00	00	02	02	00	00	02	02
		17		17		00		04		00		04	

The outcome in Group II patients with early phase CRP positivity, was expressed in terms of percentage of patients with CRP positive in early phase who developed vaso-occlusive crisis during their follow up and subsequently remain CRP positive or not. It was observed that out of 21 patients with CRP positivity in early phase 17 patients (80.9%) developed vaso-occlusive crisis during their follow up [Table 4]. All of them maintained to be CRP positivity before appearance of crisis and also when followed up for the early phase of the crisis.

Table 5: Outcome in Group II patients with early phase CRP negativity [n=29**]**

Clinical Diagnosis	No. of pts.	No. Vaso-occlusive crisis						Vaso-occlusive crisis					
		M	F	CRP Status				M	F	CRP Status			
				Positive (>6mg/l)		Negative (<6mg/l)				Positive (>6mg/l)		Negative (<6mg/l)	
				M	F	M	F			M	F	M	F
URTI	05	02	02	00	00	02	02	01	00	01	00	00	00
LRTI	04	02	01	00	00	02	01	00	01	00	01	00	00
UTI	03	01	02	00	00	01	02	00	00	00	00	00	00
Malaria	03	01	02	00	00	01	02	00	00	00	00	00	00
Diarrhoea	04	02	01	00	00	02	01	00	01	00	01	00	00
Enteric fever	03	01	01	00	00	01	01	00	01	00	01	00	00
Mumps	02	01	01	00	00	01	01	00	00	00	00	00	00
Measles	01	00	01	00	00	00	01	00	00	00	00	00	00
Pyoderma	02	01	01	00	00	01	01	00	00	00	00	00	00
Primary complex	02	01	01	00	00	01	01	00	00	00	00	00	00
Total	29	12	13	00	00	12	13	01	03	01	03	00	00
		25		00		25		04		04		00	

Outcome in Group II patients with early phase CRP negativity was demonstrated. The percentage of patients with CRP negativity in early phase who developed vaso-occlusive crisis during their follow up and subsequently their CRP status were analyzed. It showed out of 29 patients with CRP negativity in early phase only 4 patients (13.8%) developed vaso-occlusive crisis during their follow up and subsequently CRP became positive in all those 4 patients who developed vaso-occlusive crisis [Table 5].

In present study, Group II patients, when they came into clinical attention they were not in the any type of crisis but had developed some clinical problems. In this period though they were not in crisis near about 42% patients were CRP positive. Ultimately during follow up in the middle phase of clinical problem out of 21 CRP positive cases in the early phase, 17 patients developed vaso-occlusive crisis i.e. near about 80.9%. Who became (29 patients) CRP negative in the early phase of clinical problem, among them only 4 patients developed vaso-occlusive crisis and subsequently became CRP positive during the onset of crisis. Remaining 25 patients did not develop vaso-occlusive crisis and became CRP negative. So we described this early phase of clinical events as prodromal phase of vaso-occlusive crisis i.e. patients became CRP positive but not in crisis but ultimately developed vaso-occlusive crisis within the next few days.

By studying the group II patients, the utility of CRP level measurement during prodromal phase of vaso-occlusive crisis was analyzed. In this group out of 50 patients, 21 became CRP positive during early phase of their clinical problems and subsequently within few days 80.9% of them were developed vaso-occlusive crisis. Among the remaining CRP negative patients in the early phase of clinical problems, only 13.8% of them was developed vaso-occlusive crisis during their follow up and subsequently became CRP positive.

Discussion

The prevalence of sickle disease in Central India as per study of Shukla and Solanki (1985) is high in certain localities in Vidarbha region of Maharashtra.[12] In the SCD group, hs-CRP showed an inverse correlation with Hb, and a positive one with LDH, suggesting that baseline haemolytic

activity may be associated with inflammation. The correlation of hs-CRP with WBC counts further supported an increase in the baseline inflammation status. Study done by Sheika Salim AI Arrayed (1995)[13] found exposure to cold (45%) as the most frequent precipitating factor. Others were fever (35%), exhaustion and severe physical activity (35%). Study by Suba Krishnan et al (2010)[14] showed that 70 children with SCD at steady state evaluated by a broad panel of biomarkers representing previously examined mechanisms of pathogenicity in SCD, high sensitivity C-reactive protein (hs-CRP), a marker of low-grade, systemic inflammation, emerged as the most significant laboratory correlate of hospitalizations for pain or vasoocclusive (VOC) events.[14]

More significantly, hs-CRP levels showed a strong statistical association by appropriate regression modelling procedures with an important clinical endpoint – increased hospitalizations for pain. Typically, patients with homozygous HbSS/HbSβ^{0}Thal manifest the highest rate of vasocclusive events, as corroborated by our findings. Our data show that children with the more severe phenotype, HbSS/HbSβ^0 Thalassemia, have significantly higher baseline hs-CRP levels than those with HbSC disease. This finding suggests that the presence of ongoing 'significant subclinical' inflammation in HbSS/HbSβ^{0}Thal patients perhaps puts them at greater risk for experiencing acute vasocclusive events.[14]

Clinical diagnosis was confirmed after doing definitive and supportive laboratory investigation. Diarrhoea (26%), LRTI (16%), URTI (14%) and malaria (12%) were more prevalent infection in sickle cell patients in the present study. Gelpi AP et al (1982)[15] reported the common type of infections in sickle cell disease patients are respiratory tract infection (29%), malaria (17%), urinary tract infection (9%) and diarrhoea 15%. In present study also respiratory tract infection (30%) was more prevalent infections in sickle cell patients. Akinola NO, Stevens SM (1992)[21] also described similar type of prodormal phase in vaso-occlusive crisis. They reported in their study that in 60% of sickle cell patients' CRP level was increased during prodormal phase as compared to their CRP level in Steady state and these percentage of patients ultimately developed vaso-occlusive crisis 6 –7 days after the rise of CRP level and subsequently as crisis evolved, these patients' CRP level became decreased to normal steady state CRP level.

JF Dohery, A Singhal and GR Serjeant (1993)[16] also by their studies reported that during monitoring of steady state C-Reactive patients in sickle cell patients 54% developed increased levels of CRP. They followed up these patients and saw that ultimately these patients developed vaso-occlusive crisis during follow up. They also described this phase of steady state as prodromal phase of vaso–occlusive crisis. P Hernandez, Eva Svarch (2001)[17] studied 83 patients with sickle cell anaemia and they found increased C-reactive protein in 55% of them during the steady state and they found during the follow up of these patients that these 55% patients developed vaso-occlusive crisis within 4-5 days of CRP positivity.

Several lines of evidence suggest that SCD is associated with a chronic inflammatory state (Platt, 2000; Belcher et al, 2003)[18, 19]. In recent years there has been great interest in the role of high sensitivity C-reactive protein (hs-CRP) as a stable plasma biomarker of low-grade, chronic, systemic inflammation in predicting risk for cardiovascular disease in adults (Koenig et al, 1999; Ridker et al, 2000; Verma et al, 2005) [20-22].

Conclusion

The most common crisis in sickle cell disease is vaso-occlusive crisis which may have various modes of presentation starting from hand foot syndrome to ocular crisis. If early intervention is not done during tissue ischemia or infraction due to vaso-occlusive crisis, it will increase the morbidity in sickle cell disease patients by persisting sequelae of different organ damage. If vaso-occlusive crisis can be diagnosed as early as in prodromal phase, extent of tissue ischemia can

be minimized by early institution of therapy. Maximum number of vaso-occlusive crisis cases was found between 2-5 yr of age without any significant male female preponderance. There is no significant difference between steady state CRP status in sickle cell disease patients and healthy AA patients ($p > 1$). There is a significant difference of CRP status between non vaso – occlusive and vaso–occlusive crisis of sickle cell disease patients. About 96.3% patients of non vaso-occlusive crisis became CRP negative during their whole course of their crisis. Serum C-reactive protein levels are higher in cases of painful sickle cell vaso-occlusive crisis than control. C- reactive protein levels correlate positively with duration of pain in cases of painful sickle cell VOC.

Analysis of large number of cases with sickle cell vaso-occlusive crisis with quantitative serial measurement of CRP level is needed for better evaluation of patients during prodromal phase for effective and better management of these patients.

Conflict of Interest: None

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