A STUDY TO FIND OUT THE ROLE OF ZINC PROTOPORPHYRIN AS A DIAGNOSTIC TOOL IN ANAEMIA

By

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SBKS MEDICAL INSTITUTE & RESEARCH CENTRE

SUMANDEEP VIDYAPEETH, PIPARIA, VADODARA

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in

INTERNAL MEDICINE

Under the Guidance of

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PROFESSOR

M.D. (MEDICINE.)

DEPARTMENT OF MEDICINE

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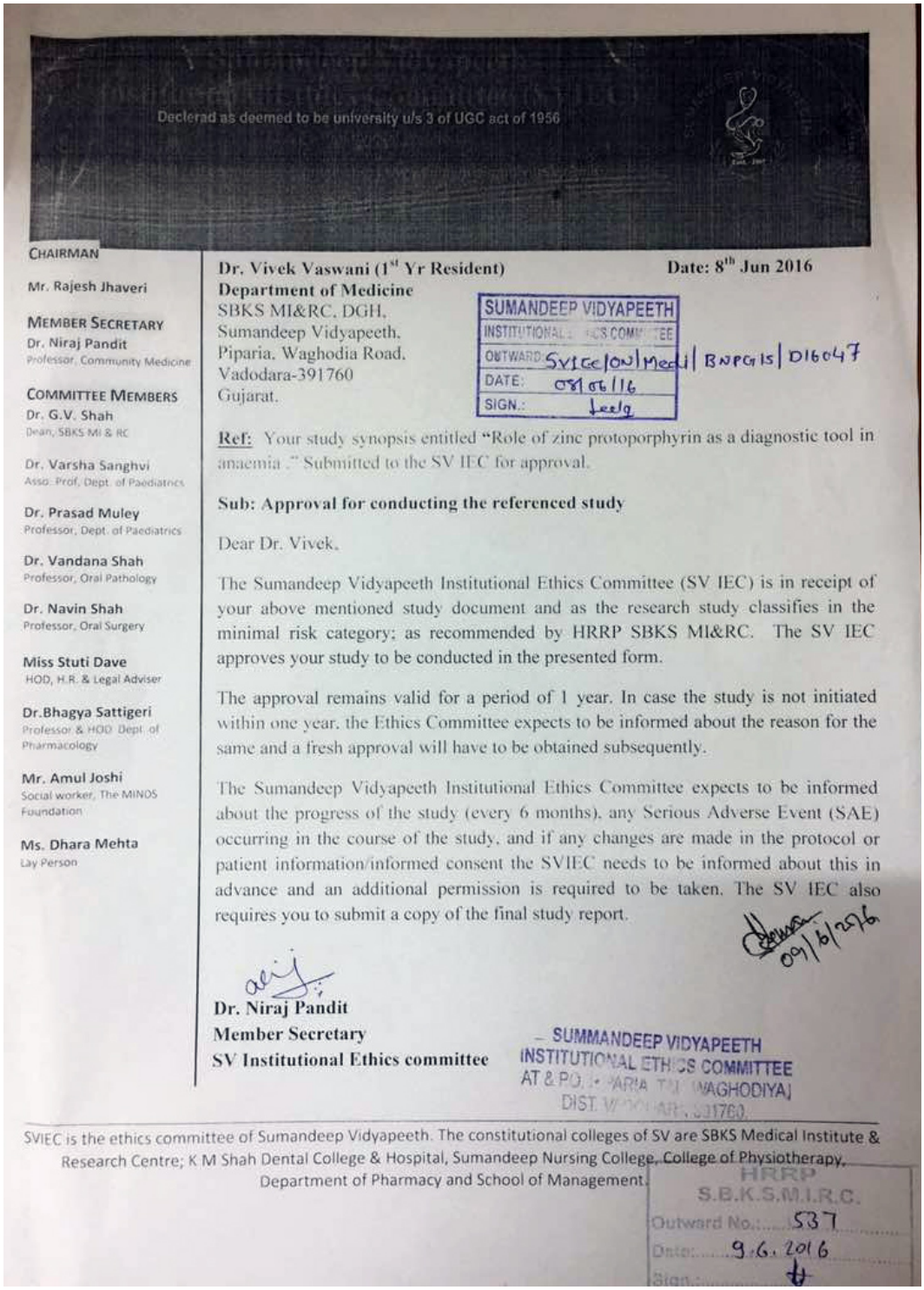

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Dr. VIVEK VASWANI

ABSTRACT

Introduction: Iron deficiency anaemia is one of the most common nutritional deficiencies in India leading to reduced work productibility, impaired mental and motor development in children and adverse outcome of pregnancy. Various and numerous tests are available for detection of Iron deficiency anaemia but some are costly and also range widely in specificity and sensitivity. Co-existence of Iron deficiency anaemia in sickle cell anaemia is often masked by the clinical features of Sickle cell disease and also because the patients are already anaemic. Zinc Protoporphyrin is a normal metabolite of heme synthesis but increases when there is depletion of Iron in the body. This study was done to determine the role of Zinc Protoporphyrin as a screening tool for the diagnosis of Iron deficiency anaemia in patients clinically presenting with features of anaemia and also in sickle cell anaemia patients.

Aim & Objective: To determine Zinc Protoporphyrin level and to correlate with other parameters of Iron Deficiency. To find out the role of ZnPP in sickling disorder.

Methodology: This study was carried out at Dhiraj hospital and consisted of 51 patients, out of which 5 patients were of sickle cell anaemia. Adult patients who were having anaemia and having clinical as well as laboratory evidence of Iron deficiency were taken for the study. They were subjected to a detailed history and clinical examination and blood investigations like CBC with indices, other haematological and biochemical investigations, sickling solubility, Iron profile and Hb electrophoresis was done. Hemoglobin analysis was done by HPLC, and Zinc Protoporphyrin was done by Haematoflourometry. Discrimination index like Mentzer and Srivastava index was also calculated.

Result: Out of 51 patients, 26 were male and 25 were female. 25(49%) out of 51 were above the age of 50 years. Out of the 5 patients of sickle cell anaemia, 3 had SCD and 2 had SCT. All the patients complained of generalized weakness and on examination had pallor. Mean Hb observed was 7.54±2.22, mean MCV was 67.56±8.49, mean MCH of 20.26±4.75, mean MCHC of 28.20±2.84, mean PCV 24.59±6.51, mean RBC

3.46±0.882, mean RDW 20.48±4.75, mean S.Iron 37.58±16.82, mean S.Ferritin 194.64±293.98 and mean TIBC was 359.63±65.54.13 patients had mild anaemia (25.4%), 18 patients had moderate anaemia (35.2%) and 20 patients had severe anaemia (39.2%). The lowest value of Zinc Protoporphyrin was 16 which was less and maximum was 577 which was more than the normal range. Out of 51 patients, 2 patients (3.92%) had Zinc Protoporphyrin in normal range, 1 patient had below normal (1.96%), 15 patients (29.4%) had in the range of 40-100, 25 patients (49%) had in the range of 101-200, 6 patients (11.7%) had in the range of 201-400 and 2 patients(3.92%) had values of more than 400. Zinc Protoporphyrin (ZnPP) represented iron deficiency in majority except in three patients in whom it was false negative. Majority of patients had blood indices in a below normal range. Mentzer Index (MI) and Srivastava Index (SI) was increased in 48 (94%) patients. In the remaining 3 patients MI was below normal in 2 and normal in 1 patient. All the three patients had SI below normal range.

Conclusion: ZnPP is a good indicator for detecting Iron deficiency particularly in patients who are hospitalized and also in those who are elderly. Though only 5 patients of Sickle cell disorder could be studied, they were iron deficient and ZnPP can be used as one of the tool to detect it.

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INTRODUCTION

Zinc Protoporphyrin (ZPP) is a compound found in red blood cells. It is a normal metabolite of heme biosynthesis. During synthesis of haemoglobin, when heme production is inhibited or decreased by lack of iron, small amount of Zinc Protoporphyrin is formed. Thus increase in zinc protoporphyrin may suggest iron deficiency. The final reaction in the biosynthetic pathway of heme is the chelation of iron with porphyrin. During periods of iron insufficiency or impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase leading to increased ZnPP *(Zinc Protoporphyrin)* formation. Evidence suggests that this metal substitution is one of the first biochemical responses to iron depletion leading to increased amount of ZnPP to appear in circulating RBC's.

The generally accepted **"gold standard"** test for iron deficiency is the determination of bone marrow iron stores, but the test is too costly and invasive for routine screening or monitoring. This limitation underscores a key benefit of ZnPP, which in fact has been shown to reflect the iron status in the bone marrow.

Serum Ferritin concentration is often considered the most suitable index for iron status. However, decreased serum ferritin concentration as a marker of iron deficiency has limitation. This is because ferritin is an acute phase protein. Like ESR, CRP and other acute phase reactant, its concentration may increase as response to acute inflammation. Thus iron deficiency with inflammatory reaction like in infection, malignancy and in other inflammatory states, it may be falsely elevated. Serum ferritin along with the determination of ZnPP/heme ratio (ZnPP/H) is considered accurate for the evaluation of many iron disorders.

Clinically ZnPP quantification is valuable as a specific & sensitive diagnostic tool for evaluating iron nutrition & metabolism. In normal persons without iron deficiency, ZPP is </=40 micromol/molheme; in patients with iron deficiency, ZPP levels are increased and show good correlation with the commonly used parameters of the iron metabolism.

There are various tests available for diagnosing iron deficiency anaemia. The “iron parameters” which are commonly used are serum iron, ferritin, saturation of transferrin, total iron binding capacity (TIBC), bone marrow smears stained with perssian blue and others. One of the simple and can be adopted as a screening test for iron deficiency is estimation of ZnPP.

Iron deficiency anaemia (IDA) is very common in India. Poor dietary intake and nutritional deficiency, multiple pregnancies, hookworm infestation, poor iron store and excessive menstruation in teenagers and young females, excessive sweating due to hot climate, mal-absorption and others may be the cause of IDA. There are reported cases of iron deficiency in sickle cell disorder. Tribal population of India has sickle cell disorder and nutritional iron deficiency; both very common, which may result in “double trouble”, contributing to severity of anaemia.

As IDA and sickle cell disorder is very common at our institute practice, this study was planned to determine role of ZnPP in patients having anaemia. Following two hypotheses was postulated

1. “Iron deficiency Anaemia could be diagnosed successfully by estimating Zinc Protoporphyrin levels”.
2. “In Sickle cell disorder patients, doing Zinc Protoporphyrin levels may suggest additional Iron deficiency Anaemia.”

AIMS AND OBJECTIVES

The Aim

To determine Zinc Protoporphyrin level and to correlate with other parameters of Iron deficiency.

Objectives

1. To estimate various haematological parameters of anaemia and to correlate with each other.

2. To find out role of ZnPP in sickling disorder.

REVIEW OF LITERATURE

A. Preamble

Numerous tests are available for detection of Iron deficiency Anaemia which are specific for different iron indices such as for storage, end product, transport & receptor.

These tests include peripheral smear examination; and looking for shape and haemoglobinization of RBC, blood indices, serum Iron, serum Ferritin, Total Iron binding capacity, soluble transferrin receptor and Zinc Protoporphyrin.

These tests range widely in specificity & sensitivity and are not able to diagnose adequately except for Bone Marrow studies which is the GOLD Standard test for detecting iron deficiency. This is a costly and an invasive test and thus it is not used for routine screening.

Serum Ferritin is considered most suitable out of all the tests used for detecting Iron deficiency but as it's an **acute phase reactant** its concentration is used in combination with the ZnPP/Heme ratio to detect iron disorders.

ZnPP is one of the tests for screening of Iron deficiency anaemia in community.

As this dissertation is concerned with Zinc Protoporphyrin, review of literature will be done first in relation to Anaemia as well as its investigatory approach and later on appraisal of Zinc Protoporphyrin as one of the test to be used in investigation of anaemia patient.

B. Anaemia and its Investigatory approach

Anaemia (an-haîma) is a Greek word meaning "without blood". It is a functional inability of the blood to supply the tissue with adequate O2 for proper metabolic function due to very less red blood cells leading to decreased amount of haemoglobin in the cells.

Anaemia may result in inability to meet the body's physiologic needs due to deficiency of haemoglobin in the blood.

Classification of Anaemia

1) Marrow production defects-Hypoproliferative
2) Red Cell maturation defects- Ineffective erythropoiesis
3) Decreased red cell survival- Blood loss or Haemolysis

If RBC's are normocytic normochromic then it is suggestive of Hypoproliferative, if they are macrocytic or microcytic then suggestive of Maturation disorder.

1) Macrocytic normochromic
    - Megaloblastic-Vitamin B12 or Folate deficiency
    - Non Megaloblastic- Alcohol, Liver disease, Hypothyroidism
2) Microcytic Hypochromic
    - Iron deficiency Anaemia
    - Thalassemia

- Anaemia of Chronic disease
- Sideroblastic Anaemia

3) Normocytic Normochromic

- Hemolytic Anaemia
- Renal failure
- Mixed deficiency
- Acute blood loss
- Aplastic Anaemia

The Approach

A proper history and physical examination is required for the evaluation of an anaemic patient. The presenting symptoms may be generalised weakness with easy fatiguability, malaise, weight loss along with other constitutional and systemic symptoms. Physical examination findings include Pallor (in mucous membranes, nail beds & palmar creases), Splenomegaly, Lymphadenopathy, Tachycardia, Koilonychia, Pedal oedema and other signs due to systemic involvement.[27]

Laboratory investigations required for the workup of anaemia are:

I. Complete blood count(CBC) which includes

- ✓ Haemoglobin
- ✓ Haematocrit

- ✓ White blood cells
- ✓ Platelets
- ✓ Red cell indices which are Mean Corpuscular Volume(MCV){if lower than normal suggestive of microcytosis, if higher then suggestive of macrocytosis}, Mean Corpuscular Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC){MCH & MCHC reflect defects in haemoglobin synthesis}
- ✓ Reticulocyte count
- ✓ Red cell distribution width (RDW)

II. Peripheral Smear: It shows the variation in the shape and size of red blood cells through which defects in red cell production can be found out. The defects that can be seen are:

- ✓ Anisocytosis
- ✓ Poikilocytosis
- ✓ Polychromasia
- ✓ Tear drop cells
- ✓ Target cells

III. Iron studies

- ✓ Serum Iron
- ✓ Total Iron binding capacity

- ✓ Serum Ferritin

IV. Bone Marrow examination

a. Aspirate

- ✓ Myeloid to Erythroid ratio
- ✓ Cell morphology
- ✓ Iron stain

b. Biopsy

- ✓ Cellularity
- ✓ Morphology

C. Zinc Protoporphyrin-Basics

Zinc Protoporphyrin (ZPP) is labelled as a "A metabolite with a mission" by Labbe RF et al. It is one of the metabolic product of heme and is found in red blood cells when heme production is inhibited or decreased by lack of iron.[1]

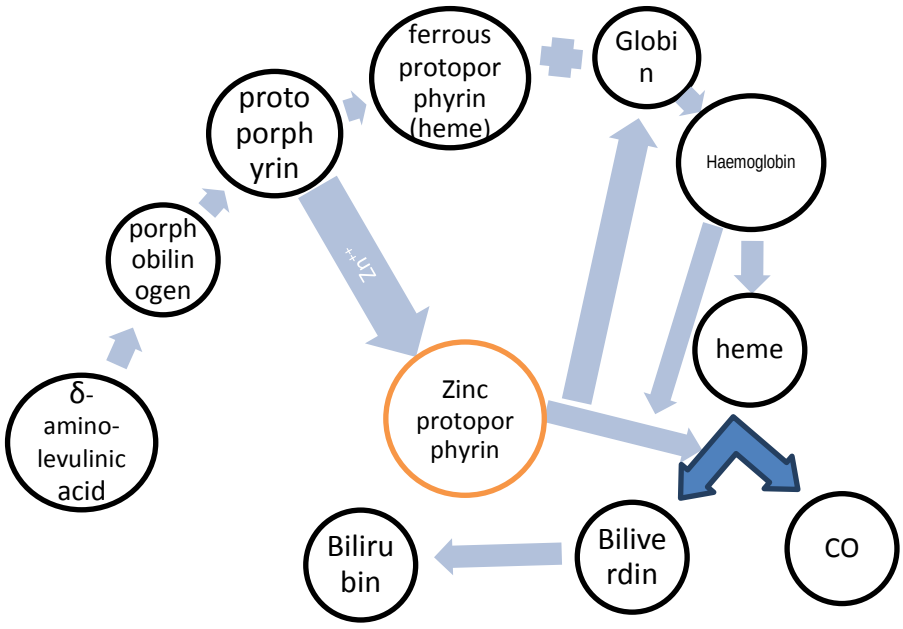
During synthesis of haemoglobin, small amount of Zinc Protoporphyrin is formed. It is a normal metabolite of heme biosynthesis and remains bound within circulating erythrocytes during their life span.[1,2]

The final reaction in the biosynthetic pathway of heme is the chelation of iron with porphyrin.

The diagram below shows relationship of heme with ZPP. Heme is also considered as ferrous Protoporphyrin which is formed from porphobilinogen in presence of enzyme

ALA dehydrogenase from delta amino levulinic acid. For production of haemoglobin ferrous Protoporphyrin is combined with globin. The haemoglobin is degraded to heme which is metabolized to biliverdin and then to bilirubin. In formation of heme apart from ALAD, zinc chelation reaction also occurs which is catalysed by ferrochelatase.

Thus ZnPP is formed in small amounts during heme biosynthesis. In Iron deficiency, zinc becomes an alternative substrate for chelation by ferrochelatase. This results into increase ZnPP formation[3]. This fact is used in diagnosis and screening of Iron deficiency anaemia.



Due to impaired heme synthesis, the presence of increased levels of ZnPP in circulating erythrocytes leads to its deposition in the spleen, and sometimes liver also.

Clinically ZnPP quantification is valuable as a specific & sensitive diagnostic tool for evaluating iron nutrition & metabolism.

In normal persons without iron deficiency, ZPP is </=40 micromol/mol heme; in patients with iron deficiency, ZPP levels are increased and show good correlation with the commonly used parameters of the iron metabolism.

Any pathological process or condition that limits the transfer of iron to or within the bone marrow or that stimulates porphyrin (heme) synthesis can potentially lead to an accumulation of ZPP.

In healthy blood donors, there is a good inverse correlation between serum ferritin and ZPP levels. Measurement of ZPP was initially done using high-performance liquid chromatography which involved separation & measurement of metal free Protoporphyrin and ZPP fluorometrically.

Later on, Hematofluorometry was developed as a consequence of observation that direct flourometric determination of ZPP was possible without prior acid extraction.

The Hematofluorometer measures the ratio of ZPP fluorescence to heme absorption as a function of the amount of light emitted by ZPP at 594 nm versus the amount of excitatory light absorbed by haemoglobin at 420 nm.[4,5]

D. Zinc Protoporphyrin & its relation to Iron deficiency Anaemia

Iron deficiency is one of the most prevalent forms of malnutrition. Globally, 50% of anaemia is attributable to iron deficiency.

The initial stage of Iron deficiency anaemia is negative iron balance in which the demand for iron does not match the body's ability of absorption of iron from the dietary sources. As a result Iron deficit is overcome by mobilization of iron stores from reticulo-endothelial storage sites. In this stage total body Iron decreases but the

haemoglobin synthesis is not impaired. The laboratory parameters of detecting Iron deficiency such as Total Iron binding capacity (TIBC), Serum Ferritin and red cell Protoporphyrin levels remain within normal limits.

Depletion of Iron stores leads to fall in serum Iron levels. Haemoglobin synthesis becomes impaired when the transferrin saturation decreases. This stage of Iron deficiency anaemia is of iron-deficient erythropoiesis.

In the final stage of Iron deficiency anaemia haemoglobin synthesis is impaired which leads to derangement of the laboratory parameters and morphological changes in the peripheral smear.[27]

Iron deficient erythropoiesis can occur in patients with inadequate (absolute iron deficiency) iron stores and also in patients with adequate iron stores.

For diagnosis of Iron deficiency anaemia Serum Ferritin is considered to be the best out of all the other laboratory parameters. But the major limitation of Serum Ferritin is that it is also an acute phase reactant and its increased levels during infection and inflammation mask iron deficiency anaemia.

A combination of a low Serum Ferritin and a low haemoglobin concentration is definitive for the diagnosis of Iron Deficiency Anaemia, but the use of Serum Ferritin alone as a marker of iron deficiency is more problematic as it may lead to a delay in diagnosis.

This is because Ferritin measures depletion of iron stores whereas ZnPP levels increase in the stage of iron deficient erythropoiesis.

Hence ZPP can be used to classify the stage of iron deficiency anaemia.[6]

During periods of iron insufficiency or impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase, leading to increased ZnPP formation.

This metal substitution is one of the first biochemical responses to iron depletion, causing increased ZnPP to appear in circulating erythrocytes.

Substitution of Zinc in place of iron predominantly occurs within the bone marrow, hence the ZnPP/heme ratio in erythrocytes reflects iron status in the bone marrow.[1,2]

Enhanced ZnPP accumulation appears in circulating erythrocytes during states of iron deficiency in the marrow. ZnPP remains bound within circulating erythrocytes during their life span.

However increase in the levels of ZPP can occur due to any condition that stimulates porphyrin synthesis. ZPP is not only the product of iron deficient erythropoiesis but also inhibits heme oxygenase which leads to slowing of heme catabolism and conservation of heme.

Detecting Iron deficiency anaemia in the stage of iron-deficient erythropoiesis requires an extensive diagnostic workup, which includes examination of Prussian blue-stained bone marrow smears and evaluation of the sideroblast count.

Though this is a **Gold Standard test**, it is an invasive, painful and a time consuming test and so cannot be performed routinely.

One of the other major laboratory marker for diagnosing Iron deficiency anaemia is soluble Transferrin receptor which also increases and detects Iron deficiency anaemia in the stage of iron deficient erythropoiesis.

Simultaneous measurement of ZPP and soluble Transferrin receptor allows in differentiation of different forms of anaemia and helps in assessment of iron metabolism as well as erythropoietic activity.

In the second stage of Iron deficiency anaemia which is iron deficient erythropoiesis levels of both, ZPP and Transferrin receptor increase.

If in anaemic patients the levels of Transferrin receptor increase and ZPP remains normal with a fall in Haemoglobin values it suggests that anaemia is not because of disturbances in iron metabolism.

It suggests that there is an increase in erythropoietic activity such as in haemolytic anaemia because levels of Transferrin receptor increase due to enhanced erythropoiesis.

The main advantage of ZPP is low cost, and it can be used as a rapid, inexpensive screening test for detecting any abnormalities in iron metabolism and in diagnosis of actual Iron deficiency anaemia.

In mild stages of Iron deficiency anaemia ZPP levels are between 41-70 µmol/molheme whereas in moderate to severe cases it goes beyond 70 µmol/mol heme and upto 180-200 µmol/mol heme in very severe cases.

Normal levels (</=40µmol/mol heme) of ZPP excludes iron deficient erythropoiesis and clinically relevant Iron deficiency.[5, 7]

E. Zinc Protoporphyrin and its relation to Sickle cell anaemia

Sickle cell anaemia is characterized by the presence of hemoglobin S (HbS) in red cells which is congenitally acquired by autosomal recessive inheritance of a pair of abnormal hemoglobin genes.

It is of two types-1] Sickle cell trait- This is the heterozygous state (HbAS), one gene from one parent is for HbS while other gene is for HbA. 2] Sickle cell disease- this is the homozygous state (HbSS), one gene inherited from each of the parents and is the more severe form.

Iron deficiency anaemia often goes unnoticed in sickle cell anaemia due to increased gastrointestinal absorption of iron which is associated with hemolysis. Also these patients receive repeated blood transfusions, which provide a sufficient source of iron.[8]

But Iron deficiency anaemia does occur in these patients because of lack of immunity to factors such as parasitic infestations, poor nutrition that leads to abnormal iron metabolism leading to Iron deficiency anaemia.

Moreover due to mucosal and submucosal infarcts and in patients with multi organ failure in sickle cell crisis iron absorption and metabolism is affected which makes sickle cell patients more susceptible to Iron deficiency anaemia.[8]

According to one study, levels of ZPP or free erythrocyte porphyrin (total porphyrin) are elevated in patients of sickle cell disease who are in steady state or in crisis. ZPP is abnormally elevated in **SS** patients with low HbF (<9%) but not in those with high HbF.

The elevated levels apart from diagnosing iron deficiency anaemia also serve as marker of the severity of the disease process.[9,10]

It has been hypothesized that iron deficiency might be beneficial to the sickle cell patient by reducing the percentage of sickle cells, thus reducing painful crisis.

F. Zinc Protoporphyrin as a diagnostic tool

There are various clinical applications of Zinc Protoporphyrin.

- Assessment of nutritional Iron status- The gold standard test for detecting iron deficiency anaemia is bone marrow studies but it is costly and an invasive procedure hence ZPP should be used as it reflects marrow iron status. A cost-effective approach for the assessment of iron status is to first determine ZnPP/H; if the result is within the reference range, then the marrow and peripheral tissues are assumed adequately supplied with iron, regardless of the ferritin concentration, which may show low iron stores in the presence of adequate tissue supplies.[11]

- Iron status in Pediatric population- Hematocrit and Haemoglobin detect iron deficiency in the stage of anaemia and misses out children who are pre-anaemic and iron depleted. Hence ZPP should be used for the early diagnosis of Iron deficient state so that early iron supplementation can be initiated.

- Detecting Iron status in pregnancy-In pregnancy because of dilution by plasma volume expansion the values of Haemoglobin, Hematocrit, Serum Iron and others may come inaccurate. ZPP has a better diagnostic sensitivity and a predictive value as ZPP and heme are equally diluted and hence avoids misinterpretation.

- Iron status in blood donors-Blood donors are routinely screened for iron deficiency, by a very simple standardized copper sulfate/hemoglobin precipitation test. Though this test detects iron-deficiency anemia, it does not accurately reflect iron status. This is because some frequent donors will have low iron stores and yet peripheral tissues can be receiving adequate iron as shown by a normal ZnPP/H. Hence using ZnPP/H for detecting iron status more volunteers become eligible for donation.

- Diagnosis of Lead toxicity- Lead leads to impairment of heme biosynthesis and also impairs iron delivery or utilization in immature erythrocytes, thereby inducing the iron deficiency like state causing increased levels of ZnPP in mature erythrocytes. Lead-intoxicated patients show the most increased ZPP levels, up to **1,000 µmol/mol heme**.

- Disorders related to impaired Iron metabolism- Patients suffering from chronic systemic inflammatory disorders develop anaemia that is associated with disturbance in iron metabolism and is called Anaemia of Chronic disease. In this disease condition, Ferritin levels and iron storage may increase and serum iron and transferrin saturation may be decreased. Low levels of serum iron leads to decreased synthesis of haemoglobin. The determination of ZPP is a simple screening procedure for the detection of iron deficiency and can be used to detect and describe the underlying pathological defect related to impaired iron metabolism.

  Patients having anaemia of chronic disease show raised levels of ZPP even as there is increase in hemosiderin levels due to decreased iron supply for erythropoiesis. This shows that increased production of ZPP does not depend on

the cause of impaired iron metabolism as it would ultimately lead to decreased Haemoglobin synthesis. Thus measurement of ZPP leads to rapid detection and quantification of impaired iron metabolism in chronic inflammatory disorders, neoplastic diseases, sideroblastic anaemia and other inflammatory disorders.[12]

- Diagnosis of Iron deficiency Anaemia in Sickle cell anaemia- Elevated ZnPP/Haem levels is an indicator of microcytic anaemia of iron deficiency. Iron deficiency Anaemia is found to be more prevalent in Sickle cell disease patients than in sickle cell trait.

G. Blood Indices and Red cell distribution width and its applicability in Anaemia.

The red blood cell indices play an important role in determining the type of anaemia. They are derived by calculating and relating haematocrit, red blood cell count and haemoglobin.[13,14]

The indices are:

- ✓ Mean Corpuscular Volume (MCV)- It measures the average size of individual of red blood cells. If it is low, the cells are said to be microcytic {smaller than normal}.If it is high the cells are said to be macrocytic {larger than normal}. The normal range is 80-94 famtolitres.

$$\text{It is calculated by:MCV (in fL)} = \frac{hematocrit \quad (inL/L)\, x\, 1000}{rbccount \quad (inmillions \quad /\mu L)}$$

- ✓ Mean Corpuscular Hemoglobin (MCH)-It measures the mean mass of haemoglobin in each RBC and is measured in picograms. The normal range is 17-31 picograms.

It is calculated by: MCH(in pg) = $\frac{hemoglobin \quad \left(in \frac{g}{dL}\right) x\ 10}{rbccount \quad (inmillions \quad /\mu L)}$

- ✓ Mean Corpuscular Hemoglobin Concentration (MCHC)-It measures the mean concentration of haemoglobin in each RBC and is expressed in g/dl. The normal range is 32-36 g/dl.

It is calculated by: MCHC (in g/dL) = $\frac{hemoglobin \quad (ing\ /dL)}{hematocrit \quad (inL\ /L)}$

Types of Anaemia according to these indices:

01. Normocytic Normochromic Anaemia- In this type of anaemia haemoglobin, haematocrit and RBC count are decreased but MCV, MCH, MCHC and RDW are normal. Causes are Anaemia due to acute blood loss, hemodilution and decreased erythropoietin secretion.

02. Microcytic Hypochromic Anaemia- In this type of anaemia there is a decrease in haemoglobin, haematocrit and RBC count along with decrease in MCV, MCH, MCHC. RDW may be increased. Causes are Iron deficiency anaemia, Anaemia of chronic disorders, disorders of globin chain synthesis (Thalassemia), Sideroblastic anaemia and lead intoxication.

03. Macrocytic Anaemia- In this type of anaemia haemoglobin, haematocrit and RBC count are decreased but the MCV and MCH are increased, MCHC is normal and RDW is increased. Macrocytic anaemia can be further divided into two types which are Megaloblastic and Non megaloblastic.

    Megaloblastic anaemia can occur due to deficiency of folic acid, vitamin B12, inherited disorders of DNA synthesis. Non megaloblastic anaemia can occur due to hypothyroidism, liver disease, alcoholism and aplastic anaemia.[13,14]

Red cell distribution width (RDW)

It is a parameter that measures variation in red blood cell size or red blood cell volume. Heterogeneity in the red cell volume is called anisocytosis. RDW quantifies the variation in red cell size and is elevated in case of anisocytosis.

It can be reported statistically in two different ways: RDW-SD (standard deviation) and RDW-CV (coefficient of variation).

RDW-SD which is expressed in famtolitres is an actual measurement of width of RBC size distribution histogram and is measured by calculating the width (in fL) at the 20% height level of the RBC size distribution histogram. It is not influenced by the average RBC size (MCV).

Normal value is 39-46 fL

RDW-CV which is expressed in % is calculated by dividing the standard deviation of the red blood cell volume by MCV.[14,15]

Normal value is 11.6%-14.6%.

One of the main factors that affects RDW is any condition that leads to change in the shape of RBC, ineffective production or increased destruction of red cells. The other main factor affecting RDW value is Erythropoietin. Low levels may increase RDW.

RDW is usually used in combination with MCV for evaluation of type of Anaemia. The conditions are as below:[15,16]

Normal RDW and low MCV is associated with the following conditions:

- Anaemia of chronic disease

- Heterozygous Thalassemia
- Haemoglobin E trait

Elevated RDW and low MCV is associated with the following conditions:

- Iron deficiency
- Sickle cell-β-Thalassemia

Normal RDW and high MCV is associated with the following conditions:

- Aplastic anemia
- Chronic liver disease
- Chemotherapy/antivirals/alcohol

Elevated RDW and high MCV is associated with the following conditions:

- Folate or vitamin B12 deficiency
- Immune hemolytic anemia
- Cytotoxic Chemotherapy
- Chronic Liver disease
- Myelodysplastic syndrome

Normal RDW and normal MCV is associated with the following conditions:

- Anemia of chronic disease
- Acute blood loss or hemolysis

- Anemia of renal disease

Elevated RDW and normal MCV is associated with the following conditions:

- Early iron, vitamin B12, or folate deficiency
- Dimorphic anemia (for example, iron and folate deficiency)
- Sickle cell disease
- Chronic liver disease
- Myelodysplastic syndrome

H. RBC discriminate indices in evaluation of Anaemia

The main purpose of using red blood cell discrimination indices is in differentiation of thalassemic and non thalassemic microcytosis. Iron deficiency anaemia and Beta Thalassemia are the two main common causes of microcytic anaemia. These indices are derived from RBC count, MCV and RDW.

There are various indices which are being used since two to three decades but Mentzer index and Srivastava index have proved to be the most accurate and reliable of all.[18]

Haematological index	**Formula**
Mentzer index (MI) (1973)	MCV/RBC
RDWI (1987)	MCV × RDW/RBC
Shine and Lal (S and L) (1977)	MCV × MCV × MCH/100
Srivastava (1973)	MCH/RBC
Green and King (G and K) (1989)	MCV × MCV × RDW/Hb × 100
Sirdah (2007)	MCV − RBC − (3 × Hb)
Ehsani (2005)	MCV − (10 × RBC)
England and Fraser (E and F) (1973)	MCV − (5 × Hb) − RBC − 3.4
Ricerca (1987)	RDW/RBC
MDHL (1999)	(MCH/MCV) × RBC
MCHD (1999)	MCH/MCV

MDHL index: mean density of Hb/liter of blood

MCHD index: mean cell Hb density.

The Mentzer index provides the highest reliabilities for differentiating β-TT from IDA. Sensitivity (98.7%), Specificity (82.3%)[19,20]

Srivastava Index: Sensitivity (IDA:79, β-TT:74%), Specificity (IDA:74% β-TT 79%)[19,20]

Index	**Iron Deficiency Anaemia**	**Beta Thalassemia**
RBC count	<5	>5
RDW-CV	>14	<14
Mentzer Index	>13	<13
Srivastava Index	>3.8	<3.8

MATERIALS & METHODS

This prospective, cross sectional study (observational) was done to determine the role of Zinc Protoporphyrin in diagnosing Iron deficiency anaemia in patients who clinically presented with signs and symptoms of anaemia which was further confirmed by preliminary investigation. Zinc Protoporphyrin was also done in Sickle cell anaemia patients who were falling in selection criteria of this study. It was carried out at Dhiraj Hospital which is attached to SBKS medical college, SumandeepVidyapeeth, Pipariya.

The study was started after getting an approval for the study from the institutional ethics committee.

The patients were enrolled in the study after taking a written informed consent for the participation in the study. First 51 patients having prescribed selection criteria and who came in my contact during various postings from Medicine department of Dhiraj hospital were taken for the study. It included outpatient as well as indoor patients. Following were the Inclusion and Exclusion criteria:

A. Inclusion Criteria

- Adult males with Hb<13g/dl.
- Adult females with Hb<12g/dl
- Those patients who were having anaemia and having clinical as well as laboratory evidence of Iron deficiency were taken for the study. Patients who had peripheral smear examination s/o Microcytic hypochromic anaemia, S.Iron<50µg/dl, MCV<92fl and Srivastava & Mentzer index more than 3.8 and 13 respectively.

B. Exclusion Criteria

- Patients below the age of 18 years
- Patients not consenting for the test & enrolment.

C. Definition of Anaemia

- Anaemia, defined as the state in which the red cell mass of blood is decreased below the normal level for the age and sex of the patient, resulting in decreased oxygen carrying capacity of blood. In this study anaemia was considered in patients who had haemoglobin less than 12g/dl in women and less than 13g/dl in men.[21]

D. Patient Selection & Data collection

- The patients who visited the OPD and those who were admitted in the wards as well as the ICU were enrolled for the study. Patients of anaemia were selected serially on "first come first choice basis", from the pool of patients whom I came in contact with as per my clinical posting. A detailed history with demographic data was taken and a thorough clinical examination was done. They were subjected to blood investigations which included Complete blood count and peripheral smear examination, RBC indices, RDW, Zinc Protoporphyrin, S.Iron, Total Iron binding capacity (TIBC), S.Ferritin, Retic Count, Sickling solubility test, Hb Electrophoresis and other haematological and biochemical tests to determine aetiology and type of anaemia. This included Coombs test, Osmotic fragility, LDH, Haptoglobin, S.bilirubin, Vitamin B12 blood levels and other tests were done in few patients as a part of further workup. Patients were also subjected to imaging such as

Chest X-ray, USG of abdomen, Endoscopy and CT scans as per requirement for diagnostic purpose of the underlying condition apart from anaemia.

- These investigations were performed in hospital's pathology, biochemistry and radiology department itself.

E. Estimation of Zinc Protoporphyrin by Haematoflourometry

- Zinc Protoporphyrin was measured by Haematoflourometry by Protoflour-Z instrument developed by Helena laboratories which is a front faced haematoflourometer designed to measure ZnPP in whole blood.

The instrument provides readings from 0-600 µmolZnPP/molheme. A sample with a greater value than this caused LED display to flash 9999. In such a case the results were reported greater than 600 µmolZnPP/molheme. Periodic calibration of the instrument was done for accuracy.

The Procedure:

Blood sample was collected in EDTA bulb from which a drop of blood was drawn using a pipette and transferred to a heparinized microhematocrit capillary tube of

50µl volume. This capillary tube was then placed in a 13x75mm test tube and after expelling the extra blood, 2 drops of Protoflour reagent was added. This mixture was used within 5 minutes of preparation. Before making a mixture of blood with reagent the instrument was warmed up for 30 minutes and was calibrated.

Method of Calibration

Calibration was done by inserting the empty sample holder alone and pressing measure button, then was removed and again inserted after placing a clean cover-slip on it and pressing blank and measure buttons on it. Then the sample holder was removed and a drop of Protoflour calibrator reagent was put on the cover slip and inserted into the instrument and Scale button was continuously pressed until the published value appeared on the LED. Then again the sample holder was removed and a drop of Protoflour low calibrator reagent was put on the cover slip and inserted into the instrument and measure button was pressed. This completed the calibration.

Measurement of Zinc Protoporphyrin

After calibration of the instrument, a drop of prepared blood sample with reagent after gently shaking was put on a new coverslip in such a way that it covered a diameter of 8-10 mm. The coverslip was placed on the sample holder and was inserted into the instrument. After pressing “measure” button, ZnPP value appeared within 5 seconds.

F. High Performance Liquid Chromatography{HPLC}

High Performance Liquid Chromatography was the test used for separation and quantification of various Haemoglobin (Hb) variants such as HbA2, HbF, HbS, HbH

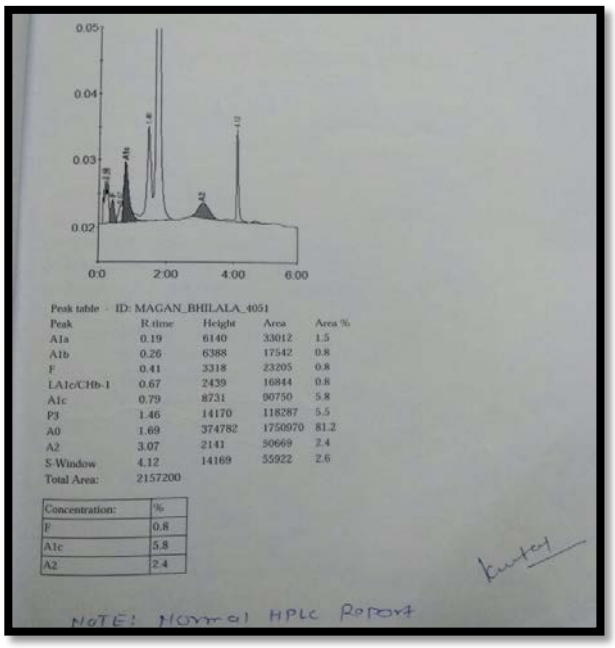
and other Hb variants in this study. HPLC testing of all the samples was done using Bio-Rad D-10 Haemoglobin analyser as shown in the photograph below.

It is based on the principle of chromatographic separation of analytes by ion exchange high performance liquid chromatography.

PROCEDURE: The amount of blood sample required was about 5 microlitres which was drawn from an EDTA bulb and mixed with lysis buffer. The sample was then injected into the analysis stream. The variants of Haemoglobin get separated by an analytical cartridge in cation exchange HPLC using a pre-programmed buffer gradient with increasing ionic strength to the cartridge. The haemoglobin fractions separate based on their ionic interaction with the cartridge. The haemoglobin fractions that are separated, pass through the flow cell of the filter photometer where changes in absorbance are measured at 415nm producing a chromatogram. Retention time of each haemoglobin was different and was measured from the time of sample injection into the HPLC to the maximum point of each peak. Unknown haemoglobin was

identified through comparison with known haemoglobin retention times. After the data was processed, a report was generated which showed the chromatogram of time against absorbance. In it various peaks are present in defined windows and their retention time, percentage and area.

Graph which was obtained of Hb analysis was like below. The values of haemoglobin sub fractions and its quantification in normal patient is shown below.



G. Sickling solubility test

This is a qualitative solubility test which detects the presence of abnormal haemoglobin (HbS) in the blood.

It was performed using Rapid Sickle Cell kit developed by BIO LAB Diagnostics.

Procedure: Blood sample was collected in EDTA bulb upto the mark of the bulb. 2ml of working reagent was put in a test tube using a reagent transfer pipette. Now one

drop of blood sample (20µl) was put in test tube and mixed. After waiting for 10 minutes interpretation of result was done.

The test tube was then placed in front of a background having black diagonal lines provided in the kit. If the background was not visible which meant that the sample had turned translucent, then the test was said to be positive.

H. The Iron Profile

This includes determination of S.Iron, S.Ferritin and TIBC.

- ✓ **Serum Ferritin**- It is an iron containing protein which is mainly found in liver and spleen. It is the storage form of Iron in the body and its measurement determines the iron status of the body.

  Low levels indicate iron deficiency anaemia whereas high levels indicate iron overload conditions such as haemochromatosis or haemosiderosis. It is also an acute phase reactant hence its levels increase in infections and inflammatory conditions.

  Procedure: It was performed by a smart card which was inserted into MISPA-i2 machine. As soon as the card was inserted R1 reagent was added to the blood sample in a curvette and placed into the curvette holder. After incubation R2 reagent was added to the curvette and result was shown on display.

  Normal value for this study in males was considered to be 20-250 ng/ml and in females it was 10-120 ng/ml.

- ✓ **Total Iron Binding Capacity**- It indirectly detects the Transferrin levels which is a glycoprotein and is responsible for binding with Iron and acts as a carrier for

Iron in bloodstream. TIBC measures the capacity of the blood to bind Iron with transferrin.

Procedure: 0.5ml of blood was mixed with 1ml of Iron chloride solution and mixed properly. After 5 minutes one tea spoon of aluminium oxide was added to it. After covering the tube was placed on a rotator for 10 minutes and at the end of 10 minutes the tube was removed and kept upright for 3 minutes. TIBC was calculated by multiplying Iron by diluent factor 3. High levels indicate Iron deficiency Anaemia.

Normal value considered for this study was 274-385 µg/dl.

- ✓ **Serum Iron-** Iron is present in haemoglobin of RBC. Its role is transportation of oxygen and cellular oxidation. Increased levels are found in haemolytic anaemia, hepatitis, lead and iron poisoning whereas decreased levels are seen in anaemia due to Iron deficiency, chronic blood loss.

  Procedure: Four test tubes were taken and Iron buffer reagents were added to these test tubes. Blood sample was added in only two tubes and distilled water was added to only one tube and Iron standard was also added only in one tube. These were kept for 5-6 minutes and then absorbance was measured by the formula.

  Normal value considered for this study was 50-150 µg/dl.

  Apart from the above mentioned tests routine blood investigations such as Complete blood count with RBC indices, RDW were also performed in all the patients.

Normal values and reference range of the blood tests taken for this study shown below:

Serial no	Test	Normal range
1	Haemoglobin	13-17 gm%
2	Total WBC count	4000-11000 cells/cumm
3	Platelets	1.5-4.5 lacs/cumm
4	RDW-CV	13-15%
5	Total RBC count	4.4-5.9 Mil/µl in males 3.8-5.2 Mil/µl in females
6	Retic count	0.5-2.5%
7	MCV	82-92 fl
8	PCV	40-53%-males 36-47%-females
9	MCH	27-31 pg
10	MCHC	32-36%
11	Zinc Protoporphyrin	20-40 µmolZnPP/molHb
12	Serum Iron	50-150 µg/dl
13	Serum Ferritin	20-250 ng/ml-males 10-120 ng/ml-females
14	TIBC	274-385 µg/dl
15	Serum LDH	150-280 U/L
16	Mentzer index	13
17	Srivastava index	3.8

RESULT

The analysis of the study is shown in a tabular form in this section. It was a observational study of 51 patients having Anaemia including Sickle cell Anaemia.

1) Age of the Patients

Age Group	Frequency	Percent
18-30	13	25.5
31-40	9	17.6
41-50	4	7.8
51-60	15	29.4
61-70	5	9.8
>70	5	9.8
Total	51	100.0

As shown in the table a total of 51 patients were taken in the study. Out of which maximum were in the age group of 51-60 years of age which was 29.4%.

2) Gender of the patients

Sex	Frequency	Percent
F	25	49.0
M	26	51.0
Total	51	100.0

This table shows that out of 51 patients, 25 were female and 26 were male patients.

3) Clinical Features of the patients

The table below shows the presenting signs and symptoms. All the patients had complaint of **generalised weakness** (100%), second most common complaint was **generalised body ache** (92.2%) and **abdominal pain** being the third most common complaint (49%). On examination **Pallor** was present in all the patients (100%). The second most common sign was **oedema** which was present in 27.5% of patients and **respiratory system** involvement which was in 25.5% of patients.

Signs & Symptoms	Frequency	Percentage (n=51)
Breathlessness	18	35.3
Pedal Oedema	14	27.5
Generalised weakness	51	100.0
Haemorrhoids	4	7.8
Generalised Bodyache	47	92.2
Abdominal Pain	25	49.0
Pallor	51	100.0
Icterus	1	2.0
Cyanosis	0	0
Clubbing	1	2.0
Lymphadenopathy	0	0
Oedema	14	27.5
RS	13	25.5
CVS	3	5.9
P/A	6	11.8
CNS	4	7.8

4) Investigatory Profile

Investigation	N	Minimum	Maximum	Mean	Std. Deviation
Hb (gm%)	51	2.0000	11.9000	7.549020	2.2267800
Total Count (cells/cumm)	51	1500	30000	8337.25	5786.777
MCV (fl)	51	53.0000	91.2000	67.56863	8.4941036
MCH (pg)	51	11.6000	36.6000	20.26471	4.7521710
MCHC (%)	51	20.6	33.1	28.204	2.8427
PCV (%)	51	8.7000	38.5000	24.59216	6.5107555
RDW-CV (%)	51	13.2000	44.2000	20.48431	4.7523204
Platelets (lacs/cumm)	51	0.20	5.8×10^5	2.62×10^5	129683.056
Retic count (%)	51	.50	7.00	1.6078	.99656
Zinc Protoporphyrin (µmolZnPP/molHb)	51	16	577	146.31	139.750
S.Iron (µg/dl)	51	14.0	145.0	37.582	16.8222
S.Ferritin (ng/ml)	51	.9000	990.0000	194.6416	293.9867467
TIBC (µg/dl)	51	135	531	359.63	65.542
RBS	51	70	229	117.02	30.688
RBC count (Mil/µl)	51	1.51	5.74	3.46	0.882

This table shows the values of Complete blood count with blood indices, RBC count, Iron profile and Zinc Protoporphyrin.

- ✓ Minimum Haemoglobin was 2gm% and maximum was 11.9gm% with a mean of 7.54±2.22
- ✓ Minimum Total count was 1500 cells/cumm and maximum was 30000 cells/cumm with a mean of 8337.2±5786.7

- ✓ Minimum Platelet count was 0.20lacs/cumm and maximum was 5.80 lacs/cumm with a mean of $2.62x10^5$±129683.05
- ✓ Minimum MCV was 53 fl and maximum was 91.2 fl with a mean of 67.56±8.49
- ✓ Minimum MCH was 11.6 pg and maximum was 36.6 pg with a mean of 20.26±4.75
- ✓ Minimum MCHC was 20.6% and maximum was 33.1% with a mean of 28.20±2.84
- ✓ Minimum PCV was 8.7% and maximum was 38.5% with a mean of 24.59±6.51
- ✓ Minimum RBC count was 1.51 Mil/µland maximum was 5.74 Mil/µl with a mean of 3.46±0.882
- ✓ Minimum RDW was 13.2% and maximum was 44.2% with a mean of 20.48±4.75
- ✓ Minimum Retic count was 0.5% and maximum was 7% with a mean of 1.6±0.99
- ✓ Minimum S.Iron level was 14µg/dl and maximum was 145 µg/dl with a mean of 37.58±16.82
- ✓ Minimum S.Ferritin was 0.9 ng/ml and maximum was 990 ng/ml with a mean of 194.64±293.98
- ✓ Minimum TIBC was 135 µg/dl and maximum was 531 µg/dl with a mean of 359.63±65.54
- ✓ The lowest value of Zinc Protoporphyrin was 16 µmolZnPP/molHb which is less than the normal range and the maximum was 577 µmolZnPP/molHb which is more than the normal range.

5) Severity of Anaemia

Grading of Anaemia	Frequency	Percentage(%)
Mild (9-12gm%)	13	25.4
Moderate (7-8.9gm%)	18	35.2
Severe (<7gm%)	20	39.2
Total	51	100

As per the above table 13 patients having haemoglobin between 9-12gm% had mild anaemia(25.4%), 18 patients having haemoglobin between 7-8.9gm% had moderate anaemia(35.2%) and 20 patients having haemoglobin less than 7gm% had severe anaemia(39.2%)

6) Range of Zinc Protoporphyrin:

Value µmolZnPP/molheme	Frequency	Percentage(%)
<20	1	1.96
20-40	2	3.92
40-100	15	29.4
101-200	25	49
201-400	6	11.7
>400	2	3.92
Total	51	100

As per the above table 2 patients(3.92%) had Zinc Protoporphyrin in normal range, 1 patient had below normal(1.96%), 15 patients(29.4%) had in the range of 40-100, 25

patients(49%) had in the range of 101-200, 6 patients(11.7%) had in the range of 201-400 and 2 patients(3.92%) had values of more than 400.

7) Range of RBC indices

A) MCV (Normal range82-92 fl)

MCV(fl)	Frequency	Percent
Below Normal (<82 fl)	48	94.1
Normal (82-92 fl)	3	5.9
High (>92 fl)	0	0.0
Total	51	100.0

As per the above table 48 patients (94%) had their MCV in below normal range and 3 patients (5.9%) had in a normal range.

B) MCH(Normal range27-31 pg)

MCH(pg)	Frequency	Percent
Below Normal (<27 pg)	47	92.2
Normal (27-31 pg)	3	5.9
High (>31 pg)	1	2.0
Total	51	100.0

As per the above table 47 patients (92.2%) had their MCH in below normal range, 3 patients (5.9%) had in a normal range and 1 patient (2%) had a higher value.

C) MCHC (Normal range32-36%)

MCHC (%)	Frequency	Percent
Below Normal (<32%)	48	94.1
Normal (32-36%)	3	5.9
High (>36%)	0	0.0
Total	51	100.0

As per the above table 48 patients (94.1%) had their MCHC in below normal range, 3 patients (5.9%) had in a normal range.

D PCV(Normal range:40-53%-Males , 36-47%-Females)

PCV(%)	Frequency	Percent
Below normal (<40%-M,<36%-F)	51	100.0
Normal (40-53%-M,36-47%-F)	0	0.0
High(>53%-M, >47%-F)	0	0.0
Total	51	100.0

As per the above table all the patients (100%) had their PCV in below normal range.

8) Range of Iron profile parameters

A) S.Iron:(Normal range 50-150 µg/dl)

S.Iron(µg/dl)	Frequency	Percent
Below Normal (<50 µg/dl)	50	98.0
Normal (50-150 µg/dl)	1	2.0
High (>150 µg/dl)	0	0.0
Total	51	100.0

As per the above table, S.Iron of 50 patients (98%) were in a below normal range and 1 patient (2%) had in a normal range.

B) S.Ferritin:(Normal range:20-250 ng/ml-Males,10-120 ng/ml-Females)

S.Ferritin(ng/ml)	Frequency	Percent
Below Normal (<20 ng/ml-M, <10 ng/ml-F)	11	21.5
Normal (20-250 ng/ml-M, 10-120 ng/ml-F)	25	49.0
High(>250 ng/ml-M, >120 ng/ml-F)	15	29.4
Total	51	100.0

As per the above table, S.Ferritin of 11 patients (21.5%) were in a below normal range, 25 patients (49%) had in a normal range and 15 patients had higher values.

C) TIBC:(Normal range;274-385 µg/dl)

TIBC(µg/dl)	Frequency	Percent
Below Normal (<274 µg/dl)	6	11.8
Normal (274-385 µg/dl)	21	41.2
High (>385 µg/dl)	24	47.1
Total	51	100.0

As per the above table, TIBC of 6 patients (11.8%) were in a below normal range, 21 patients (41.2%) had in a normal range and 24 patients (47.1%) had higher values.

9) Correlation of Zinc Protoporphyrin with RBC indices in non-sickle patients.

		MCV	MCH	MCHC	PCV
Zinc Protoporphyrin	Pearson Correlation	-.093	-.054	-.018	-.124
	P-value	.537	.719	.904	.413
	N	46	46	46	46

As per the above table there is a weak correlation between Zinc Protoporphyrin and RBC indices as the value is less than 0.4.

10) Correlation of Zinc Protoporphyrin with Iron profile parameters in non-sickle patients

		Zinc Protoporphyrin
S.Iron	Pearson Correlation	0.005
	P-value	0.976
	N	46
TIBC	Pearson Correlation	-0.234
	P-value	0.117
	N	46
S.Ferritin	Pearson Correlation	-.030
	P-value	.842
	N	46

As per the above table there is a weak and a positive correlation between Zinc Protoporphyrin and S.Iron and a weak and negative correlation between TIBC and Zinc Protoporphyrin and Ferritin and Zinc Protoporphyrin.

11) Correlation of RBC count and RBC indices with Iron profile parameters in non-sickle patients

The table below shows non-correlation or non-significant correlation between RBC count and RBC indices with Iron profile parameters in non-sickle patients

		S.Iron	**S.Ferritin**	**TIBC**
RBC	Pearson Correlation	-.220	-.100	-.065
	P-value	.142	.509	.670
	N	46	46	46
MCV	Pearson Correlation	.227	.197	.065
	P-value	.130	.189	.669
	N	46	46	46
MCH	Pearson Correlation	.265	.162	-.264
	P-value	.076	.282	.076
	N	46	46	46
MCHC	Pearson Correlation	.290	.087	.020
	P-value	.050	.565	.897
	N	46	46	46
PCV	Pearson Correlation	-.107	.056	.065
	P-value	.478	.710	.669
	N	46	46	46

12) <u>Comparison of Iron profile parameters in sickle and non-sickle patients.</u>

As per the table,

- ✓ Mean value of S.Iron in non-sickle patients (N=46) is 37.711±17.6613 and in sickle patients (N=5) it is 36.4±5.1284 with a p-value of 0.871.
- ✓ Mean value of S.Ferritin in non-sickle patients (N=46) is 193.255±290.979 and in sickle patients(N=5) it is 207.4±357.207 with a p-value of 0.920
- ✓ Mean value of TIBC in non-sickle patients (N=46) is 358.80±67.715 and in sickle patients (N=5) it is 367.20±45.085 with a p-value of 0.789.

Test		N	Mean	Std. Deviation	t-value	p-value
S.Iron	Sickle	5	36.400	5.1284	-.164	.871
	Non Sickle	46	37.711	17.6613		
S.Ferritin	Sickle	5	207.400	357.207	.101	.920
	Non Sickle	46	193.255	290.979		
TIBC	Sickle	5	367.20	45.085	.269	.789
	Non Sickle	46	358.80	67.715		

13) Comparison of RBC count and RDW in sickle and non-sickle patients

Test		N	Mean	Std. Deviation	t-value	p-value
RBC	Sickle	5	3.238000	.5356958	-.611	.544
	Non Sickle	46	3.493261	.9125156		
RDW	Sickle	5	27.360000	10.3299564	3.848	<0.001
	Non Sickle	46	19.736957	3.1281062		

As per the above table,

- ✓ Mean value of RBC count in sickle patients(N=5) is 3.238±0.5356958 and that in non-sickle patients(N=46) it is 3.493261±0.9125156 with a p-value of 0.544
- ✓ Mean value of RDW (Red cell distribution width) in sickle patients (N=5) is 27.36±10.3299564 and that in non-sickle patients (N=46) it is 19.736957±3.1281062 with a p-value of <0.001.

14) Comparison of Iron profile parameters in Sickle cell patients

Test		N	Mean	Std. Deviation
S.Iron	SCD	3	36.000	6.000
	SCT	2	37.000	5.656
	Total	5	37.582	16.822
S.Ferritin	SCD	3	331.667	444.167
	SCT	2	21.000	1.4142
	Total	5	194.641	293.986
TIBC	SCD	3	354.00	58.404
	SCT	2	387.00	1.414
	Total	5	359.63	65.542

As per the above table,

- ✓ Mean value of S.Iron in Sickle cell disease (SCD) patients is 36±6 whereas in Sickle cell trait (SCT) patients it is 37±5.656
- ✓ Mean value of S.Ferritin in Sickle cell disease patients is 331.667±444.167 whereas in Sickle cell trait it is 21±1.4142
- ✓ Mean value of TIBC in sickle cell disease patients is 354±58.404 whereas in sickle cell trait patients it is 387±1.414

15) Correlation of RBC indices with Iron profile parameters and Zinc Protoporphyrin in sickle cell patients

As per the below table, there is a negative and weak correlation between MCV and Zinc Protoporphyrin, and a negative and good correlation between MCH and Zinc Protoporphyrin, MCHC and Zinc Protoporphyrin and PCV and Zinc Protoporphyrin.

		S.Iron	**S.Ferritin**	**TIBC**	**Zinc Protoporphyrin**
MCV	Pearson Correlation	-.296	-.228	.142	-.223
	P-value	.629	.712	.819	.719
	N	5	5	5	5
MCH	Pearson Correlation	-.198	-.023	-.410	-.518
	P-value	.749	.971	.493	.371
	N	5	5	5	5
MCHC	Pearson Correlation	-.221	.524	-.692	-.550
	P-value	.721	.365	.196	.337
	N	5	5	5	5
PCV	Pearson Correlation	.081	.613	-.677	-.904*
	P-value	.897	.272	.209	.035
	N	5	5	5	5

16) Correlation of Iron profile parameters with Zinc Protoporphyrin in sickle patients

As per the below table there is a good correlation between S.Ferritin and Zinc Protoporphyrin and between TIBC and Zinc Protoporphyrin and a weak correlation between S.Iron and Zinc Protoporphyrin.

Test		**Zinc Protoporphyrin**
S.Iron	Pearson Correlation	.062
	P-value	.921
	N	5
S.Ferritin	Pearson Correlation	-.533
	P-value	.355
	N	5
TIBC	Pearson Correlation	.552
	P-value	.335
	N	5

DISCUSSION

1. Why the need for this study?

Anaemia is considered to be an important health problem worldwide and especially in India . As per WHO, two billion people in the world suffer from anaemia and half of them suffer from Iron deficiency anaemia. Anaemia is a late indicator of Iron deficiency. The estimated prevalence of anaemia in India as per National Family health Survey carried out in 2005-2006, 55% of females and 24% of males were detected to have anaemia.[22,23]

Moreover Iron deficiency anaemia has been a major cause for disability in people of a younger age group as well as in the older people which leads to decreased productivity, cognitive impairment, and poor pregnancy outcome affecting the general health of the person.

Hence detection of Iron deficiency anaemia at the earliest with minimal investigations is very important to prevent disability and improve health status of the people.

This study was carried out with an intention to discover a newer, cheaper and a specific tool to diagnose iron deficiency in the early stages of Iron deficiency anaemia.

Various tools for diagnosis of Iron deficiency anaemia are a Complete blood count with RBC indices, S.Iron, S.Ferritin and TIBC. Zinc Protoporphyrin (ZnPP) is normally produced in small amounts during synthesis of heme. When there is decrease in the amount of Iron the concentration of ZnPP in erythrocytes is increased.

This increase in the amount of ZnPP is considered to be the first biochemical response to depletion of Iron.

As a result this study was done to find out the role of ZnPP as a screening tool in early diagnosis of Iron deficiency anaemia.

2. Discussion on Methodology

In this study patients who had clinical signs and symptoms of anaemia as well as laboratory evidence of Iron deficiency anaemia who presented in the OPD and admitted in the wards and ICU were taken for the study. Purpose of such selection criteria was to carry out iron profile in suspected cases of Iron deficiency anaemia and to correlate with ZnPP. Clinical suspicion of Iron deficiency may include history of "Pica" meaning cravings for non-nutritive substances. This may include history of pagophagia (ice chewing), geophagia (eating clay or mud), chewing dirt, paper or starch and other substances. Reason for such perverted appetite is not known. Clinical signs which are related with iron deficiency anaemia are Koilonychia and brittle nails. Plummer–Vinson syndrome (Paterson–Brown–Kelly syndrome) may be associated with Iron deficiency which results into dysphagia. This is also known as sideropenic dysphagia which is due to web formation in oesophagus and related with iron deficiency.

In patients who had symptoms of anaemia including symptoms specific for iron deficiency, laboratory evidence of Iron deficiency were looked for, like peripheral smear examination s/o Microcytic hypochromic anaemia, S.Iron value less than 50,MCV less than 92fl and Srivastava & Mentzer index more than 3.8 and 13 respectively. This combined criteria was taken as one investigation but cannot be made a “gold standard” for the diagnosis. Microcytosis can also be present in

Thalassemia and to differentiate Thalassemia from Iron deficiency, Srivastava & Mentzer index were calculated.

Laboratory investigations included Complete blood count with RBC indices, RDW, Zinc Protoporphyrin, S.Iron, S.Ferritin and TIBC, Retic count, Sickling solubility, Hb electrophoresis and other haematological investigations as per requirement for diagnosis of other underlying conditions. Though study was pertaining to iron deficiency, we may not be sure of other causes and thus these investigations were carried out. Out of 51 patients, 46 patients were thought to have iron deficiency. These are included as "non-sickle group in this study". As per the study hypothesis that sickle cell disorder patients also have iron deficiency, 5 patients falling in inclusion criteria and had positive sickling solubility test and HPLC proven sickle cell disease or trait were also taken in the study. Out of the 51 patients, 5 patients had sickle cell haemoglobinopathy out of which 3 had sickle cell disease and 2 had sickle cell trait.

HPLC (High Performance Liquid Chromatography) has become the method of choice as it is an accurate and a cost effective method for the diagnosis of Haemoglobinopathies and also used nowadays for the measurement of HbA1C levels. HPLC is also considered better for accurate quantification of haemoglobin concentration especially like Hb F and Hb A2. Other advantage is of complete automation and is less time consuming.[24]

Estimation of ZnPP was done by Haematoflourometry using a Protoflour Z instrument. This instrument works on the principle that after placing the coverslip on the sample holder and inserted into the instrument, light from a quartz-halogen lamp is collected and filtered by a lens filter system to produce light at 415nm and is

focussed onto the sample. When the sample is exposed to 415nm light ZnPP is excited and emits light at 595nm. A second lens filter system collects, filters and focuses the 595nm light onto a photomultiplier tube (PMT). The PMT produces a current level in response to the light reaching it which is proportional to the ZnPP/Heme ratio. 1000 light level readings are taken within 5 seconds of pressing measure button and averaged by the microcomputer and a value is displayed. However there are limitations which includes following:

A. Blood samples which have raised levels of bilirubin should not be used as they would create a positive inference leading to high values due to its spectral qualities.

B. Blood samples which have increased levels of Riboflavin (10 times greater than normal) may also lead to false high levels. This may occur in patients receiving vitamin supplementations.

Normal value of Zinc Protoporphyrin in this study was considered between 20-40µmol zinc Protoporphyrin/molHb. This range was established by our laboratory and is one of the tests in menu of investigation offered by the Central Laboratory.

3. Age, Gender and Clinical profile of patients in this study.

In this study 51 patients were studied out of which maximum patients were in the age group of 51-60 years of age followed by 18-30 years of age.

There were 26 male patients and 25 female patients. The most common presenting symptom was generalised weakness followed by generalised bodyache. **(Table-1,2,3)**

According to various studies carried out in the past prevalence of anaemia has been found more in younger age girls than in males but in this study the prevalence is more in males than in females which may be attributed to more number of patients being middle aged. According to WHO (World Health Organization), the prevalence of anaemia increases after the age of 50 by 10%. It also suggests that one third of older adults having anaemia is because of nutritional deficiency which is mostly due to Iron deficiency and other causes because of folate or Vitamin B12. The remaining third have because of renal insufficiency and chronic inflammatory conditions.

Also according to a nationwide survey the most common symptom was weakness along with decreased work productivity which correlates with this study also.

4. Haemoglobin and blood indices in study patients

In our study there was a great variation in the haemoglobin values of the patients.

The minimum haemoglobin was 2gm% which was in a 46 year old female who was suffering from a Left maxillary tumour. The maximum haemoglobin was 11.9gm% which was in a 20 year old male patient.**(Table-4)**

The mean haemoglobin found was 7.54±2.22. Out of the 51 patients, 20 patients (39.2%)had haemoglobin levels less than 7gm% which was considered as severe anaemia, 18 patients(35.2%) had haemoglobin in the range of 7gm%-9gm% which was considered as moderate anaemia and 13 patients(25.4%) had haemoglobin in the range of 9gm%-12gm% which was considered as mild anaemia.**(Table-5)**

Minimum MCV observed was 53 and the maximum was 91.2 with a mean of 67.56±8.49.**(Table-4)**48 patients(94%) had their MCV in below normal range and 3 patients(5.9%) had in a normal range**(Table-7A)**

Minimum MCH observed was 11.6 and the maximum was 36.6 with a mean of 20.26±4.75.**(Table-4)**47 patients(92.2%) had their MCH in below normal range, 3 patients(5.9%) had in a normal range and 1 patient(2%) had a higher value.**(Table-7B)**

Minimum MCHC observed was 20.6 and the maximum was 33.1 with a mean of 28.20±2.84.**(Table-4)**48 patients(94.1%) had their MCHC in below normal range, 3 patients(5.9%) had in a normal range**(Table-7C)**

Minimum PCV observed was 8.7 and the maximum was 38.5 with a mean of 24.59±6.51.**(Table-4)**All the patients(100%) had their PCV in below normal range.**(Table-7D)**

All the minimum RBC indices were in the same patient whose haemoglobin was 2gm%. This suggests that as the age increases the incidence of inflammatory conditions and debilitating conditions increase which leads to increased tendency of developing anaemia particularly Iron deficiency anaemia.

As patient's selection criteria included MCV, we did not find higher MCV value in study patients. Red Blood Cell (RBC) Indices are helpful in quantification, diagnosis and classification of anaemia. The RBC indices describe the size, shape & Hb content of RBC. Conflicting reports are available in literature regarding values of blood indices in sickle cell disorder. Most of the reported cases of sickle cell disorder have documented normal or increased MCV. Few of the reports especially in India have suggested decreased in MCV. As we had taken patients of sickle cell disorder having decrease MCV values, we have missed iron deficiency in patients having sickle cell disorder and high MCV. There is paucity of data on blood indices of SCD from India

5. Zinc Protoporphyrin and its relation with Iron profile and RBC indices

The usefulness of ZnPP has not been properly studied. The detection of Iron deficiency anaemia may be difficult in case of hospitalized patients who usually have multiple disorders that could affect the interpretation of routinely performed investigations for Anaemia. ZnPP has been used for monitoring the iron status in pregnancy, lead poisoning, and blood donation in healthy individuals but widespread applications in detecting Iron deficiency anaemia have been limited.

Hastka et al. have suggested that ZnPP should be performed with other tests also to classify the degree of iron deficiency.

In another study, all patients were having MCV levels in a below normal range. Iron profile was also done where S.Iron was not considered very reliable as the hospitalized patients might have received Iron supplementations. Out of the three parameters Ferritin was considered to be the most reliable but as it is an acute phase reactant it may be falsely elevated in the presence of illness. Hence ZnPP was done for the diagnosis of Iron deficiency anaemia. The usefulness of ZPP is not limited to the detection of IDA. In the normocytic population, the patients with low ferritin who also have high ZnPP presumably are in the stage of iron depletion.

In our study also the indices of almost all the patients were in a below normal range, S.Iron levels of 50 patients were in a below normal range, S.Ferritin of 11 patients(21.5%) were in a below normal range, 25 patients(49%) had in a normal range and 15 patients had higher values and TIBC of 6 patients(11.8%) were in a below normal range, 21 patients(41.2%) had in a normal range and 24 patients(47.1%) had higher values.(Table-8A,B,C)

Zinc Protoporphyrin levels of 48 patients were increased which is suggestive that the patients either were in a stage of Iron deficient erythropoiesis or had completely developed Iron deficiency anaemia.

6. Role of Zinc Protoporphyrin in Iron deficiency Anaemia

Central theme of this study was to evaluate ZnPP as a marker for Iron deficiency. If we conclude that all 51 patients had iron deficiency, then in 2 patients (3.92%) Zinc Protoporphyrin was in normal range which was 20-40 µmolZnPP/molheme. In one patient (1.96%) it was below normal. Thus in these 3 patients out of 51(5.88%), it gave false negative results. Incomplete oxygenation of haemoglobin may produce falsely low ZnPP values because of a shift in haemoglobin absorption[30]. A reagent that converts haemoglobin to cyanomethemoglobin is available, but washing the cells to remove interfering substances oxygenates haemoglobin and eliminates the need for the reagent. False positive result can be obtained if ZnPP is directly measured in whole blood with a hematofluorometer. This may be because of interfering substances in plasma produced by acute inflammation and haemolysis which can increase ZnPP concentrations 3–4-fold times in the absence of iron deficiency[28]. These interfering substances can be removed by washing the erythrocytes, which markedly improves assay specificity[28,29] .

Though precautions were taken to avoid technical shortfall, this can be considered as limitation of falsely low ZnPP values because of a shift in haemoglobin absorption[6].

ZnPP in 15 patients(29.4%) was in the range of 40-100,in 25 patients(49%) in the range of 101-200, 6 patients(11.7%) had in the range of 201-400 and 2 patients(3.92%) had values of more than 400 (Table-6).Thus of 51,in 48 it was higher giving good prediction of iron deficiency.

There was a weak correlation between Zinc Protoporphyrin and RBC indices and Zinc Protoporphyrin and Iron profile parameters in Iron deficiency anaemia patients. In fact ZnPP will be elevated in pre-anaemic stage and it will reflect iron status as a whole and not only serum iron. It can reflect bone marrow iron status as well. In comparison, haematocrit and haemoglobin can, by definition, diagnose iron deficiency only at the stage of anaemia. In fact ZnPP is recommended for screening for iron status. If we know them earlier of iron depletion state, iron supplementation may help them before stage of alter physiology and anaemia sets in[31,32]. In children it may promote growth. On the contrary, iron depletion may cause impaired motor and cognitive development[33].In sickle cell disorder and in other haemoglobinopathies where iron depletion rather than iron overload is reported, this iron supplementation may improve anaemia. Although haemoglobin and haematocrit are simple tests to perform, they are neither as sensitive nor as specific as ZnPP/H, which can be used effectively in routine practice[34,35]

In this study, there was a good correlation between S.Ferritin and Zinc Protoporphyrin and between TIBC and Zinc Protoporphyrin in sickle cell anaemia patients. As number of patients was only 5, it can be said that in sickle cell anaemia ZnPP may indicate iron deficient state like other iron parameters.

Many times the conclusion is based on one test which is head-on compared with other. Ferritin as a marker may be compared with ZnPP. We did not get significant correlation in decrease in Ferritin with degree of increase in ZnPP. This may occur as both may be affected at different grade and level. If both are taken as complimentary tests, it may give more idea of iron status and underlying pathophysiological disturbances.

Following table shows value of both as complimentary investigation rather than competitive.

Interpretation of ZnPP/H in the diagnosis of Iron disorders[36]

Lab result	Interpretation	Recommendations
Low ZnPP/H(<60 µmolZnPP/molheme)	Adequate systemic iron supply	Iron stores can be determined using S.Ferritin
Mid range ZnPP/H(60-80 µmolZnPP/molheme)	Possible non replete iron status	Hb or Haematocrit from CBC may support a state of Iron depletion
High ZnPP/H-low Ferritin(>80 µmolZnPP/molheme plus low to normal Ferritin)	An indication of iron deficient erythropoiesis attributable to low marrow iron supply possible related to depleted iron stores	Iron supplementation indicated
High ZnPP/H-high Ferritin(>80 µmolZnPP/molheme plus normal to high Ferritin)	Severe inflammatory block, anaemia of chronic disease	Correct cause of impaired iron utilization, effectiveness of iron supplementation limited.

7. Role of Zinc Protoporphyrin in Sickle Cell disorder

One of the most interesting finding which Indian workers have observed and are observing is presence of Iron deficiency anaemia in patients having haemoglobinopathies. This is truer with sickle cell disorder as they come from tribal area and their nutrition may be affected by poverty and illiteracy. In the Western literature they mention more of Iron overload in patients having haemolytic anaemia. This may be true in patients of Thalassemia who receive repeated blood transfusions however as far as sickle cell disorder is concerned they may be iron deficient. Giving

Iron to such patients may improve their haemoglobin and patient's gravity of symptoms can be decreased.

Zinc Protoporphyrin is basically a marker of Iron depletion in the body which can detect Iron deficiency anaemia in its second stage.

Iron deficiency in sickle cell anaemia is not generally considered as a major problem as these patients receive repeated blood transfusions and also there is increased absorption of iron from the gastrointestinal tract. But there are various factors such as poor nutrition, parasitic infestations and decreased immunity which can lead to Iron deficiency in SCA patients. Moreover there is excessive urinary Iron loss and mucosal and submucosal infarcts which causes decreased gastrointestinal absorption of Iron which makes SCA patients susceptible to IDA.[37]

The routine investigations which are done for diagnosis of Iron deficiency anaemia such as S.Iron, S.Ferritin and TIBC may be abnormal as S.Iron and S.Ferritin are acute phase reactants and SCD patients mostly present in crisis which is characterized by acute inflammatory state preceded by infection. As a result ZnPP levels are to be determined for the diagnosis of IDA in SCA patients.[37, 38]

According to a study conducted in 2011-2013 at Surat, Gujarat in 155 patients of Sickle cell anaemia, ZnPP was elevated in 45 patients and out of these 45 patients, 7 met the criteria of Iron deficiency which were low serum iron, low MCV, low S.Ferritin and increased level of ZnPP.

Also in a study conducted by Mohanty et al. using the single criteria of ZPP/heme ratio (ZPP/heme) for diagnosis in Indian subjects reported iron deficiency in 67.7% of SCDs patients and 26.2% of sickle cell trait patients.[39]

In another study conducted at Heredity Clinic of the Comprehensive Sickle Cell Center of the Albert Einstein College of Medicine at New York they found that ZPP is abnormally elevated in SCD patients with low HbF (<9%) and not in those with HbF>9%.

Our study consisted of 51 patients, out of which 5 patients were of Sickle cell haemoglobinopathy. Out of those 5 patients, 3 were of SCD and 2 of SCT.

ZnPP was increased in 3 of the 5 patients and out of those 3 patients 2 were of SCT and 1 was of SCD. In the remaining 2 patients 1 had in the normal range and other had below the normal value. All the 5 patients had S.Iron levels, MCV levels, MI and SI which met the criteria for the diagnosis of IDA according to my study. Also HbF was less than 9% in 4 out of 5 patients and was 10.1 in 1 patient which was of SCD. Many more patients could have been enrolled if our selection criteria was not based on S.Iron level as ZnPP not only reflects S.Iron but also bone marrow Iron and tissue Iron and doing ZnPP screening test in sickle cell disorder may become pointer towards Iron deficiency and treating such patients with Iron supplements may decrease their morbidity.

8. Importance of Mentzer and Srivastava index.

There are various discrimination indices which are used to differentiate between β-Thalassemia and Iron deficiency anaemia as these two conditions are very frequent and common cause of Microcytic anaemia.

It is important to differentiate between these two conditions as to find out the cause of the condition as the further workup and management depends upon the etiology.[25]

A study of 384 patients was carried out in Tunisia in 2011 to determine the diagnostic reliability of various discrimination indices in as compared to RBC indices and RDW in differentiating β-Thalassemia from Iron deficiency anaemia and differentiating Sickle cell disease from Sickle cell Thalassemia.

They found out that Mentzer index and Srivastava index were the most reliable indices in differentiating between β-Thalassemia and Iron deficiency anaemia and other indices were reliable in differentiating between Sickle cell disease and Sickle cell Thalassemia.

In our study both the indices were increased in 48 patients(94%) pointing towards Iron deficiency anaemia. In the remaining 3 patients Mentzer index was below normal in 2 patients and normal in one patient and all the three patients had Srivastava index in a below normal range. Though these indices are considered to be very much reliable for distinguishing between Thalassemia and Iron deficiency anaemia patients with low or normal indices were proved to have Iron deficiency anaemia as per the other investigatory parameters.

SUMMARY AND CONCLUSION

This study was carried out to determine the role of Zinc Protoporphyrin as a screening tool for diagnosis of Iron deficiency anaemia in patients who presented with clinical signs and symptoms of anaemia and also to find out the presence of co-existing Iron deficiency anaemia in sickle cell anaemia patients.

According to studies carried out in the past, there was a belief that zinc Protoporphyrin levels are elevated in case of Iron depletion hence the study was done to find out the role of ZnPP as compared to routine investigations such as blood indices and Iron profile parameters.

51 patients were analysed in the study, out of which 26 were male and 25 were female, 46 patients were of Iron deficiency anaemia and 5 were of sickle cell anaemia. Out of those 5, 3 had sickle cell disease and 2 had sickle cell trait.

The most common presenting complaints were generalised weakness, fatigue and generalised bodyache. All the patients presented with these complaints. The other complains were abdominal pain, breathlessness, pedal oedema and others.

The minimum haemoglobin was 2gm% and the maximum was 11.9gm% with a mean of 7.54±2.22. 39.2% patients had severe anaemia, 35.2% had moderate anaemia and 25.4% had mild anaemia. The RBC indices of most of the patients were in a below normal range and had microcytic hypochromic picture in blood smear.

There was a weak correlation between Zinc Protoporphyrin and RBC indices and Zinc Protoporphyrin and Iron profile parameters in Iron deficiency anaemia patients

whereas there was a good correlation between S.Ferritin and Zinc Protoporphyrin and between TIBC and Zinc Protoporphyrin in sickle cell anaemia patients.

S.Iron levels of 50 patients were in a below normal range, S.Ferritin levels of 11 patients were below normal, 25 had in a normal range and 15 had increased levels. TIBC was high in 24 patients, 21 patients had in a normal range and 6 patients had in a below normal range.

But Zinc Protoporphyrin was elevated in 48 patients and was normal and below normal in 2 patients and 1 patient respectively. Thus in 3 out of 51 patients (5.88%) it gave false negative results.

The values of Mentzer and Srivastava index were raised in 48 patients. This suggested Iron deficiency anaemia and ruled out associated Thalassemia.

Hence it can be concluded by stating that ZnPP is good indicator of detecting Iron deficiency particularly in patients who are hospitalized and also in those who are elderly. Though only 5 patients of Sickle cell disorder could be studied, they were iron deficient and ZnPP can be used as one of the tool to detect it.

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LIST OF ABBREVIATIONS

ALAD-Amino Levulinic acid Dehydrogenase

ALA- Amino Levulinic acid

β-TT- Beta Thalassemia trait

CBC-Complete Blood Count

Hb-Haemoglobin

HbA- Haemoglobin A

HbF- Fetal haemoglobin

HbH- Haemoglobin H

HbSS- Haemoglobin SS (Homozygous state)

HbAS- Haemoglobin AS (heterozygous state)

HPLC-High Performance Liquid Chromatography

IDA- Iron deficiency anaemia

LDH- Lactate dehydrogenase

MCV-Mean Corpuscular Volume

MCH-Mean Corpuscular Haemoglobin

MCHC-Mean Cell Haemoglobin Concentration

MI-Mentzer Index

PCV-Packed cell volume

PMT- Photo Multiplier tube

RBC-Red blood cell

RDW-CV-Red cell distribution width coefficient of variation

RDW-SD-Red cell distribution width standard deviation

RDWI- Red cell distribution width Index

SI-Srivastava Index

SCA-Sickle cell Anaemia

SCD-Sickle cell disease

SCT-Sickle cell trait

TC-Total count

TIBC-Total Iron binding capacity

ZnPP/ZPP-Zinc Protoporphyrin

KEY TO MASTER CHART

1-Present

2-Absent

1-Abnormal

2-Normal

1-Positive

2-Negative

N/A-Not available

SCD-Sickle cell disease

SCT-Sickle cell trait

LIST OF TABLES

PROFORMA/QUESTIONNAIRE

Name of the patient:

Age:

Sex:

Address:

Occupation:

Education:

Marital status:

Income:

Date of admission:

Date of examination:

IPD/OPD number

C/C:

Breathlessness- Y___N___

Pedal Oedema- Y___N___

Generalised Weakness- Y___N___

Giddiness- Y___N___

Generalised bodyache- Y___N___

To be specified if others:

O/E:

General Examination:-

Pallor: Y___N___

Icterus: Y___N___

Cyanosis: Y___N___

Clubbing: Y___N___

Lymphadenopathy: Y___N___

Oedema: Y___N___

Koilonychia: Y___N___

To be specified if others:

Systemic Examination:-

P/A:

Spleenomegaly Y___N___

Hepatomegaly Y___N___

Ascites Y___N___

To be specified if others:

Investigations;

Hb-

TC-

Platelets-

MCV-

MCH-

RDW-

Retic Count-

Sickling- Y___N___

Zinc Protoporphyrin-

Iron Profile:-

S.Iron-

S.Ferritin-

TIBC-

PARTICIPANT INFORMATION SHEET

Study Title: Role of Zinc Protoporphyrin as a Diagnostic tool for Anaemia

1. Introduction of study

   This is a research based study which would be conducted in a hospital with a medical college.

   It is a study which would help diagnose Anaemia in a cost effective manner & at an earlier stage. Few blood tests would be conducted as a part of the study which would lead to the diagnosis.

2. What is the purpose of this study?

   The purpose of the study is to diagnose Anaemia in a cost effective manner & at an earlier stage.

3. Why have I been chosen?

   The reason behind choosing you is you have the signs & symptoms of Anaemia your participation would be beneficial for you as well as for others.

4. Do I have to take part?

   Participation is voluntary.

5. How long will the study last?

   Study would last for 2.5 years.

6. What are the benefits of the study?

   The prevalence of Iron deficiency in sickle cell can be known & diagnosis of Anaemia in a cost effective manner & at an early stage.

7. What if new information becomes available?

   If some new information is available your participation would be credited for that.

8. Will my taking part be kept confidential?

   Participation will be kept confidential

9. What else should I know?

   All the necessary details & information would be given if anything else is to be informed we will surely inform you.

10. Who to call with questions?

    Dr. Vivek Vaswani (9825022387)

ભાગીદારીમાહિતીશીટ

1. પરિચય

આ એક મેડિકલ કોલેજ સાથે હોસ્પિટલમાં હાથ ધરવામાં આવશે, જે એક સંશોધન આધારિત અભ્યાસ છે.તે ખર્ચ અસરકારક રીતે અને પહેલાંની તબક્કે Anaemia તે તપાસ કરવામાં મદદ કરશે, જે એક અભ્યાસ છે. થોડા રક્ત પરીક્ષણો નિદાન તરફ દોરી જાય છે, જે અભ્યાસ એક ભાગ તરીકે હાથ ધરવામાં આવશે.

2. આ અભ્યાસ હેતુ શું છે?

આ અભ્યાસના હેતુ ખર્ચ અસરકારક રીતે અને પહેલાંની તબક્કે Anaemia નિદાન છે.

3. હું શા માટે પસંદ કરવામાં આવી છે?

તમે પસંદ પાછળ કારણ તમે ચિહ્નો અને Anaemia લક્ષણો તમારી ભાગીદારી માટે તમે તેમજ અન્ય લોકો માટે લાભદાયી રહેશે હોય છે.

4. મારે ભાગ લેવા માટે હોય છે?

ભાગીદારી સ્વૈચ્છિક છે.

5. લાંબા કેવી રીતે અભ્યાસ ચાલશે?

અભ્યાસ 2.5 વર્ષ માટે રહે કરશે.

6.અભ્યાસમાં શું ફાયદા છે.?

સિકલ સેલ માં આયર્ન ઉણપ વ્યાપ ખર્ચ અસરકારક રીતે અને પ્રારંભિક તબક્કે ઓળખાય &Anaemia નિદાન કરી શકાય છે.

7. નવી માહિતી ઉપલબ્ધ બને તો શું?

કેટલાક નવી માહિતી ઉપલબ્ધ હોય તો તમારી ભાગીદારી તે માટે જમા કરવામાં આવશે.

8. મારા ભાગ લેવા ગુપ્ત રાખવામાં આવશે?

ભાગીદારી ગુપ્ત રાખવામાં આવશે

9. હું બીજું શું જાણવું જોઈએ?

તમામ જરૂરી વિગતો અને માહિતી અન્ય કંઈપણ જાણ કરી છે તો અમે ચોક્કસ તમે જાણ કરશે આપવામાં આવશે.

10. પ્રશ્નો સાથે કોને કૉલ કરવા માટે?

ડૉ. વિવેક વસ્વાણી (9825022387).

Informed Consent Form (ICF) for Participants in Research Programmes involving studies on human beings

Study Title: -

Please initial box (Subject)

1. I confirm that I have read and understood the information sheet dated ……....….for the above study and have had the opportunity to ask questions. ☐

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. ☐

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) ☐

(v) I agree to take part in the above study. ☐

Signature (or Thumb impression) of the Subject/LAR: _____

Date: ____/____/____

Signatory's Name: _____

Signature of the Investigator: _____

Date: ____/____/_____

Study Investigator's Name: _____

Signature of the Witness _____

Date: ____/____/_____

Name of the Witness:

ઇન્ફોર્મડકન્સેન્ટફોર્મ

અભ્યાસનુંશીર્ષક: -

(i) હું ખાતરી કરી રહ્યો છુંકે હું ખાતરી વાંચી અને તારીખક્ક માહિતી શીટ સમજી છે તેની ખાતરી .... ઉપર અભ્યાસ માટે અને પ્રશ્નો પૂછી કરવાની તક મળી છે.
(ii) હું અભ્યાસ મારો સહયોગ સ્વૈચ્છિક છે અને હું મારા તબીબી સંભાળ કર્યા વગર , કોઇ કારણ આપ્યા વગર , કોઈપણ સમયે પાછી ખેંચી મુક્ત છું અથવા કાનૂની અધિકારો પર અસર થઈ રહી છે કે જે સમજે છે.

(iii) હું સમજી કે ક્લિનિકલ ટ્રાયલ સ્પોન્સર , અન્યસ્પોન્સર વતી કામ કરતા એથિક્સ સમિતિ અને નિયમનકારી સત્તાવાળાઓ બંને વર્તમાન અભ્યાસ અને તે સંબંધમાં હાથ ધરવામાં કરી શકાય છે કે જે કોઈપણ વધુ સંશોધન બાબતમાં મારા આરોગ્ય રેકોર્ડ જોવા માટે મારા પરવાનગી જરૂર નથી , પણ હું ટ્રાયલ ખસી તો . હું આ ઍક્સેસ કરવા માટે સંમત થાય છે. જો કે , હું મારી ઓળખ તૃતીય પક્ષો રજૂ અથવા પ્રકાશિત કોઈપણ માહિતી જાહેર કરવામાં આવશે નહીં કે સમજે છે.

(iv) હું આ અભ્યાસ પરથી ઊભી છે કે જે કોઈપણ માહિતી અથવા પરિણામો ઉપયોગ પ્રતિબંધિત માત્ર વૈજ્ઞાનિક હેતુ માટે (ઓ) આવા ઉપયોગ છે પૂરું પાડવામાં આવેલ નહિં સંમત

(v) હું ઉપર અભ્યાસ માં ભાગ લેવા માટે સંમત થાય છે.

આ વિષય / હસ્તાક્ષર (અથવા અંગૂઠાની છાપ):

તારીખ: / /

સહી નામ:

તપાસનીસ ની સહી:

તારીખ: / /

તપાસનીશ નામ અભ્યાસ:

સાક્ષી હસ્તાક્ષર

તારીખ: / _ /
સાક્ષી નામ:

Primary Data							Clinical Features															Investigatory Profile																														Index	
Sr No	Name	Age	Sex	Date of Admission	Occupation	Address	Breathlessness	Pedal Oedema	Generalised weakness	Haemorrhoids	Generalised bodyache	Abdominal Pain	Pallor	Cyanosis	Clubbing	Lymphadenopathy	Oedema	Icterus	CVS	P/A	CNS	BS	Hb	MCV	MCH	MCHC	PCV	RDW	Platelets	Total Count	Zinc Protoporphyrin	S.Iron	S.Ferritin	TIBC	Sickling	RBC	SGOT	SGPT	S.Creatinine	Hb Electrophoresis	HbS	HbF	HbA2	Retic count	Vitamin B12	RBS	Serology(HIV/HBsAg/HCV)	S.Bilirubin	S.LDH	Stool for Occult blood	Mentzer Index	Srivastava Index
1	A.M	19	M	28-11-16	Student	Badvani,MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		5.2	79.9	26.3	32.9	15.5	13.2	20000	1500	110	145	926	295	2	1.94	24	33	0.6	Normal				1%		99	2	0.5		2	41	13.55
2	P.R	38	M	28-11-16	Farmer	Khargon,MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		7.5	55.6	13.1	23.5	31.9	20.5	120000	7500	93	29	26	388	N/A	**5.74**	21	35	0.6	N/A				1.50%		110	2	0.4			**9**	**2.28**
3	H.D	32	F	21-11-16	Farmer	Ratlam,MP	2	2	1	1	1	1	1	2	2	2	2	2	2	1	2		8	75.7	19.1	25.3	26.5	21.2	480000	6800	212	46	211	403	N/A	4.1	20	24	0.7	N/A				3%		99	2	0.5		2	18	4.65
4	D.V	22	F	28-11-16	Home-maker	Waghodiya,Gujarat	2	2	1	2	1	1	1	2	2	2	2	2	2	1	2		6.6	88.4	23.2	26.2	25.2	30.2	150000	11200	58	36	55	399	1	2.85	25	19	0.6	**SCD**	78.1	10.1	4.1	2%		100	2	2.1			31	8.14
5	S.R	70	F	07-01-17	Retired	Ujjain,MP	1	2	1	2	1	1	1	2	2	2	1	2	1	1	2		5.3	61.8	16.2	26.2	20.2	14.6	80000	2400	125	40	12.6	387	2	3.27	31	30	0.6	N/A				1%	208	90	2	1.2	325		19	4.95
6	M.R	40	M	23-01-17	Farmer	Dhar,MP	1	1	1	1	1	2	1	2	2	2	1	2	2	2	2		2.5	57.6	14.6	25.3	8.7	20.8	100000	4000	219	14	4.3	392	2	**1.51**	14	15	0.6	N/A				1.50%	1152.7	113	2	0.6	280	2	38	9.66
7	S.R	40	M	21-01-17	Farmer	Dhar,MP	2	1	1	2	1	1	1	2	2	2	1	2	2	1	2		7.6	73.3	22.2	30.3	24.1	21.1	246000	5300	577	42	35	326		3.34	91	23	0.8	N/A				0.50%		120	2	0.8		2	22	6.64
8	H.M	63	M	12-04-07	Farmer	Mansaur,MP	1	1	1	2	1	2	1	2	1	2	1	2	1	2	2		5.9	70.2	17.4	24.7	18.6	20.1	150000	8200	151	26	<1.0	265	2	2.11	24	21	0.8	N/A				1%		114	2	0.5			33.2	8.24
9	C.R	55	M	14-02-17	Farmer	Savli,Vadodara	1	1	1	2	1	2	1	2	2	2	1	2	2	2	2		8.4	73	20.4	28	30	22.1	160000	9600	89	32	21	388		4.11	145	187	0.7	N/A				1.50%		162	2	1.7			17.76	4.96
10	K.T	32	F	02-05-17	Farmer	Agar,MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		7.3	69.1	19.5	28.2	25.9	16.9	580000	7700	109	27	<1.0	321		3.75			0.7	N/A				2%		111	2				18.42	5.2
11	R.P	46	F	01-05-17	Farmer	Khargon,MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		2	53	11.6	21.8	8.7	23.5	416000	5400	206	35	13	396		1.64	19	16	0.6	N/A				3%		97	2	0.3			32.3	7.07
12	M.B	35	M	23-05-17	Farmer	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		7	59.6	15.6	26.2	26.7	16.8	120000	5300	135	41	23	225	2	4.48	21	26	1	N/A				0.50%		117	2	0.7			13.3	**3.48**
13	S.S	18	F	25-05-17	Student	MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		6.8	56.1	16.3	29.1	21.8	20	355000	5900	470	39	425	234		3.89				N/A				1%		100	2				14.42	4.19
14	M.S	60	F	29-05-17	Home-maker	Ratlam,MP	2	2	1	2	1	2	1	2	2	2	1	2	2	2	2		8	69.9	21.7	31	25.8	15	210000	6800	57	39	29	236		3.69		17	0.9	N/A				1%	221	164	2				19	5.88
15	B.Y	47	M	12-06-17	Clerk	Badvani,MP	2	2	1	1	1	1	1	2	2	2	2	2	2	2	2		8.3	69.6	29.8	29.3	23.2	18.5	536000	12600	65	40	35	370		4.17			1	N/A				1%		122	2			2	16.69	7.14
16	J.T	18	M	16-06-17	Student	Sankheda,Gujarat	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		6.3	79.2	23.4	29.6	21.2	44.2	249000	5900	162	33	20	388	1	2.69	33	31	0.6	**β thal trait and SCT**	19	3.5	4.9	2%	150.2	110	2	0.5	879	1	29.4	8.69
17	A.G	21	F	12-06-17	Home-maker	Mansaur,MP	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2		5.4	73.3	20.3	27.7	19.6	23.4	386000	4200	216	25	<1.50	443	2	2.68	19	21	0.6	Normal				1%		106	2			2	27.35	7.57
18	J.M	60	F	26-06-17	Home-maker	Nanded,Gujarat	1	2	1	2	1	2	1	2	2	2	2	2	1	2	2		6.6	53.8	14.2	26.3	24.2	22	291000	5200	160	41	22	386	1	3.55			0.9	**SCT**	2.5	<0.8	2.6	1.50%		181	2			2	15.15	3.94
19	D.B	55	f	28-08-17	Farmer	Ratlam,MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		10.7	63.4	19.2	30.3	35.8	23.4	322000	12000	186	20	26	321	1	5.64	43	57	0.6	Normal				1%		107	2	0.5			**11.24**	**3.4**
20	P.C	60	M	28-08-17	Retired	Jhabuva,MP	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2		9.9	64.3	20	31	21.8	21.3	284000	6400	67	38	25.7	394		4.95	40	48	0.6	N/A				1%		120	2	0.6		2	**13**	4.04
21	P.P	34	F	28-08-17	Home-maker	MP	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2		8.5	69.1	20.7	30	28.5	17.3	295000	4800	118	32	21	384		4.12				N/A				2%		90	2			2	16.77	5.02
22	M.B	39	M	21-08-07	Farmer	Dhar,MP	2	2	1	1	1	1	1	2	2	2	2	2	2	1	2		6	61.2	18.9	30.9	19.4	19.6	203000	6400	137	28	16	394	1	3.17	56	32	0.9	Normal				2%		112	2	1.5			19.3	5.69
23	U.P	72	F	21-08-17	Retired	Savli,Vadodara	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2		5.6	64.6	18.2	28.1	19.5	21	336000	8200	213	36	17.9	240		3.08		26	0.6	N/A				2%	215	153	2	1		1	21	5.9
24	M.P	66	M	21-08-17	Farmer	Khargon,MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		7.1	63.6	18.2	20.6	24.8	25.4	212000	8400	80	31	84	349		3.91	45	28	1.3	N/A				1.50%		136	2	0.8		1	16.26	4.65
25	K.P	45	F	14-08-17	Farmer	MP	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		5.8	63.3	36.6	26.2	22.1	16.5	381000	9400	259	45	110	135	2	3.49	13	11	0.6	Normal				2%	321.5	112	2	0.4	301	1	18.13	10.48
26	S.B	22	M	15-08-17	Student	Waghodiya,Gujarat	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		9.9	71.3	24.8	32	30.9	21.6	358000	16300	16	42	96	288	1	4	76	131	0.6	**SCD**	86.5	3.4	5.9	2%		108	2	3.5			17.8	6.2
27	R.M	72	F	04-06-07	Home-maker	Ankleshwar,Bharuch	2	1	1	2	1	2	1	2	2	2	1	2	2	2	2		7.4	71.2	21.6	30.3	24.4	22	390000	11200	74	40	45	368		4.11	67	26	1.7	N/A				2.50%		132	2	0.8			17.32	5.25
28	S.T	20	M	04-08-17	Student	Savli,Vadodara	2	2	1	2	1	1	1	2	2	2	2	2	2	2	2		11.9	64.5	20	30.9	38.5	20.1	140000	4500	59	44	65	389	2	3.26	25	21	0.8	Normal				2%		110	2	0.6		2	19.78	6.13
29	R.K	52	M	28-08-17	Farmer	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		3.9	86.8	27.1	31.2	12.5	29.4	60000	2600	36	28	308	444	2	4.58	20	17	0.8	Normal				3.00%		101	2	1.2			19	5.91
30	S.D	25	F	03-07-17	Farmer	MP	1	2	1	2	1	2	1	2	2	2	1	2	2	2	2		4	57.6	13.9	24.1	16.6	23.5	322000	6600	142	36	<1.0	332	1	2.15	18	16	0.9	N/A				1%		98	2	0.8	445		26.79	6.46
31	N.P	65	M	01-02-17	Farmer	MP	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2		11.7	91.2	30.2	33.1	35.4	14.3	270000	7700	63	32	16	388		4.38		25	2.2	N/A				1%		166	2			2	20.82	6.89
32	A.J	55	M	29-05-17	Farmer	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		9.8	72	22	30.5	32.1	18.6	355000	6100	131	38	24	377		3.48	16	14	2.1	N/A				2%		113	2	0.4			20.68	6.32
33	G.B	55	F	19-06-17	Home-maker	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		8.2	61.1	17.2	28.2	29.1	18.8	374000	5000	48	41	91	380		3.23	23	21	0.8	N/A				0.50%		229	2	0.6			19	5.32
34	S.V	47	F	10-07-17	Farmer	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		5	58.8	17.3	29.5	17.1	22.2	20000	7100	70	35	48	399		2.7	17	10	1	N/A				2%		110	2	0.6	841		21.78	6.4
35	V.M	58	M	05-06-17	Farmer	MP	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		8.6	70.8	18.8	26.3	32.7	17.7	361000	7900	113	26	22	437		3.78	21	19	1.2	N/A				1%		98	2	0.8		1	18.73	4.97
36	J.R	22	M	12-04-17	Student	Gujarat	1	2	1	2	1	1	1	2	2	2	2	2	2	2	2		9.6	65.9	20.7	31.5	30.5	18.8	450000	28000	25	30	844	375	1	3.1	26	23	0.7	**SCD**	82.4	5.8	4.4	7%		101	2	1.9		2	21.25	6.67
37	P.D	26	F	03-04-17	Home-maker	MP	2	1	1	2	1	1	1	2	2	2	2	2	2	2	2		5.7	56.7	16	28.2	20.2	23.9	333000	4800	135	35	21	401	1	2.45	53	56	1.3	N/A				1%		90	2	0.2			23.14	6.53
38	S.Y	30	F	OPD	Home-maker	Darjipura,Vadodara	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2		7.5	58.8	17.3	30.1	25.5	19.7	410000	5400	140	45.7	9.12	531	2	2.88	20	24		Normal				1%		98	2				20.41	6
39	P.R	20	F	15-12-16	Student	Vadodara	1	2	1	2	1	1	1	2	2	2	2	2	2	2	2		9.5	70.8	21.1	29.6	31.4	18.2	304000	13200	120	38	334	390	2	3.48	82	76	0.8	N/A				1.50%		70	2			2	20.34	6.06
40	K.P	65	F	13-12-16	Home-maker	Vadodara	2	1	1	2	1	2	1	2	2	2	1	2	2	2	2		9.1	68.8	20.2	27.6	29.5	17	150000	7000	162	47	76	355		3.28	32	38	3.2	N/A				1%		88	2				21	6.15
41	D.S	74	M	16-12-16	Retired	Vadodara	2	1	1	2	1	2	1	2	2	2	2	2	2	1	2		8.5	65.7	17.4	25.6	26.8	19.6	201000	30000	189	36	900	378		2.55	74	54	8.1	N/A				2%		110	2				25.76	6.82
42	M.J	35	F	13-12-16	Farmer	Dabhoi	2	2	1	2	1	2	1	2	2	2	2	2	2	1	2		10.3	74.6	25.8	30.1	22.3	18.5	100000	4300	101	32	292	344		4.12	89	76	1	N/A				1%		180	2				18.1	6.26
43	K.P	79	M	13-12-16	Retired	Godhra	2	2	1	2	1	1	1	2	2	2	2	2	2	1	2		10.5	75.2	26.3	29.6	21.8	18.2	150000	6700	94	38	510	382		3.98	28	26	0.9	N/A				2%		95	2				18.89	6.6
44	J.T	58	M	16-12-16	tickshaw drive	Vadodara	1	1	1	2	1	1	1	2	2	2	1	2	2	2	2		8	63.6	16.7	23.6	31.1	17.2	168000	8500	134	40	700	390		2.88	65	54	6.1	N/A				1.50%		104	2	0.4			22	5.79
45	J.S	58	F	16-12-16	Home-maker	Vadodara	2	2	1	2	1	2	1	2	2	2	2	2	2	2	1		10.3	73.8	25.5	30.8	22.6	18.2	177000	6800	118	47	111	344		4	44	41	2.2	N/A				1%		194	2				18.45	6.37
46	M.P	55	M	15-12-16	Farmer	Vadodara	1	2	1	2	1	1	1	2	2	2	2	2	2	2	2		10.5	74.5	25.9	31.1	22.1	18.8	360000	20200	90	39	950	310		3.88	28	32	1.5	N/A				1%		88	2				19.2	6.67
47	P.P	71	F	22-12-16	Home-maker	Dabhoi	1	1	1	2	2	1	1	2	2	2	1	2	2	2	2		9	66.7	20.4	26.7	28.8	17.8	269000	9700	108	27	311	370		3.3	22	28	2.1	N/A				1.50%		102	2				20.21	6.18
48	M.P	51	M	21-12-16	Farmer	Alirajpur,MP	1	1	1	2	1	1	1	2	2	2	1	2	2	2	2		6.6	60.4	18.2	29.6	22.1	24.6	160000	4000	216	36	670	394		2.92	20	24	9.6	N/A				2%		118	2				20.68	6.23
49	A.C	58	M	22-12-16	tickshaw drive	Vadodara	1	1	1	2	2	1	1	2	2	2	1	2	2	2	2		8.1	62.8	17.1	28.7	27.8	18.9	181000	3900	155	32	319	335		4.04	46	42	3.2	N/A				1%		94	2				15.54	4.27
50	N.B	28	M	23-12-16	Clerk	Khargon,MP	1	2	1	2	1	1	1	2	2	2	1	2	2	1	2		8.6	68.8	19.2	26.1	34.5	18.4	330000	21800	98	38	990	388		3.68	75	68	1.3	N/A				2%		130	2				18.69	5.21
51	H.R	60	F	09-04-17	Home-maker	MP	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2		4.5	65	16.1	24.7	18.2	20.1	262000	4800	151	34	12	393		2.8	22	24	0.6	N/A				2%		96	2				23.21	5.75