"EFFECT OF SEVERITY OF ANEMIA ON HbA1c IN NON DIABETIC PATIENTS – ONE YEAR HOSPITAL BASED STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE "

By

REG NO. BG0117007

Dissertation

Submitted to the
KAHER, Belagavi, Karnataka
In partial fulfillment
of the requirements for the degree of

M. D.

in

GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

APRIL – 2020

KLE Academy of Higher Education and Research, Belagavi, Karnataka

ENDORSEMENT

This is to certify that the dissertation entitled **"EFFECT OF SEVERITY OF ANEMIA ON HbA1c IN NON DIABETIC PATIENTS – ONE YEAR HOSPITAL BASED STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE"** is a bonafide research work done by **(REG NO. BG0117007)**.

Dr. Arathi Darshan MD
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi

Dr. N. S. Mahantshetti MD
Principal,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi

PLAGIARISM CERTIFICATE

JAWAHARLAL NEHRU MEDICAL COLLEGE
(A constituent unit of KLE Academy of Higher Education & Research Deemed-to-be University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category "A" by MHRD (GoI)
Nehru Nagar, Belagavi-590 010, Karnataka-India

Website : http/www.jnmc.edu
E-Mail : Principal@jnmc.edu
☎ Office :+91-(0)831 2471350
FAX :+91 (0)831-2470759

Ref. No. : MDC|PG|2105 Date : 17|9|2019

To,

REG NO. BG0117007

Sub: Acceptance Letter

Sir/Madam,

The softcopy of thesis entitled " EFFECT OF SEVERITY OF ANEMIA ON HbA1c IN NON-DIABETIC PATIENTS – ONE YEAR HOSPITAL BASED STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL & MRC, BELAGAVI ", has been submitted for Anti-Plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of 7% (Seven percentage) which is within the acceptable limits of 10% as per the guidelines given by UGC.

Thanking you,

Yours sincerely,

Coordinator
Department of General Medicine
J. N. M. C. Belagavi.

Chairman,
Antiplagiarism Committee

LIST OF ABBREVIATIONS

WHO	:	World Health Organisation
Hb	:	Hemoglobin
YLD	:	Years Living with Disability
HbA1c	:	Hemoglobin A1c
GRADE	:	Grading of Recommendations, Assessment, Development and Evaluation
FBG	:	Fasting Blood Glucose
IDA	:	Iron Deficiency Anemia
RBC	:	Red Blood Cell
GBD	:	Global Burden of Disease
DLHS	:	District Level Housing Survey
AHS	:	Annual Health Survey
FAO	:	Food and Agricultural Organisation
NFHS	:	National Family Health Survey
ICMR	:	Indian Council of Medical Research
NNMB	:	National Nutrition Monitoring Bureau
DA	:	Dimorphic Anemia
IDF	:	International Diabetes Association
EASD	:	European Association for Study of Diabetes
DCCT	:	Diabetes Control and Complication Trial
UKPDS	:	United Kingdom Prospective DiabetesStudy
ACE	:	American College of Endocrinology
CBC	:	Complete Blood Count

HCT	:	Hematocrit
MCH	:	Mean Corpuscular Hemoglobin
MCV	:	Mean Corpuscular Volume
MCHC	:	Mean Corpuscular Hemoglobin Concentration
CINAHL	:	Cumulative Index to Nursing and Allied Health Literature
EMBASE	:	Excerpta Medica Database
OGTT	:	Oral Glucose Tolerance Test
PPBS	:	Post Prandial Blood Sugars

ABSTRACT

Background and Objectives

Anemia continues to be a medical and financial burden for both developed and developing countries, and affects all age groups. The normal value of hemoglobin varies with age, gender, ethnicity and physiologic status of the population. Diabetes mellitus is another condition with increased global prevalence along with anemia and depends on postprandial blood sugar, fasting blood sugar and hemoglobin A1c (HbA1c) for its diagnosis. The use of HbA1c for the diagnosis of diabetes mellitus is widely advocated despite caveats to its use with anemia being cited as a major confounding factor. The value of HbA1c may be erroneous in those with anemia, thus mandating its evaluation prior to using HbA1c as a guide for diagnostic or therapeutic decisions of glycemic status. The objective of this study was to identify a correlation between severity of different types of anemia and the derangement of HbA1c levels in an adult non-diabetic population.

Materials and Methods

The present study was conducted on non-diabetic anemic patients admitted in the Department of General Medicine of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre from January 2018 to December 2018. Relevant data was collected by a detailed interview with the patient, clinical examination and blood investigations. The fasting blood sugars and post prandial blood sugars were measured in order to rule out a diagnosis of diabetes mellitus. Following this, the patients were categorised into the 3 categories of severity of anemia, based on the WHO Grading, as per the patient's hemoglobin values. Peripheral smear and HbA1c

was measured and recorded and a correlation of severity of different types of anemia with HbA1c values was studied. Statistical tests such as Chi Square test and ANOVA were used for analysis.

Results

In the 100 non diabetic anemic patients, age ranged from 18-86 years. The number of female patients was slightly more than males. The commonest symptom of patient presentation was generalized weakness, and the most common sign was pallor. Majority of our patients were found to have iron deficiency anemia. It was observed that that the HbA1c was significantly higher in females as compared to males (p value 0.0106). No correlation was found on comparison of age, types of anemia or severity of anemia with HbA1c.

Conclusion

HbA1c may be affected by variables such as age, sex, anemia, chronic illnesses or drugs. We feel it is worthwhile to study these confounding factors with large sample sizes for ascertaining a correlation of HbA1c with these variables before diagnosing and treating diabetes mellitus based on it alone.

Keywords: ANEMIA, HbA1c, DIABETES MELLITUS

CONTENTS

SL. NO.	TOPIC	PAGE NO.
1.	INTRODUCTION	1-5
2.	OBJECTIVES	6
3.	REVIEW OF LITERATURE	7-28
4.	METHODOLOGY	29-33
5.	RESULTS	34-55
6.	DISCUSSION	56-66
7.	CONCLUSION	67-68
8.	SUMMARY	69
9.	BIBLIOGRAPHY	70-80
10.	ANNEXURES	
	ANNEXURE I – CONSENT FORM	81-84
	ANNEXURE II – PROFORMA	85-87
	ANNEXURE III – ETHICAL CLEARANCE LETTER	88
	ANNEXURE IV – MASTER CHART	89-91

LIST OF TABLES

TABLE NO.	DESCRIPTION	PAGE NO.
1	Age Distribution	**35**
2	Sex Distribution	**37**
3	Clinical Presentation	**38**
4	Clinical Signs	**39**
5	Type of Anemia	**40**
6	Severity of Anemia (WHO Criteria)	**41**
7	Hemoglobin Percentage (WHO Criteria)	**42**
8	Peripheral Smear	**43**
9	Fasting Blood Sugar	**44**
10	Post Prandial Blood Sugar	**45**
11	HbA1c	**46**
12	Comparison of Age with Severity of Anemia	**47**
13	Comparison of Age with HbA1c	**48**
14	Comparison of Sex with Severity of Anemia	**49**
15	Comparison of Sex with HbA1c	**50**
16	Comparison of Type of Anemia with Severity of Anemia	**51**
17	Comparison of Type of Anemia with HbA1c	**52**
18	Comparison of Severity of Anemia with HbA1c in Males	**53**

19	Comparison of Severity of Anemia with HbA1c in Females	**53**
20	Comparison of Iron Deficiency Anemia with HbA1c	**54**
21	Comparison of Dimorphic Anemia with HbA1c	**54**
22	Comparison of Megaloblastic Anemia with HbA1c	**55**

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
1	Age Distribution	**36**
2	Sex Distribution	**37**
3	Clinical Presentation	**38**
4	Clinical Signs	**39**
5	Type of Anemia	**40**
6	Severity of Anemia (WHO Criteria)	**41**
7	Hemoglobin Percentage (WHO Criteria)	**42**
8	Peripheral Smear	**43**
9	Fasting Blood Sugar	**44**
10	Post Prandial Blood Sugar	**45**
11	HbA1c	**46**
12	Comparison of Age with Severity of Anemia	**47**
13	Comparison of Age with HbA1c	**48**
14	Comparison of Sex with Severity of Anemia	**49**
15	Comparison of Sex with HbA1c	**50**
16	Comparison of Type of Anemia with Severity of Anemia	**51**
17	Comparison of Type of Anemia and HbA1c	**52**

INTRODUCTION

Globally, anemia continues to be a crucial health issue for countries which are developed as well as those which are still developing, and affects all age groups. According to the WHO, definition of anemia is hemoglobin (Hb) levels <13.0 g/dL in males and <12.0 g/dL in females.[1] The normal values of hemoglobin may vary with gender, ethnicity and the physiologic status of the population. Hence, a lesser limit of normal Hb values is proposed to match the ethnicity, gender and age. Various etiological factors such as physiological and pathological mechanisms account to anemia. The classification and diagnosis of anemia is mainly based on the history of patient, hematologic parameters and thorough physical examination.[2] During morphological presentation; in examination of a peripheral blood smear, anemia can be macrocytic type, microcytic hypochromic type, normocytic, dimorphic type and normochromic type. Varied etiologies are present with each of these morphological types.

According to the World Health Organization's 2008 report, 24.8% of the world's population was anemic.[3] Recent global reports by Kassebaum et al.[4] estimated that the worldwide anemia prevalence in 2010 was 32.9% and has resulted in 68.4 million years living with disability (YLD) since then.[4] The reports in India from 2014-15, has recorded anemia among all ages and gender with a prevalence ranging between 57.1% and 89.3%. Hence, the prevalence of anemia in India is significantly greater across all groups.[5]

Diabetes mellitus is another condition with increased global prevalence along with anemia. The most important diagnostic values in evaluating glycemia are postprandial blood sugar, fasting blood sugar and hemoglobin A1c (HbA1c). The report of WHO in 2011 summarised the evidence on the usage of HbA1c for the diagnosis of Diabetes Mellitus and its quality with the help of GRADE methodology. The report says that for diabetes mellitus, HbA1c can be used as a diagnostic test with strict quality assurance tests in place along with assays standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.[6] The measure of HbA1c is the most widely accepted diagnostic test used, especially in community-based studies, as it needs no fasting blood glucose and has lesser inter-observer variability.[7] The World Health Organisation along with the American Diabetes Association has promoted the use of HbA1c for diagnosing type 2 diabetes mellitus with 6.5% or higher considered as a cut off value.[6,8]

The value of HbA1c can be altered by conditions unrelated to diabetes mellitus. Factors which influence an increased HbA1c value include iron deficiency, decreased erythropoiesis and vitamin B_{12} deficiency, whereas low values of HbA1c could be influenced by administration of erythropoietin, vitamin B_{12} or iron and reticulocytosis or chronic liver disease, while genetic or chemical alterations in hemoglobin such as hemoglobinopathies, fetal hemoglobin (HbF), methemoglobin can decrease or increase HbA1c.[9] The hemolytic anemias, because of reduction in erythrocyte lifespan can cause a decrease in HbA1c value. Whereas, iron deficiency anemia results in increased HbA1c estimates, contrasting to the previous types of anemia.[7]

The measures of HbA1c and fasting blood glucose among large population cannot group diabetes identically, as these values reproduce the glycemic status over different time phases.[10,11] Around 1.6 billion people[12] around the globe are affected by anemia, in particular with iron deficiency anemia.[13] Hence, there is an increased chance for diabetes to be misdiagnosed across populations globally. The mechanism of iron deficiency and anemia as two separate entities influencing the values of HbA1c is not completely explained.[14,15] Few epidemiological studies[14,16,17] results suggest that iron deficiency anemia can cause an increased HbA1c value. However, few studies showed the presence of lower HbA1c values among people with IDA[18] or anemia[19].

The contrasting conclusions of these previous researches maybe because of the different etiologies of anemia. These include iron deficiency, sickle cell disease, thalassemia's, vitamin B_{12} deficiency or folate deficiency.[13]

Deficiency of iron occurs due to decreased intake of iron, reduced absorption of iron, loss of blood, menstrual bleeding or pregnancy.[13] Thus with several etiologies, iron deficiency anemia characterises as two separate disease processes; mainly anemia and iron deficiency. Further, the severity of iron deficiency against anemia might differ by gender due to menstrual cycles in females.

The lower dietary intake of the vitamin (i.e., a low intake of animal-source foods) and malabsorption are considered as most commonly given explanations for low vitamin B_{12} levels. According to past research studies, population consuming strict vegetarian diet (vegans) are more prone to development deficiency of vitamin B_{12}. Newer pieces of evidence show that lower consumptions of animal foods, like those occurring in some lacto-ovo vegetarian diet consumers and many other less-

industrialised nations cause depletion of vitamin B_{12}. Consumption of green leafy vegetables and legumes, which are among rich sources of vitamin, may explain why folate level can be sufficient in relatively poor population. Lactation and alcoholism are the conditions where an increase in the risk of folate deficiency can be observed.[20]

Works of literature have examined iron deficiency and anemia as two separate entities independently and studied the impact of diabetes and prediabetes prevalence using HbA1c values. An extensive longitudinal study by China Health and Nutrition Survey utilised covariate and descriptive adjusted models to evaluate the relative risk of diabetes as well as prediabetes using only Fasting Blood Glucose (FBG), only hemoglobin A1c and FBG and hemoglobin A1c, in normal, anemia exclusively, and in IDA study population. The study population was 7308 adults aged eighteen to seventy-five years. The analysis showed that the prediabetes adjusted prevalence using HbA1c alone was twenty-two per cent for male with anemia alone, but thirteen per cent for male population with iron/hemoglobin at normal levels. Oppositely, the pre-estimated prediabetes' widespread presence using only hemoglobin A1c came to be eight per cent for female population having only IDA, compared with thirteen per cent for females with iron/hemoglobin at normal levels. Hence, such findings recommend, the prevalence of diabetes was underdiagnosed in females with deficiency of iron using HbA1c alone and over diagnosed in males with anemia. Therefore, there is an increased chance of misdiagnosing the population to be diabetic using HbA1c, especially in region of highly prevalent deficiency of iron and anemia.[15] A Systematic review [21] results also suggest HbA1c could be affected by iron deficiency and iron deficiency anemia with a false rise in HbA1c. Whereas, subjects without iron deficiency anemia may generate a lower HbA1c value. This further leads to chaos in the diagnosis of diabetes with HbA1c.[21]

Need for the study:

The evidence from studies[21,22] strongly suggest that iron deficiency and IDA influence the HbA1c values. However, to what extent, the severity of iron deficiency anemia affects the HbA1c is less calculated. Further, majority of the studies have made an attempt to evaluate the effects of only iron deficiency and iron deficiency anemia on HbA1c, leaving a void in the understanding of the effects of other types of anemia such as dimorphic anemia and megaloblastic anemia, on HbA1c. In an attempt to test this hypothesis surrounding IDA and shed some light on the effects of other types of anemia on HbA1c, our study intends to assess the effects of severity of different types of anemia on HbA1c among non -diabetic adult population.

OBJECTIVES

The objective of the present study was

- To study the correlation between severity of different types of anemia and the derangement of HbA1c levels in an adult non-diabetic population.

REVIEW OF LITERATURE

- Anemia is referred to as a condition where the number of red blood cells (RBC) and consequently, their oxygen-carrying capacity is inadequate to fulfil the body's physiologic requirements.
- The physiological needs differ from an individual, age, gender, geographic region (high altitude or at sea level), habit of smoking and during gestational periods.
- The common etiology of anemia across the globe is iron deficiency followed by vitamin B_{12}, vitamin A and folate.
- Pathological effects of anemia include chronic inflammation, acute inflammation, inherited or acquired disorders, and parasitic infections. These physiological factors affect the synthesis of hemoglobin, red blood cell survival or red blood cell production.
- The prevalence of anemia acts as a vital health indicator and reflects the iron status of the body. Hemoglobin concentration can provide information about the severity in deficiency of vitamin B_{12}, folate or iron.[23]

Hemoglobin levels to diagnose anemia at sea level (g/dl)[23]

Age	Non-anemia (g/dl)	Anemia (g/dl)		
		Mild	Moderate	Severe
6-59 months	11 or higher	10 - 10.9	7 - 9.9	< 7
5-11 years	11.5 or higher	11-11.4	8-10.9	< 8
12-14 years	12 or higher	11-11.9	8-10.9	< 8
Non-pregnant women (>15 years)	12 or higher	11 - 11.9	8 - 10.9	< 8
Pregnant women	11 or higher	10 - 10.9	7 - 9.9	< 7
Men (> 15 years)	13 or higher	11 - 12.9	8 - 10.9	< 8

Burden statistics of Anemia - Global

According to the WHO reports in 2008, around 24.8% of the global community suffered from anemia. The estimates included forty two per cent of pregnant females, thirty per cent of non-pregnant females, and forty seven per cent of preschool children.[24] Latest reports estimated the prevalence of anemia as twenty nine per cent in females who were pregnant, thirty eight per cent among females who were not pregnant, and in children it was forty three per cent. As per the report of the Global Burden of Disease (GBD) 2000, estimation of anemia came up to two per cent of all YLD, and for disability-adjusted life-years it was one per cent.[1] Similar results were found in the GBD 2004 update.[25] The worldwide anemia prevalence in year 2010 was estimated to be 32.9%, which was leading to 68.4 million years YLD.[4] The increased prevalence of anemia has increased the overall global burden of disease. The global anemia burden caused by South Asia accounted for 37.5%, whereas the contribution of sub-Saharan Africa was 23.9% of it.

Burden statistics of Anemia - India

India has continuously witnessed the burden of anemia since decades, despite various measures taken up by national and state health programs in reducing its prevalence. The DLHS4 (District Level Household Survey) 2014 and AHS (Annual Health Survey) 2015 reports records reveal the highest prevalence of anemia in India, which is highest in and around the globe.[5]

In India during 2014 - 2016, 190.7 million (14.5%) population was deficiently nourished as per the report published by the FAO (Food and Agriculture Organization) on the condition of nutrition and food security.[26] The Global Burden of Disease Study 2016,[27] reported for disability-adjusted life years for female population, iron-deficiency anemia as among its top 10 causes. Ministry of Health and Family Welfare conducted latest, the National Family Health Survey (NFHS4), which stated anemia prevalence as 53.1, 58.6, 22.7 and 50.4 per cent, among children between the age group of six to fifty nine months, females aged 15 - 49 years, pregnant females aged 15 - 49 years and males aged 15 - 49 years.[28]

A task force study was conducted by the Indian Council of Medical Research (ICMR), New Delhi, across sixteen districts of eleven states. As per the study findings, 84.9 and 90.1 per cent prevalence of anemia was observed in a study group of eleven thousand two hundred and sixty which included pregnant females (n=6923) and girls in adolescent age groups (n=4337). [29]

A survey conducted by NNMB (National Nutrition Monitoring Bureau) (under the aegis of ICMR) in 8 States has reported anemia to be sixty-seven to seventy-eight per cent among preschool children, pregnant and lactating females and girls in

adolescent age group residing in the rural areas.[30] Reports among the rural population of Maharashtra showed the prevalence rate of anemia as 91%,[31] whereas in rural Telangana, females in between age groups of fifteen to thirty-five years (n=979) reported less anemia prevalence (28.4%) than other micronutrient deficiencies like ferritin (46.3%), folate (56.8%) and vitamin B_{12} (44.4%).[32]

Nutritional anemia is caused by deficiencies such as micronutrients including folic acid, vitamin B_{12} and iron. No national data on the level of these micronutrients is available. However, studies conducted have recently stressed on deficiencies existing until now in our community of India[30] with as high as 70-100 per cent deficiency of vitamin B_{12}. This may also due to, twenty nine per cent of the Indian population following a vegetarian diet.[30] The folate deficiency does not have higher prevalence as compared to vitamin B_{12} deficiency; However, studies conducted at Maharashtra and New Delhi with adolescents and preschool children as study subjects have reported deficiency of about forty to sixty per cent. Studies have concluded the prevalence of low ferritin to be almost sixty to seventy percent of the total population in community.[32]

Dimorphic anemia (DA) is one of the common anemias in India. Bentley M. E et al. conducted a study, showing the incidence of DA to be 12.5%.[33] Research by Garg P. et al.[34] in 2017 reported the prevalence of dimorphic anemia and spoke of the presence of dual cell populations in a peripheral smear of DA of which one population is microcytic hypochromic and other is either normocytic or macrocytic. In his study of peripheral blood smears, dual community was seen in 17.5% of people with dimorphic anemia (n=178). Out of 178, in 37.1% of subjects, normocytic

normochromic with microcytic hypochromic red blood cells were observed whereas macrocytes were seen in 62.9% of subjects.

The etiology of low hemoglobin concentrations can be due to genetic traits such as thalassaemia or sickle-cell anemia.[35] Other factors are inadequate bioavailability of consumable iron in foods, vitamin B_{12} or folate, [36,37] malaria,[38] schistosomiasis,[39] hookworm infection,[40] HIV infection and other non-communicable diseases.

HbA1c and its role in the diagnosis of Diabetes Mellitus

Glycated hemoglobin (HbA1c) is recognised as "unusual" hemoglobin associated with subjects suffering from diabetes, for forty years.[9] Since its discovery, many studies have attempted to correlate the glucose measurements with HbA1c value so that its estimates could be used in estimating the glycemic control. One such study is the A1c-Derived Average Glucose (ADAG) study which included six hundred and forty three subjects who presented with a range of A1c levels. This study estimated a validated relationship between A1c and average glucose across types of diabetes and patient population.[41] Further in the 1980s and henceforth, its use in clinical practice proved to be stronger.[42] The value of HbA1c reveals average plasma glucose over eight to twelve weeks. [43].

In 2009, i.e. a decade later, a hemoglobin A1c cut-off value of 6.5% for diabetes mellitus diagnosis was recommended by the International Expert Committee with members appointed by the ADA, the International Diabetes Federation (IDF) and the European Association for the Study of Diabetes (EASD).[44] The analysis conducted by DETECT-2 study formed the basis for these values. This database

analysis of thirteen studies examined the interrelation of retinopathy with measures of glycemia which was assessed and graded by data comprising of fundus photography of 28,898 participants aged twenty to seventy nine years .[44]

A year later in 2010, Clinical Practice Recommendations and the ADA, and World Health Organization in 2011, incorporated an HbA1c of 6.5% or greater as a diagnostic criterion for diabetes. The HbA1c testing offers many advantages over FBG or 2-hour blood glucose tests for the diagnosis of diabetes.[44] The advantages according to the Diabetes Control and Complications Trial (DCCT) / United Kingdom Prospective Diabetes Study (UKPDS) are as follows

- Overall glycemic exposure's better index;
- Similar or higher risk prediction of complications (retinopathy) of long term nature;
- Biologic variability significantly less;
- Short-term intra-individual variability lower;[45]
- Pre-analytical stability higher (up to one week at 4°C);
- Ability to sample blood at any time — fasting samples no more required;
- Relatively unaffected by acute fluctuations in glucose levels (e.g., due to stress or dietary intake);
- Established for guiding diabetes management and decision support;
- Expected faster results than Oral Glucose Tolerance Test.

Pathophysiology of formation of HbA1c

The glucose estimates and complications of diabetes have a direct relationship with HbA1c.[43] HbA1c measurement has been recommended by international guidelines in order to estimate the overall control of diabetes and patient's risk for complications.[46] The mechanism of formation of glycated hemoglobin is as follows:

- It is mainly by a non-enzymatic, post-translational process which depends on substrate concentration. Formation of HbA1c is an irreversible process, in which the aldehyde group of glucose and other hexoses combines with the amino-terminal valine of the β-chain of hemoglobin.
- Hemoglobin forms glycated hemoglobin. Therefore, as the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases predictably.
- While glycation of hemoglobin occurs over the entire 120 day life span of the red blood cell, within these 120 days, recent glycemia has the largest influence on the HbA1c value.[43,46]
- Hence, the mean blood glucose levels during the past one month, two months and three months contribute fifty per cent, forty per cent and ten per cent respectively to the final HbA1c value.
- The estimation of the t½ of HbA1c is done in 35.2 days by mathematical modelling.[47] HbA1c is firstly considered as a marker with reliability, for the excessive rate of glycation, overall glucose exposure and its direct consequences[48,49] and secondly, as an integrator of both post prandial and fasting glycemic disorders.

As an end result, it is not shocking that post prandial and fasting hyperglycemia were recognised differently or as independent risk factors leading to complications associated with diabetes mellitus. The UKPDS demonstrated that substantial decreases in the risk of all diabetes related end-points were complimented by reductions in HbA1c and FBG levels, mainly the microvascular complications and myocardial infarction risks, which were reduced by fourteen per cent and thirty seven per cent respectively, for each one per cent decrease in HbA1c.[43] The International Diabetes Federation (IDF) and the American College of Endocrinology (ACE) recommend HbA1c values below 6.5%, while ADA recommends control below 7% for most patients.[44]

While setting a target HbA1c level in single subject, his/her health, risk of hypoglycemia and particular health risks must be given due consideration. Subjects at greater risk of microvascular complications may further benefit from lowering HbA1c less than 7% as long as hypoglycemia can be avoided, because the patients themselves are liable for avoiding or responding to their episodes of hypoglycemia. Recent position statement of ADA suggested that the patient's input and the assessment of a doctor on patient's self-care skills both act as deciding factors for the target HbA1c in an individual.[49] ADA guidelines also recommends that patients who are meeting treatment goals/have stable glycemic control need to undergo the glycosylated hemoglobin test at least two times in a year. The quarterly check is recommended for those patients with diabetes who are not meeting glycemic goals or those whose therapy has changed. [48]

Factors influencing HbA1c and its measurement

1. Abnormal hemoglobin: Patients with hemoglobinopathies and associated anemia can also have reduced red cell survival that will have an impact on all measurements of HbA1c. Any condition responsible for changes in red cell turnover also cause false HbA1c results such as major blood loss, chronic malaria, haemolytic anemia, glucose-6-phosphate dehydrogenase deficiency and sickle cell anemia. It has been demonstrated that erythrocyte survival is shorter at chronic high glucose levels and this hyperglycaemia-related decrease in erythrocyte survival improves on control of hyperglycaemia which results in an exponential underestimation of the severity of hyperglycaemia at higher HbA1c levels.[50]

2. Ethnicity & Age: It has been observed that non-diabetic subjects who are older in age have higher HbA1c values than individuals of younger age groups. Approximately 0.4% higher at seventy years than at forty years, even after adjusting for fasting and 2-hour glucose is seen. The consistent differences in the HbA1c have also been observed between populations of different races.[51]

3. Analytical considerations: Clinically, there are significant differences between different instruments from different manufacturers and laboratories using them for evaluating HbA1c. HbA1c estimation does not require a fasting state, and the analytical variability is < 2%.[43]

4. Drugs: High-dose salicylates, ribavirin, dapsone, Antiretrovirals, Trimethoprim-sulphamethoxazole, vitamins C and E have been reported to be interfering substances in the measurement of HBA1c.[9,52]

5. Erythropoiesis: Increased HbA1c results due to iron, vitamin B_{12} deficiency in cases of decreased erythropoiesis. Whereas low HbA1c has been noted when there is administration of erythropoietin, iron or vitamin B_{12}.[50]

Association of Hemoglobin and HbA1c: Pathophysiological Mechanisms

Hemoglobin A1c (HbA1c) or glycated hemoglobin is formed by an irreversible, slow non-enzymatic catalysis of the β chain of globin in mature hemoglobin (Hb).[53] Monitoring of glycemic status in diabetic patients can be done by HbA1c, which is a gold standard.[54] Integrated measure of glycemia is the advantage of HbA1c because it is less vulnerable to transient change of blood glucose levels. Individuals who are suffering from diabetes mellitus can use this as a tracking tool for diabetic treatment. HbA1c as a diagnostic test for type 2 diabetes patients was approved by WHO and ADA.[6] Four to six per cent is the normal range of HbA1c in a healthy person.[55]

Clinically, HbA1c levels depend on three major factors:

- HbA1c in reticulocytes when released from the bone marrow;
- Hb glycation rate as RBCs become older, a function of glucose concentration to which Hb is exposed; and
- The mean age of RBCs in the circulation.[56]

Various factors like structural hemoglobinopathies, thalassemia syndrome, and alteration in the quaternary structure of Hb can affect HbA1c levels.[57] Different types of anemia also change HbA1c levels.[21] Both developed countries and counties in developing state have a higher prevalence of anemia. Iron deficiency alone contributes to 50% of the global anemic burden.[58,59] Iron can regulate the clinical

profile of many systemic diseases,[60] because it is involved in important metabolic processes such as DNA synthesis, oxygen transportation, electron transport, regulation of cell growth and differentiation.[61] Red blood cell turnover can be raised in iron deficiency anemia (IDA), which leads to a rise in hemoglobin glycation and thus, HbA1c values will rise, as in myelodysplastic disease, hemolysis, red cell disorders, blood loss and hemoglobinopathies.[62] In literature few studies are in agreement with the fact that the changes in the iron levels of body can influence HbA1c.[16] Rise in the glycation of Hb has also been linked to the low serum iron and serum ferritin levels.[63] Iron metabolism and glucose homeostasis both have bidirectional relationship. Secretion of insulin and its action, both were modulated by higher iron levels.[60] Both in the non-diabetic and diabetic population, false rise of HbA1c values was because of low iron levels and higher glycation of HbA1c.[64]

A study done by Brooks et al.[65] shows that there was excessive glycation of the beta-globin because of the relative absence of iron which resulted in the alteration of the quaternary structure of the Hb molecule. Sluiter et al.[66] study findings state that with the ageing of RBC, there was a rise in HbA1c in the erythrocyte. Glycation of Hb is a unidirectional process which is irreversible.

Higher HbA1c values were reported in IDA patients in a study by Coban et al.[64] He also observed drastic fall in HbA1c values on treatment with iron. This was supported by studies by Mudenha et al.[67] Other studies by Silva et al.[68] and Rajagopal et al.[56] observed that HbA1c values would differ among diabetic and non-diabetic patients with the severity of IDA.

Association of Hemoglobin and HbA1c: Diabetic Population

A cross-sectional study by Soloman et al.[69](2019), aimed to examine the influence of IDA on HbA1c among diabetic patients. The study included 174 diabetic patients of which 87 were diagnosed with IDA and 87 without IDA. A structured questionnaire was used in evaluating socio-demographic data and clinical conditions. Cell Dyn 1800 haematology analyser was used for collection of venous blood for performing complete blood count (CBC). The results depicted lesser hematocrit (HCT), Mean Cell Hemoglobin (MCH), Mean Cell Volume (MCV), mean hemoglobin, Mean Cell Hemoglobin Concentration (MCHC) in IDA group compared to non-IDA in participants without diabetes. These results suggested that HbA1c values were comparatively lower in diabetic patients with IDA in comparison with non-diabetic patients with IDA.

A cross-sectional study by Abbas AE et al.[55](2017) researched the association between HbA1c and Hb / RBC count and RBC indices; mainly in pregnant females without diabetes. A total of one twenty three pregnant non-diabetic females were recruited for analysis. FBG, hemoglobin A1c and two hour postprandial sugars were recorded. Lab parameters of Red Blood Cells were performed and included Hb, HCT, MCH, MCHC and MCV. The mean body mass index and age of participants were 27.65±6.8 kg/m2 and 28±5.6 years respectively. There was significant positive correlation between HbA1c and Hb (r=0.174, p=0.037), Hct (r=0.174, p=0.037) and MCHC (r=0.180, p=0.031). The study concluded that there was a significant positive correlation between HbA1c value with Hct, Hb, and MCHC, whereas between HbA1c and other RBC parameters there was no significant correlation.

The effect of IDA on HbA1c levels was studied by Christy AL et al.[63] (2014) in a case control study. A total of 120 diabetic iron deficient individuals with anemia and controlled plasma glucose levels with similar number of iron-sufficient non-anemic subjects as controls were examined. Student's t-test was used for statistical analysis along with Pearson's coefficient of regression and Chi-square test. HbA1c (6.8 ± 1.4%) among iron-deficient individuals was increased as per findings as compared with control groups. The females (7.02 ± 1.58%) showed significantly higher elevation than the males. Diabetes diagnosis made based on FBG levels showed A1c elevation in subjects with fasting glucose levels between hundred to one twenty six mg/dl (7.33 ± 1.55%) compared to the those with normal plasma glucose levels (<100 mg/dl). However, no significant correlation was found between HbA1c and ferritin and hemoglobin. The study concluded a positive correlation between increased A1c levels and iron deficiency anemia, especially in controlled diabetic women and individuals having FBG between hundred to one twenty six mg/dl.

A population based study in China by Attard S et al.[15] (2015) aimed to determine how anemia alone, iron-deficiency anemia (IDA) and only IDA were each related with prevalence of pre-diabetes and diabetes using FBG versus HbA1c in a community-based study of adult individuals with endemic IDA. This longitudinal study used data from the China Health and Nutrition Survey. The final analysis included seven thousand three hundred and eight adults from a 2009 survey aged eighteen to seventy five years, to examine relative risk of prediabetes and diabetes with the help of descriptive and covariate-adjusted models using FBG alone, HbA1c alone, FBG and HbA1c, or neither (normoglycemia) by anemia alone, IDA or normal iron/hemoglobin, iron deficiency alone. Within the sample size, 65% of individuals were identified as people with diabetes using both HbA1c and FBG while the other

thirty five per cent had a conflicting classification of diabetes and were classified using either FBG or HbA1c or, but not both. Fewer, with normal iron/hemoglobin versus iron deficiency alone were grouped as diabetes using HbA1c only. The regression analysis showed the adjusted prevalence of prediabetes using HbA1c only was twenty two per cent for male individuals having anemia alone, but thirteen per cent for males with normal levels of iron/hemoglobin. Conversely, the prediabetes predicted prevalence using HbA1c only was eight per cent for females with ID only, compared to thirteen per cent for females with l iron/hemoglobin normal levels. Hence, the results suggested a greater chance of misdiagnosing diabetes in anemic patients using HbA1c.

Perisammy et al.'s [71] (2016) study aimed to find the incidence of anaemia in patients of type 2 diabetes mellitus among the rural population, and its prognostic value. This is prospective observational study included 250 patients diagnosed with type 2 diabetic mellitus. Inclusion of patients greater than 18 years of age with risk factors such as hypertension and coronary artery disease was considered. An exclusion of age of below 18 years with anaemia due to chronic blood loss, or other secondary causes and patient on treatment for anaemia were considered. Lab investigations done included HbA1c, complete blood picture and peripheral smear study. This study had a majority of female participants of 160 (64%) with mean age of 60±13 years with mean duration of diabetes being 4.1±3 years and mean hemoglobin level of 11.3 ±2 g/dl. Co-morbid condition such as coronary artery disease and hypertension was present in 90 and 110 patients respectively. Mean HbA1c value was 8.4±2.1%. Normocytic Normochromic anemia was found in 25% of anemic patients; microcytic hypochromic anemia in 35%, macrocytic hypochromic in 32% and dimorphic anemia was seen in 8%. Hence in this study, anemia was present in 74% of

the study population. This prevalence of anemia in diabetic population points toward the need for haematological evaluations and care for the diabetic patients to prevent confounding factors like anemia being the cause for spuriously high levels if HbA1c for better outcome of patients.

Association of Hemoglobin and HbA1c: Non-Diabetic Population

Paralapally RP et al.[70] (2016) conducted a study to assess the effects of anemia on HbA1c among iron deficient non-diabetic subjects. The study involved 63 non-diabetic, anemic patients, and 63 age-matched healthy subjects. The results of the study showed greater mean HbA1c (6.13% ± 0.6%) level in patients with iron deficiency anemia (IDA) as compared to the control group (5.12% ± 0.5%) (p value < 0.001). The incidence of IDA was greater among reproductive age group of females, and the HbA1c levels were primarily on higher side, between 6.0% and 6.5%.

A systematic review by English et al.[21] (2015) reviewed 12 articles which studied the effects of anemia on HbA1c values. The search engines used for the purpose of the study were the Cumulative Index to Nursing & Allied Health Literature (CINAHL), EMBASE, MEDLINE, Cochrane Library and was conducted for related studies published from January 1990 to May 2014. The studies with one measurement of, and one index of hematinic deficiency, HbA1c and glucose, negative diabetes mellitus history involving non-pregnant adults were searched. A total of twelve articles were finally selected. The included studies mainly focused on IDA and presence of iron deficiency with or without anemia demonstrating an increased HbA1c levels as compared to controls. The influence of other indices of erythrocyte abnormalities on HbA1c was partial but showed a possible decrease in HbA1c values with non-iron deficiency forms of anemia.

Shanthi et al.'s[17] (2013) study aimed to determine the effect of IDA on the HbA1c levels in non-diabetic patients. Study subjects of 100 were included, where 50 were non-diabetic anemic patients who were the cases, and the other 50 were healthy subjects as controls. Both groups were age-matched. The study population was subjected to hematologic investigations, fasting and post prandial glucose and HbA1c levels. The results of the study showed higher mean HbA1c levels (7.6 ± 0.5%) in the subjects with IDA as compared to that of the control group (5.5% ± 0.8) ($p < 0.001$). However, there was no statistical significance in the levels of fasting and post prandial glucose between the IDA group and the control groups ($p > 0.05$). The hemoglobin, serum ferritin, fasting and post prandial glucose, and the HbA1c levels were normal in the control group ($p > 0.05$). Hence, the measurement of HbA1c solely does not depend on the blood glucose levels but are impacted by confounding factors, especially anemia.

Hardikar PS et al.[14] (2012), examined the impact of non-glycemic and glycemic parameters on HbA1c concentrations among young non-diabetic adults. The analysis also included comparing the diagnosis of normal glucose tolerance in diabetics and prediabetics with a standard Oral Glucose Tolerance Test (OGTT) and HbA1c concentrations in 116 young adults whose average age was 21.6 years. The OGTT showed 2.6% population in diabetic stage and 7.8% of participants in prediabetic stage. By ADA HbA1c criteria, 2.6% were diabetic, and 23.3% were prediabetic. The prevalence of anemia was 34%, of which iron-deficiency was 37%, Vitamin B_{12} was 40 % and folate deficiency accounted for about 22%. The multiple regression analysis of HbA1c predicted lower hemoglobin ($R2 = 7.7\%$) and higher 2-h glucose ($R2 = 25.6\%$). Hence, this study added to the evidence that use of HbA1c

alone to diagnose prediabetes and diabetes in iron-deficient populations may cause an overstated prevalence.

Hansen G et al.'s[72] 1990 study measured glycosylated hemoglobin (HbA1c) in 10 patients with iron deficiency anemia, ten patients with vitamin B_{12} deficiency anemia and ten healthy controls. The results in the initial period showed no significant differences between the groups (p value > 0.4), but after treatment with iron and vitamin B_{12} for 3 and 6 weeks, the glycosylated hemoglobin concentration decreased significantly ($p < 0.01$). This study's results concluded that glycosylated hemoglobin is a sensitive marker of the changes in the erythrocyte population, which are observed when predominantly immature erythrocytes are being produced.

Ford, E. S. et al.[73] (2011) examined the effect of iron and hemoglobin (Hb) status on HbA1c and on the association between concentrations of fasting glucose and HbA1c in a national sample of adults in the United States of America. The prevalence of low Hb (defined as < 120 g/L and < 118 g/L in women aged 20 – 69 years and ≥ 70 years respectively, and < 137 g/L, < 133 g/L and < 124 g/L in men aged 20 – 49 years, 50 – 69 years and ≥ 70 years respectively) was 5.5%. There was a significant positive correlation between Hb concentrations and HbA1c concentrations after adjusting for age, race or ethnicity, gender, with HbA1c rising from a mean of 5.28% among study subjects with Hb < 100 g/L to 5.72% among participants with Hb ≥ 170 g/L. The study concluded that while diagnosing prediabetes and diabetes among population with a high or low Hb when the HbA1c level is near 6.5% or 5.7%, caution should be used, as changes in erythrocyte turnover may reduce the test result.

Association of severity of Anemia & HbA1c

Manjhavar SK et al.[74] (2017) in their prospective observational research had the aim to analyse the effect of iron deficiency anemia on glycated hemoglobin (HbA1c) in non-diabetic Indians. The study comprised of five hundred iron deficiency anemia patients. For analysis of the data appropriate descriptive statistics were used. They observed an increased level of HbA1c with severity of IDA. The total number of women was three hundred and seventy-nine out of five hundred. A total of 73 women had mild anemia; moderate anemia was seen in 242 women, and severe anemias in 64 women. The male subjects were 121 in number. Mildly anemic were 34, moderately anemic were 73, and severely anemic were 14 males. It was observed that IDA was more common among the reproductive age group in females. It was closely related with a shift in the HbA1c levels to higher side with severity of anemia.

Takeuchi M et al.[75] (2018) studied the effects of HbA1c levels with degree of anemia. The study included subjects aged 18 – 75 years. They found lower HbA1c levels were associated with low Hb levels, but these values differed among populations. In the cohort of 55,420 people with type-2 diabetes mellitus, the curve indicating the relationship between Hb and HbA1c was generally a plateau in the persons with Hb value < 12 – 13 g/dL. Whereas, in a cohort of 18,478 people with HbA1c around 6.5%, a linear trend was noticed. Among the 6253 people on oral hypoglycemic agents, a right upward curve was observed. These results suggest that the relationship between HbA1c and Hb are not direct, but varied among individuals with dissimilar clinical interest.

Elhabbash M. [76] (2018), in her study aimed to find the association of IDA with HbA1c levels among patients without diabetes before and after treatment with iron supplements. A total of 100 female subjects, of which were 50 women subjects with IDA and 50 healthy female subjects of 20 - 40 years were included. The lab investigations included fasting blood glucose, HbA1c, complete blood count, serum iron, total iron-binding capacity and serum ferritin; measured in all females before treatment and after treatment. The treatment for all IDA women included oral intake of 325 mg ferrous sulfate three times a day for three months. The mean percentage of the HbA1c in the non-anemic patients (i.e. control group) was 5.2%. The mean percentage of HbA1c in patients with mild anemia was 6% and moderate anemia was 5.9%.

The HbA1c was 3.2% in severely anemic women which when compared to normal females was low. Post treatment with iron supplements, in severely anemic females the HbA1c levels rose to 5.2% whereas a decrease in HbA1c was observed in females with mild and moderate anemia (5.2% and 5.3%) respectively which was significant (p value < 0.001). The study results inferred that greater the severity of anemia lesser the HbA1c levels and that with intervention with iron supplements, the HbA1c levels increased.

Maheshwari VD et al.[77] (2017), studied the effects of vitamin B_{12} and iron deficiency anemia on HbA1c levels in a tertiary centre. The study included 60 patients, out of which 30 were of iron deficiency anemia, and the remaining 30 had Vitamin B_{12} deficiency anemia. Control group of 30 non anemic, non-diabetic were included. In the iron deficiency anemia group, among the 30 participants 36.67% were male and 63.33% were female, while 53.33% were male and 46.67% were

female in the Vitamin B_{12} deficiency group. The participants of control group were 60% male and 40% female. In iron deficiency anemia, ten out of thirty had Hba1c in the range of impaired glucose homeostasis (i.e. 5.6% - 6.5%), while in Vitamin B_{12} deficiency anemia twelve out of thirty individuals had Hba1c in the range of impaired glucose homeostasis. The study reported two patients of iron deficiency anemia with Hba1c in diabetic range of ≥ 6.5%. The study results thus concluded that lower hemoglobin levels were associated with higher HbA1c values.

Rajagopal L et al.'s[56] study evaluated and aimed to find a correlation of HbA1c values with the grades of anemia. The study was a cross-sectional study which included one fifty non-diabetics (seventy five with IDA and seventy five without IDA) as study subjects. The results of the study showed greater mean HbA1C among IDA patients than the non-anemic group. Further the HbA1c values increased with severity of anemia. This study showed that a positive correlation exists between IDA and elevated HbA1c level in non-diabetics.

Another study by Rajagopal L et al.[78] (2017); a cross-sectional study, compared the effects of IDA on HbA1C levels among controlled diabetics (FBG < 126mg/dl since last six months) and non-diabetics, and its variation according to the degree of anemia. This study involved 150 controlled diabetics where 75 were with IDA and 75 without IDA, and 150 non-diabetics where 75 were with IDA and 75 were without IDA. The necessary lab investigations of HbA1c, complete hemogram, iron profile and FBG were done. The mean HbA1c in controlled diabetics with and without IDA was 8.81±0.13% and 5.79±0.01% respectively ($p < 0.05$). The mean HbA1c among non-diabetics with and without IDA was 6.84±0.07% and 5.12±0.04% respectively ($p < 0.05$). The difference between non anemics, mildly, moderately and

severely anemics in both diabetics and non-diabetics was statistically significant ($p < 0.05$). Groups with severe anemia showed greater mean HbA1c. Hence, these results infer that IDA falsely elevates HbA1c levels, independent of blood glucose concentrations in both controlled diabetics and non-diabetics. This further mandates a thorough evaluation of anemia to minimise the misdiagnosis of diabetes among the highly prevalent anemic population.

A case control study by Silva et al.[68] in 2016, investigated the effect of IDA on HbA1c levels in non-diabetic individuals. The study included 122 patients where, sixty one patients were with IDA and sixty one patients without anemia. Two methods were employed to assess HbA1c which were ion-exchange HPLC Variant II Turbo BioRad and Immunoturbidimetry (IT) Tina Quant II Roche Diagnostics for each sample. This eliminated any bias created by the method of testing HbA1c in the laboratory. The results showed statistical difference between HbA1c in patients with IDA (Mean HbA1c by HPLC 5.6 ± 0.4% and IT 5.7 ± 0.4%) and without IDA (Mean HbA1c by HPLC 5.3 ± 0.4% and IT 5.3 ± 0.3%) with a p value of < 0.001. The results of this study infer that with moderate and severe grade of anemia, the HbA1c values increased, whereas mild grade of anemia had no significant effects on HbA1c values.

Lacunae in Literature

Anemia and diabetes are the two of the most prevalent conditions adding to the burden of disease across the world. The relationship of HbA1c with iron deficiency anemia has been studied extensively in literature. But the studies stating what degree of anemia does to HbA1c are few, with different opinions. Not only the severity of anemia but the etiological profile and type of anemia may also be a key

factor influencing HbA1c levels. Hence apart from iron deficiency, the relationship between other important causes of anemia like vitamin B_{12} deficiency and folic acid deficiency with HbA1c level needs to be explored. But the volume of literature studying this aspect is scarce, especially in Indian population. The current study was aimed at fulfilling the above specified lacunae, especially the association between vitamin B_{12} deficiency and severity of different types of anemia and HbA1c levels.

METHODOLOGY

The present study was conducted in the Department of General Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

Study Design

This study was a hospital based cross-sectional study.

Study Period

It was conducted over a period of one year from January 2018 to December 2018

Study Site

The present study was carried out at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. A tertiary care teaching hospital attached to Jawaharlal Nehru Medical College, Belagavi.

Study Population

All patients admitted to the wards of Department of General Medicine at KLES Dr. Prabhakar Kore Hospital, Belagavi fulfilling the inclusion criteria.

Sample Size

A total of 100 patients with anemia were studied.

Sampling Method

The following formula was used for calculation of the sample size

$$n = \frac{z_\alpha{}^2 P(1-P)}{d^2}$$

Where:

z_α = 1.96 (at 95% confidence interval)

P = percentage of prevalence

d = absolute error

Inclusion Criteria

- Males and females above the age of 18 years
- Male subjects with hemoglobin value less than 13.0 g/dl
- Female subjects with hemoglobin value less than 12.0 g/dl
- Patients who are non-diabetic [based on fasting and post prandial blood sugars].

Exclusion Criteria

- Cases of diabetes and prediabetes
- Pregnant women
- Patients with known hemoglobinopathies
- Patients with hemolytic anemias
- Patients with chronic renal failure
- Patients with chronic liver disease

- ❖ Subjects on drugs such as
    - Aspirin,
    - Ribavirin,
    - Dapsone,
    - Anti Retrovirals,
    - Trimethoprim-Sulphamethoxazole,
    - Hydroxyurea

Ethical Clearance

Prior to the commencement, the study was cleared by the Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belagavi.

Informed Consent

Informed consent was obtained from all the study participants and only those participants who willingly signed the informed consent were included in the study. The risks and benefits involved in the study, and the voluntary nature of participation were explained to the participants before obtaining consent. Confidentiality of the study participants was maintained.

Data Collection

All relevant parameters were documented in a structured Study Proforma

Methodology

Inpatient, non-diabetic anaemic patients above the age of 18 years and fulfilling the inclusion criteria were enrolled for the study after obtaining written

informed consent in their own vernacular language. Demographic data and detailed history was recorded. A thorough physical examination included the recording of Vital Data (Pulse Rate, Blood Pressure, Temperature and Respiratory Rate), general physical examination and a thorough systemic examination.

The fasting blood sugars and post prandial blood sugars were measured in order to rule out a diagnosis of diabetes mellitus. Following this, the patients were categorised into the 3 categories of severity of anemia, based on the WHO Grading, as per the patient's hemoglobin values. Peripheral smear and HbA1c was measured and recorded and a correlation of severity of different types of anemia with HbA1c values was studied.

Investigations

Venous blood samples were collected and subjected to the following investigations

- Hemoglobin
- Peripheral smear
- Fasting blood sugar
- Post prandial blood sugar
- HbA1c

Severity Grading of Anemia

The WHO Grading for Anemia was employed in this study, categorising patients into mild, moderate and severe anemia based on the hemoglobin levels. The grading system is as below.

❖ **WHO Grading of Anemia**

Population	Non Anemia	Mild Anemia	Moderate Anemia	Severe Anemia
Non Pregnant Women (15 years of age and older)	12 g/dl or higher	11-11.9 g/dl	8-10.9 g/dl	Below 8 g/dl
Men (15 years of age and older)	13 g/dl or higher	11-12.9 g/dl	8-10.9 g/dl	Below 8 g/dl

Statistical Methods

Data was analyzed using R i386 3.6.0 statistical software. Continuous variables are represented by mean ± SD form and categorical variables by a frequency table. Chi Square test was used to check the association between different categorical variables. ANOVA was used to analyze the relationship between severity and HbA1c. In the tables below p-value <0.05 indicates statistical significance of variables.

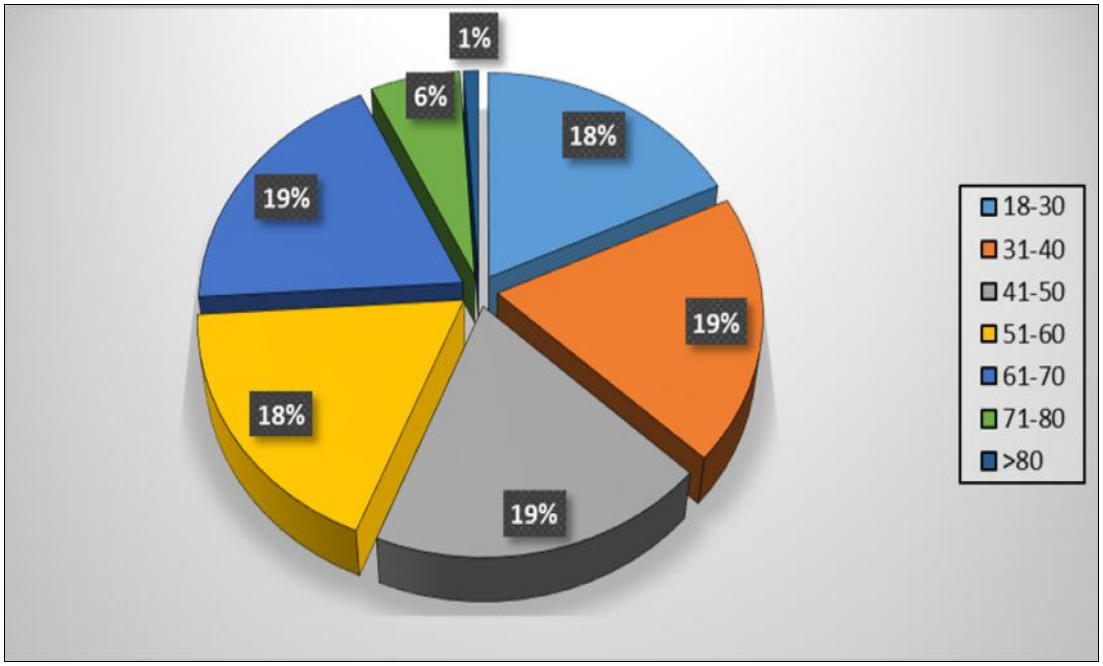
RESULTS

The present cross sectional study titled “A Study on the effect of Severity of Anemia on HbA1c in non-diabetic Patients – A one year hospital based study in KLES Dr. Prabhakar Kore Hospital and Medical Research Centre” was carried out in the Department of General Medicine. During the study period from January 2018 to December 2018, a total of 100 non-diabetic anemic patients were studied. The findings / observations and final results are as tabulated below.

Table 1: Age Distribution

Age (years)	Number of Patients	Percentage
18-30	18	18%
31-40	19	19%
41-50	19	19%
51-60	18	18%
61-70	19	19%
71-80	6	6%
>80	1	1%
Total	100	100%
Mean Age (years)		48.12±16.47

Figure 1: Age Distribution

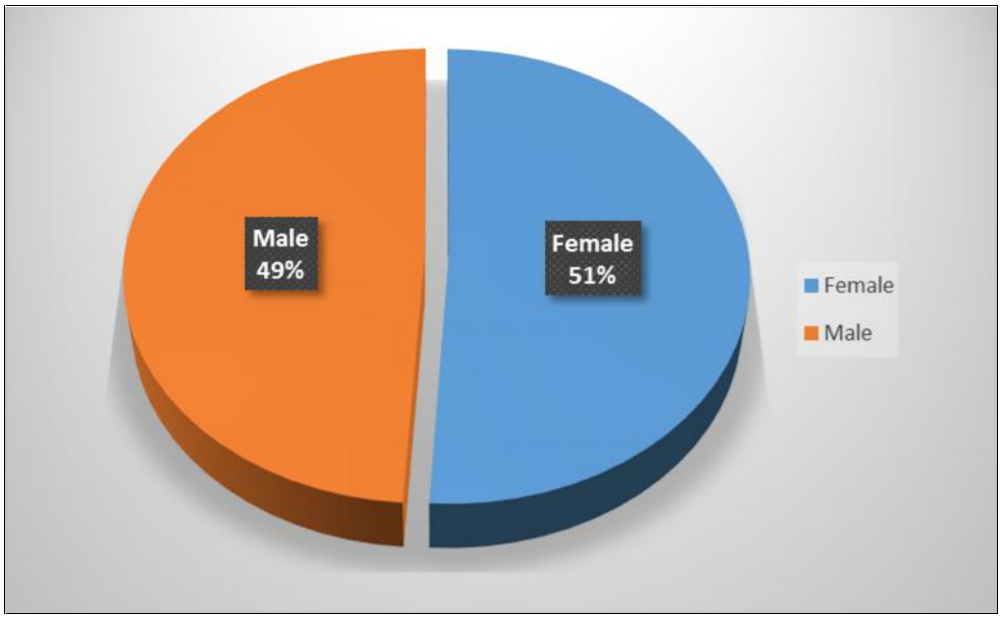


In the present study of 100 patients, we observed patient age ranged from 18-86 years with 19 patients (19%) each between the ages of 31-40 years, 41-50 years and 61-70 years, followed by 18 patients (18%) in the age group of 18-30 years and 51-60 years. Only 1 patient (1%) was in the age group of above 80 years. The mean age was 48.12±16.47 years.

Table 2: Sex Distribution

Gender	Number of Patients	Percentage
Female	51	51%
Male	49	49%
Total	100	100%

Figure 2: Sex Distribution

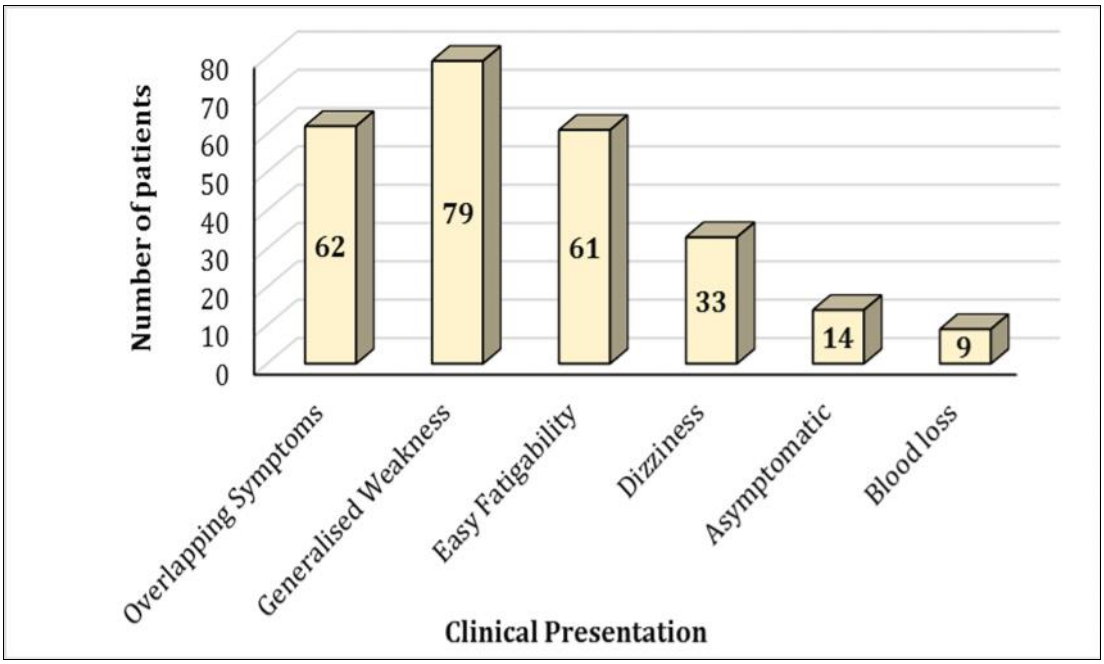


In our study of 100 patients, we observed 51 female patients (51%) and 49 male patients (49%), with M:F ratio of 0.96:1, showing a slight female preponderance.

Table 3: Clinical Presentation

Clinical Presentation	Number of Patients	Percentage
Overlapping Symptoms	62	62%
Generalised Weakness	79	79%
Easy Fatigability	61	61%
Dizziness	33	33%
Asymptomatic	14	14%
Blood loss	9	9%

Figure 3: Clinical Presentation

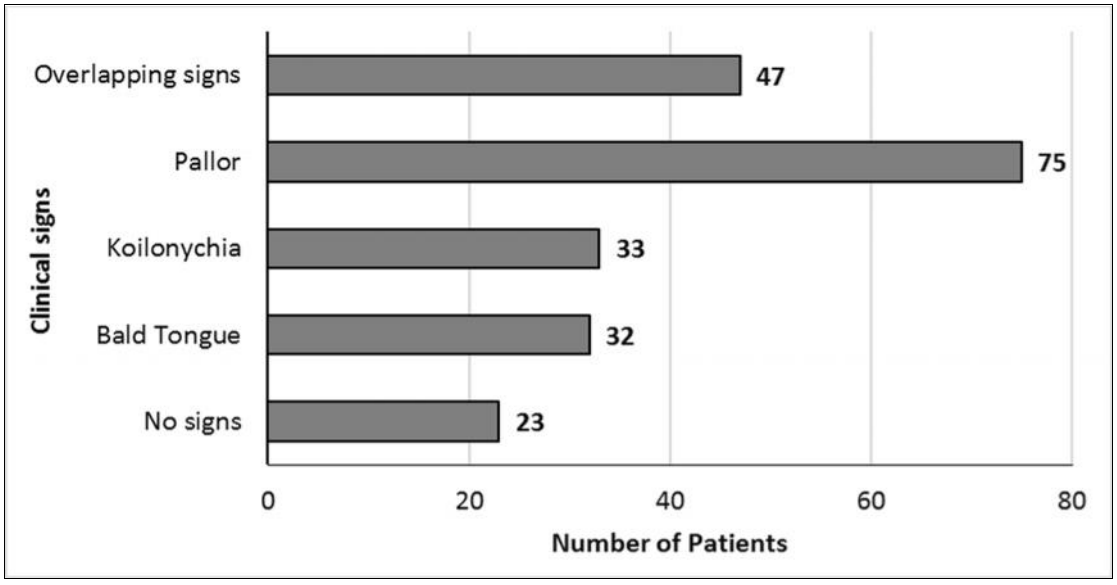


We observed various clinical manifestations of patients, with a majority of 79 patients (79%) with generalised weakness symptom, followed by overlapping symptoms in 62 patients (62%). Other patients had easy fatigability i.e. 61 patients (61%), dizziness in 33 patients (33%) while 9 patients (9%) gave history of blood loss. There were 14 patients (14%) who were asymptomatic.

Table 4: Clinical Signs

Clinical signs	Number of Patients	Percentage
Overlapping Signs	47	47%
Pallor	75	75%
Koilonychia	33	33%
Bald Tongue	32	32%
No Signs	23	23%

Figure 4: Clinical Signs

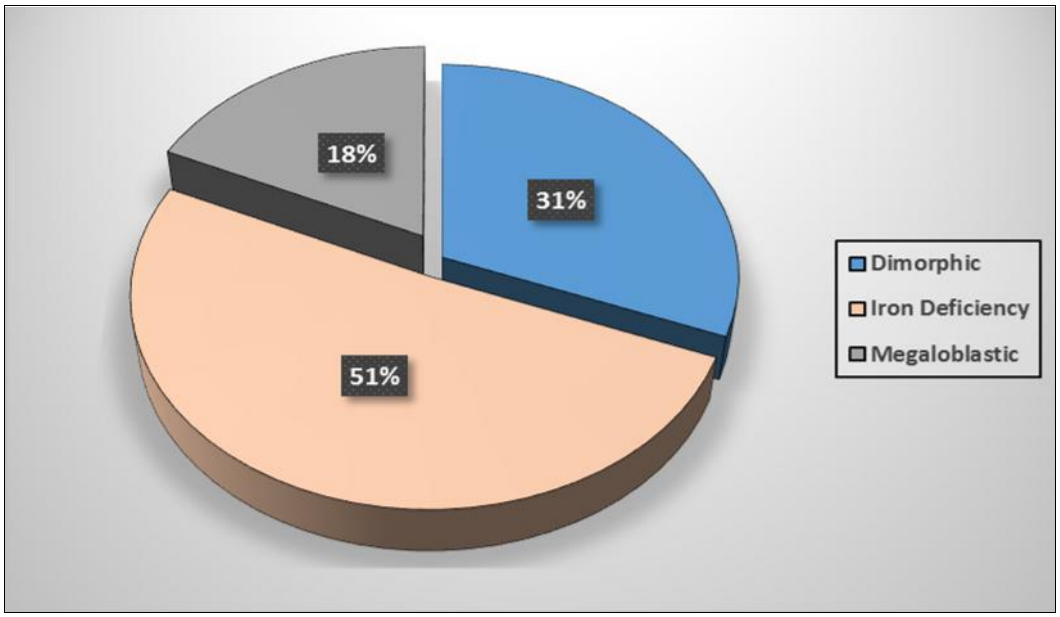


Majority of our patients had signs in the form of pallor (75 patients, i.e. 75%), followed by koilonychia (33 patients, i.e. 33%) and bald tongue (32 patients, i.e. 2%). We found 47 patients (47%) had overlapping signs while 23 patients (23%) didn't have signs.

Table 5: Type of Anemia

Type of Anemia	Number of Patients	Percentage
Iron Deficiency	51	51%
Dimorphic	31	31%
Megaloblastic	18	18%
Total	100	100%

Figure 5: Type of Anemia

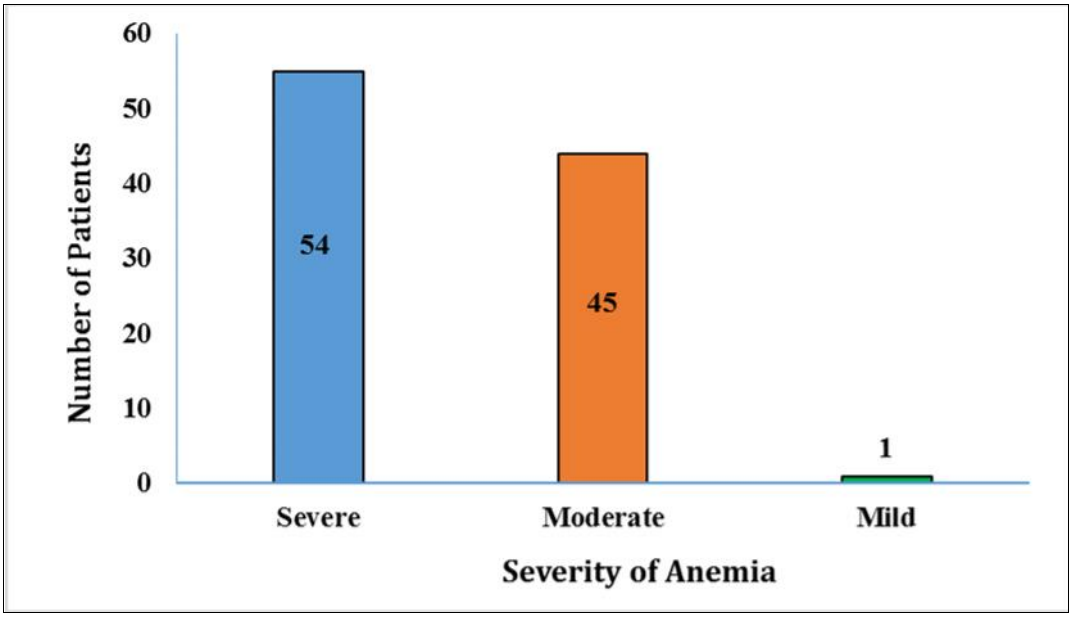


Out of 100 patients, we observed majority had iron deficiency anemia (51 patients i.e. 51%), followed by dimorphic anemia (31 patients i.e. 31%) while the remaining 18 patients (18%) had megaloblastic anemia.

Table 6: Severity of Anemia (WHO Criteria)

Severity (Hb in g/dl)	Number of Patients	Percentage
Severe (<8)	54	54%
Moderate (8-10.9)	45	45%
Mild (11-12.9)	1	1%
Total	100	100%

Figure 6: Severity of Anemia (WHO Criteria)



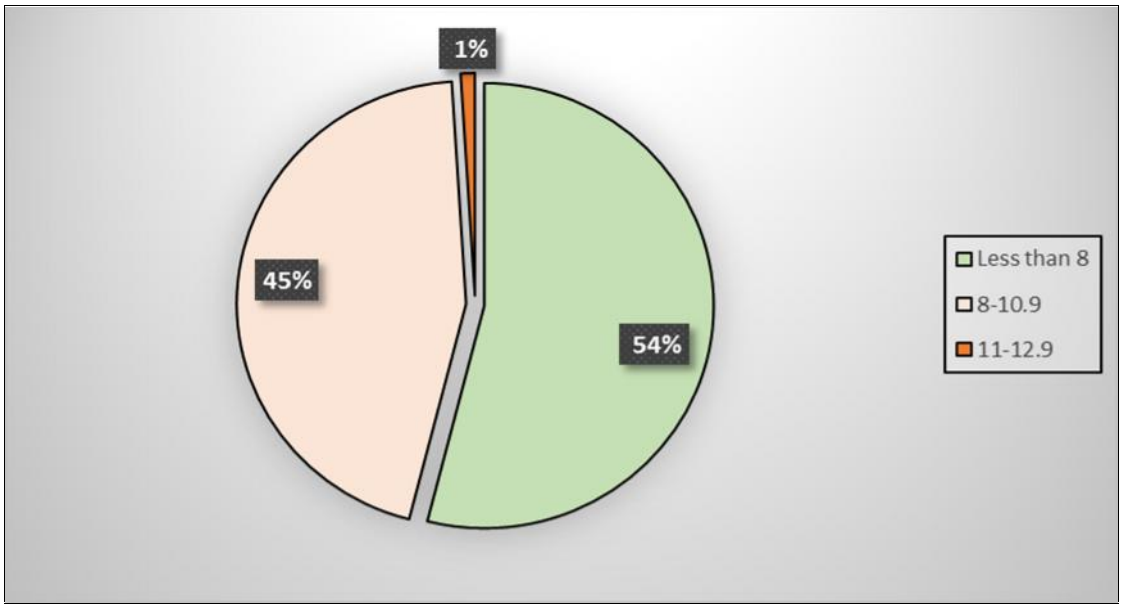
The our study, we observed that 54 patients (54%) had severe anemia, while 45 patients (45%) had moderate anemia and only 1 patient (1%) had mild anemia.

LAB PARAMETERS

Table 7: Hemoglobin Percentage (WHO Criteria)

Hemoglobin (g/dl)	Number of patients	Percentage
< 8	54	54%
8 - 10.9	45	45%
11 - 12.9	1	1%
Total	100	100%
Mean Hb (g/dl)	7.56±2.00	

Figure 7: Hemoglobin Percentage (WHO Criteria)

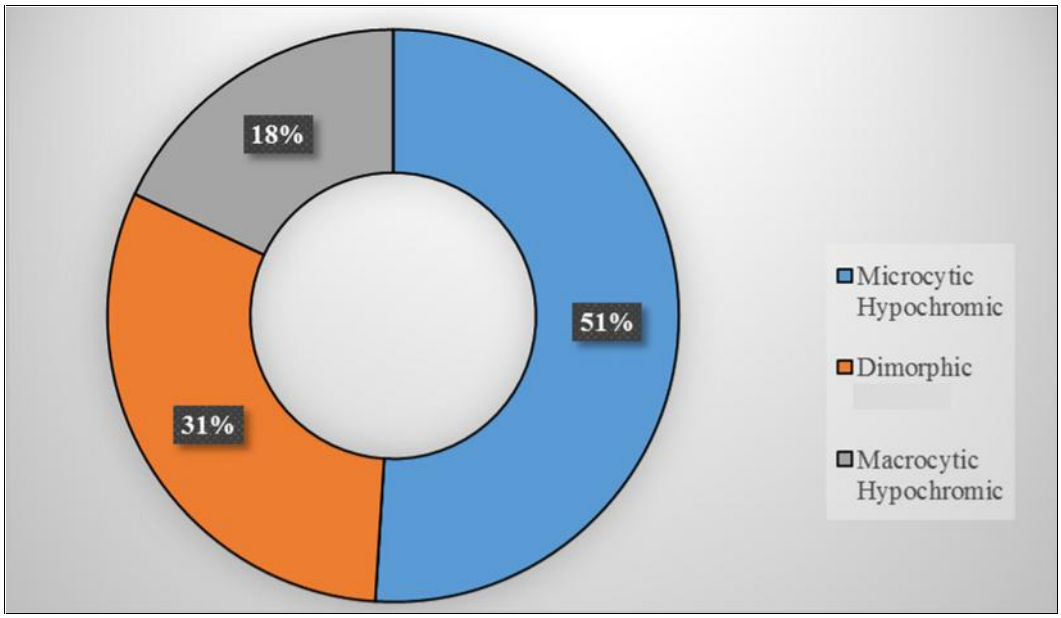


Based on WHO Criteria, 54 patients (54%) had Severe Anemia where Hemoglobin was less than 8 g/dl. 45 patients (45%) had moderate anemia with hemoglobin from 8-10.9 g/dl whereas only 1 patient (1%) had mild anemia as depicted in the above table. The mean hemoglobin in our study was 7.56±2.00 g/dl.

Table 8: Peripheral Smear

Peripheral Smear	Number of patients	Percentage
Microcyctic Hypochromic	51	51%
Dimorphic	31	31%
Macrocyctic Hypochromic	18	18%
Total	100	100%

Figure 8: Peripheral Smear

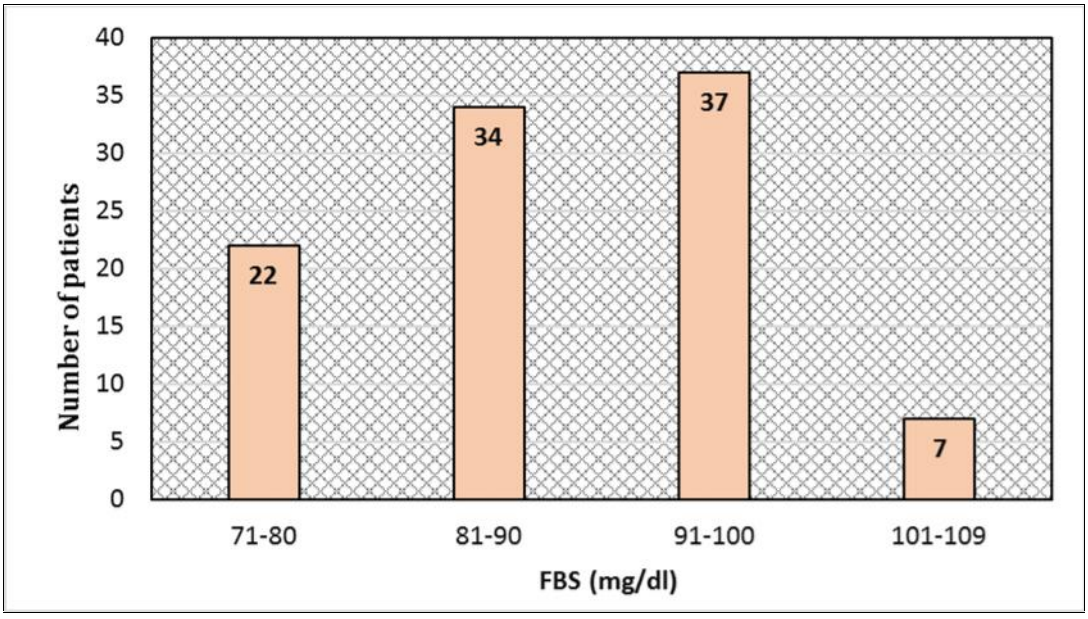


Peripheral smear study revealed that 51 patients (51%) had microcytic hypochromic picture, dimorphic picture was present in 31 patients (31%) and 18 patients (18%) with macrocyctic hypochromic (megaloblastic) blood picture.

Table 9: Fasting Blood Sugar

FBS (mg/dl)	Number of Patients	Percentage
71-80	22	22%
81-90	34	34%
91-100	37	37%
101-109	7	7%
Total	100	100%
Mean FBS (mg/dl)	86.23±8.2	

Figure 9: Fasting Blood Sugar

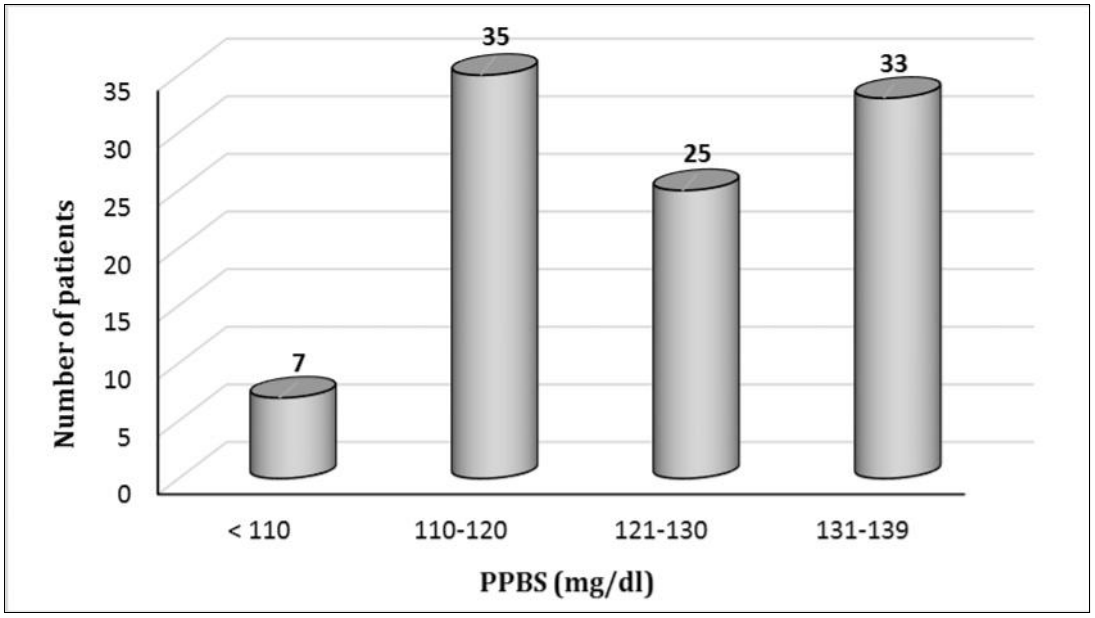


In our patients, we observed that all except 7 patient's (93%) fasting blood sugars ranged from 71-100 mg/dl. The remaining 7 had fasting blood sugars ranging from 101-109 mg/dl (greater than 100 mg/dl but less than 110 mg/dl). The mean FBS was 86.23±8.2 mg/dl.

Table 10: Post Prandial Blood Sugar

PPBS (mg/dl)	Number of Patients	Percentage
< 110	7	7%
110-120	35	35%
121-130	25	25%
131-139	33	33%
Total	100	100%
Mean PPBS (mg/dl)	121.93±10.52	

Figure 10: Post Prandial Blood Sugar

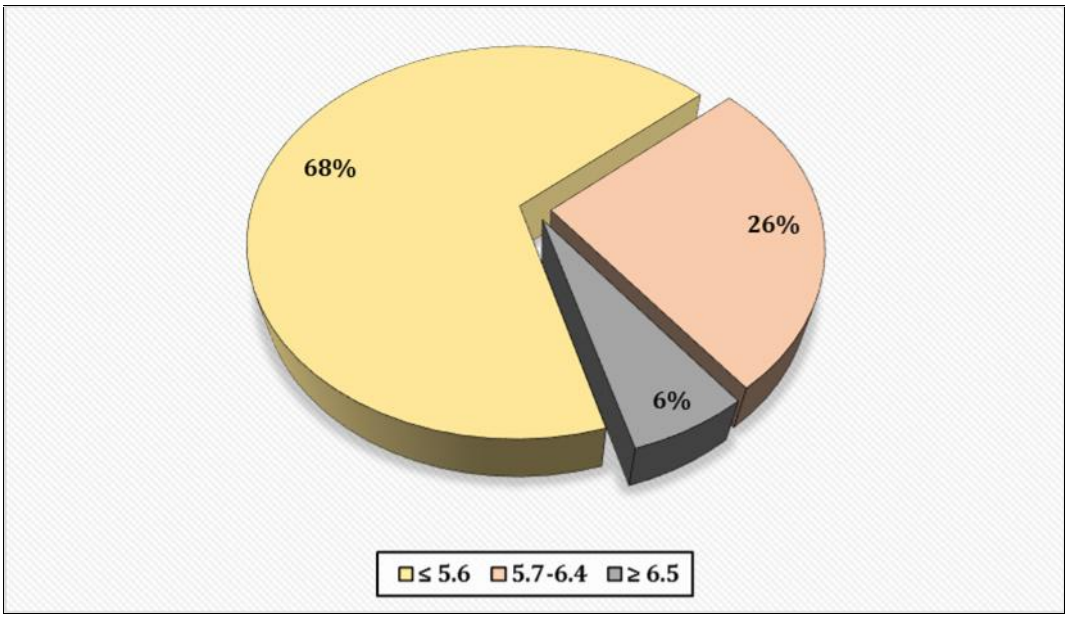


The above table depicts the post prandial blood sugars in our patients. None of the patients had post prandial sugars ≥ 140 mg/dl (33 patients, i.e. 33% had PPBS of 131-139 mg/dl). The mean PPBS was 121.93±10.52 mg/dl.

Table 11: HbA1c

HbA1c (%)	Number of Patients	Percentage
≤ 5.6	68	68%
5.7-6.4	26	26%
≥ 6.5	6	6%
Total	100	100%
Mean HbA1c (mg/dl)	5.34±0.68	

Figure 11: HbA1c

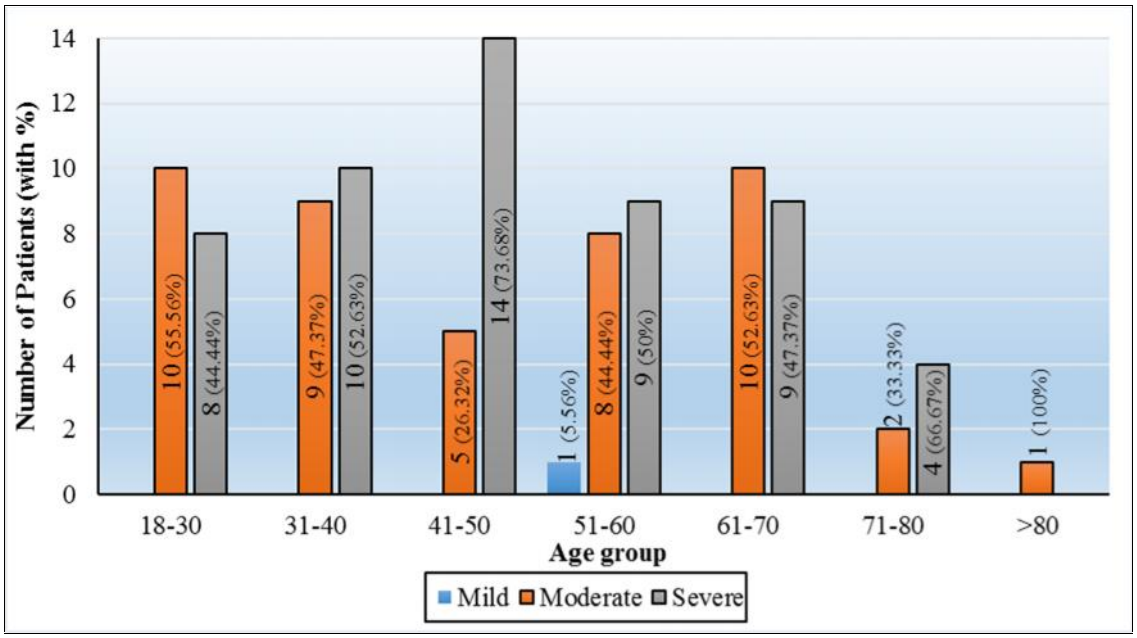


All patients enrolled in our study were subjected to HbA1c estimation. Only 6 patients (6%) had HbA1c ≥ 6.5%. The remaining patients HbA1c is depicted in the above table. The mean HbA1c was 5.34±0.68 %.

Table 12: Comparison of Age with Severity of Anemia

Age (years)	Severity of Anemia			p-value
	Mild	Moderate	Severe	
18-30	0	10	8	**0.4883**
31-40	0	9	10	
41-50	0	5	14	
51-60	1	8	9	
61-70	0	10	9	
71-80	0	2	4	
> 80	0	1	0	

Figure 12: Comparison of Age with Severity of Anemia

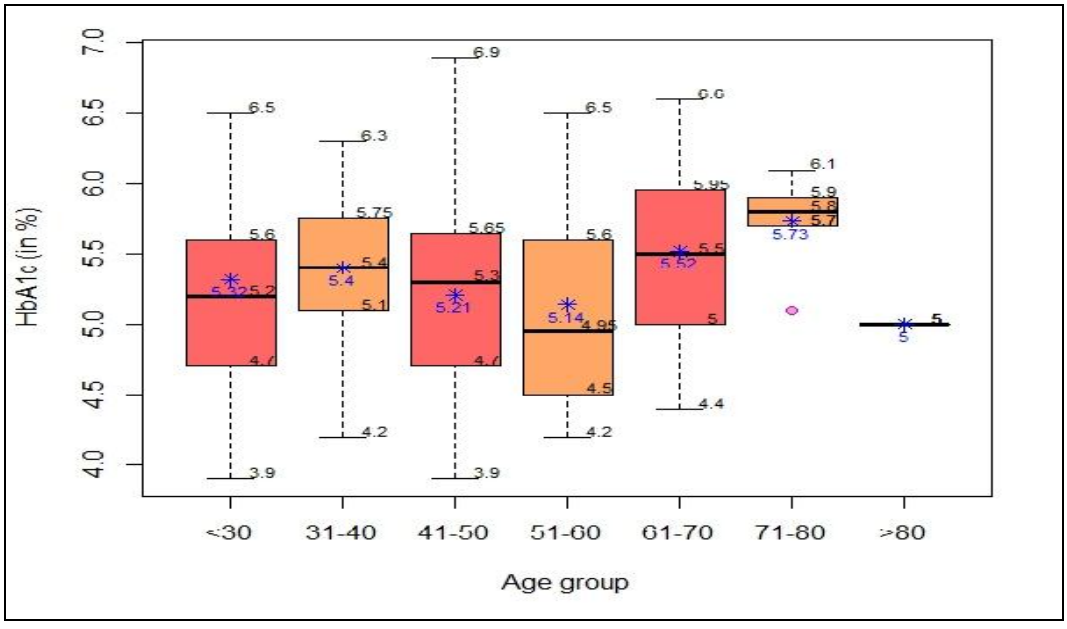


The above table depicts the comparison of age with the severity of anemia. In our study we found no statistical significance of the comparison (p value 0.4883).

Table 13: Comparison of Age with HbA1c

Age (years)	Mean HbA1c (%)	p-value
18-30	5.32±0.74	
31-40	5.40±0.55	
41-50	5.21±0.76	
51-60	5.14±0.75	**0.4115**
61-70	5.52±0.63	
71-80	5.73±0.34	
≥ 80	5(single observation)	

Figure 13: Comparison of Age with HbA1c

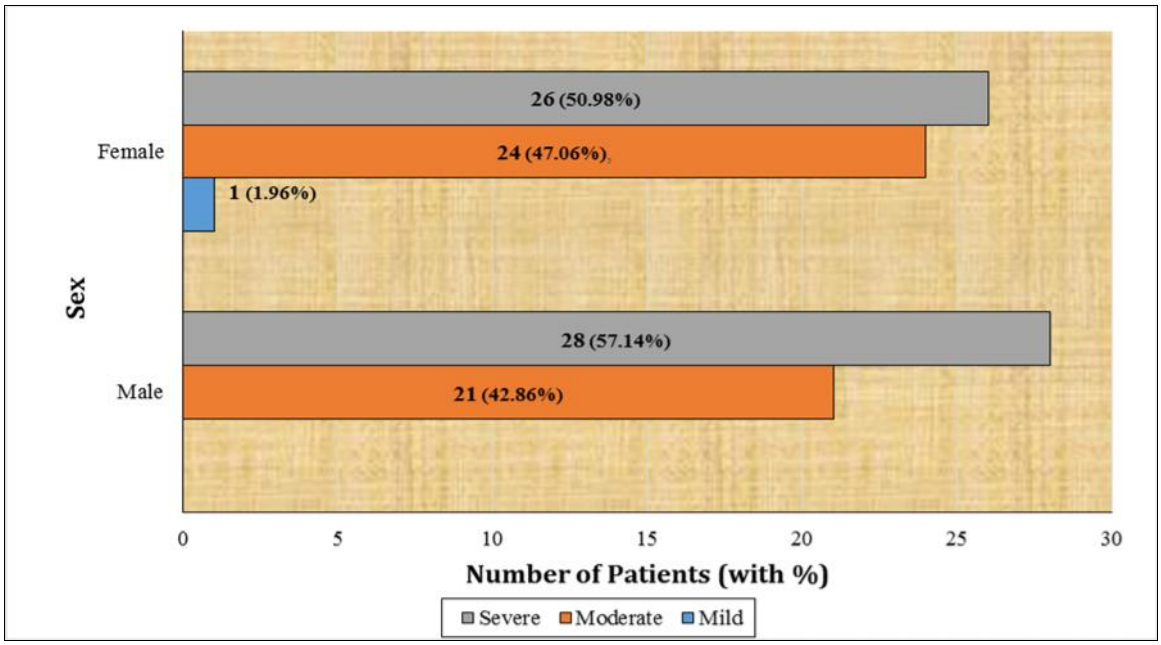


Similarly, comparison of age with HbA1c did not have any statistical significance (p value 0.4115).

Table 14: Comparison of Sex with Severity of Anemia

	Severity of Anemia			
Sex	**Mild**	**Moderate**	**Severe**	**p-value**
Male	0(0%)	21(42.86%)	28(57.14%)	
Female	1(1.96%)	24(47.06%)	26(50.98%)	**0.6957**

Figure 14: Comparison of Sex with Severity of Anemia

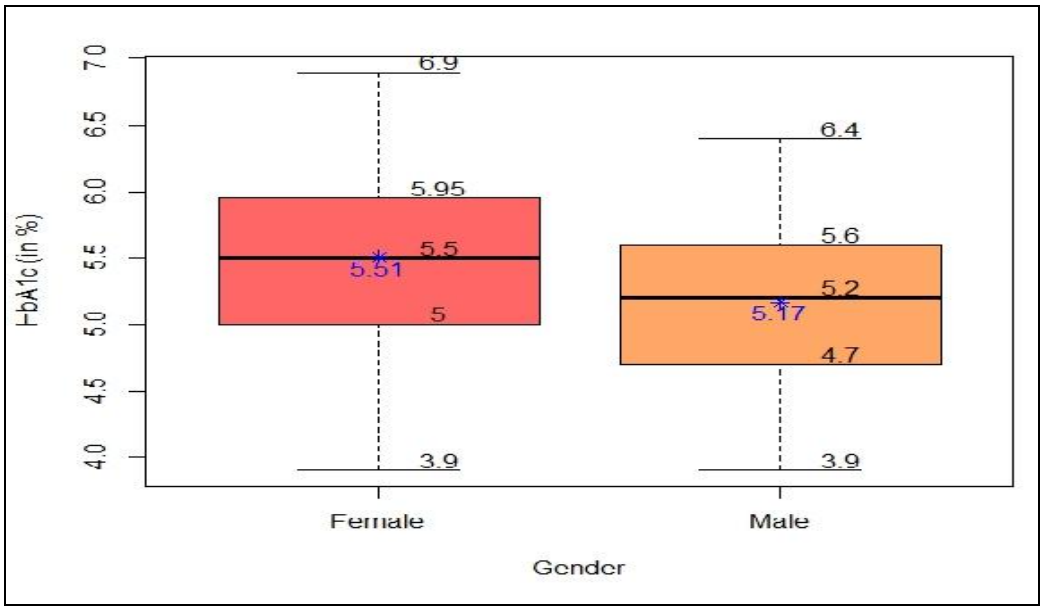


The above table depicts the comparison of severity of anemia with gender. It was found that there was no statistical significance in the severity of anemia compared to sex, both in moderate as well as severe anemia (p value 0.6957). The number of patients in both groups was almost same.

Table 15: Comparison of Sex with HbA1c

Sex	Mean HbA1c (%)	p-value
Male	5.17±0.61	**0.0106**
Female	5.51±0.70	

Figure 15: Comparison of Sex with HbA1c

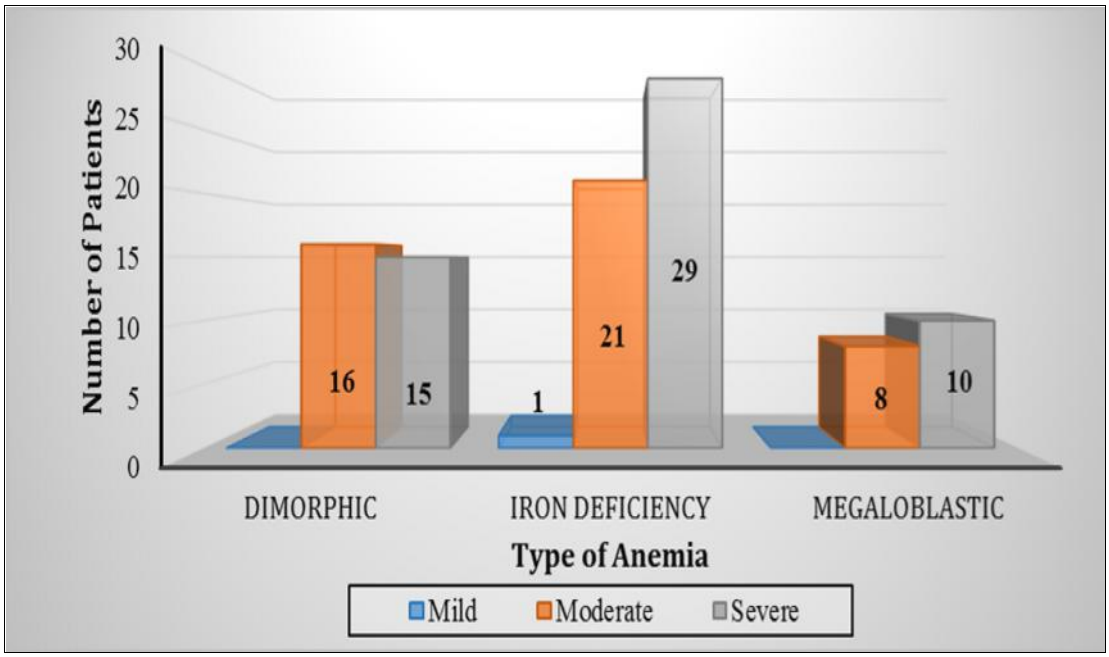


Similarly, comparison of gender with HbA1c was studied in our patients and we found significant correlation between gender and HbA1c (p value 0.0106). It was observed that HbA1c was slightly higher in females as compared to males as shown in the table above.

Table 16: Comparison of Types of Anemia with Severity of Anemia

Type	Severity			p-value
	Mild	**Moderate**	**Severe**	
Dimorphic	0 (0%)	16 (51.61%)	15 (48.39%)	**0.7916**
Iron Deficiency	1 (1.96%)	21 (41.18%)	29 (56.86%)	
Megaloblastic	0 (0%)	8 (44.44%)	10 (55.56%)	

Figure 16: Comparison of Types of Anemia with Severity of Anemia

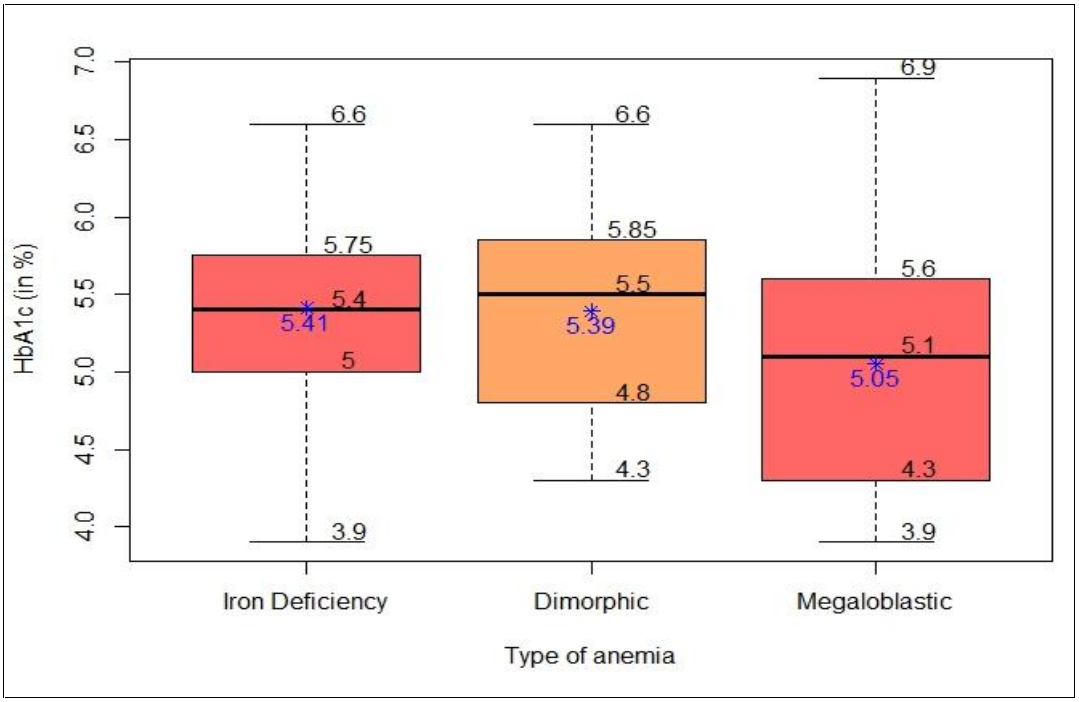


In our study of 100 patients, comparison of types of anemia with severity did not have any significant correlation and was also statistically insignificant (p value 0.7916)

Table 17: Comparison of Types of Anemia with HbA1c

Type of Anemia	Mean HbA1c(%)	p-value
Iron Deficiency	5.41±0.66	**0.1314**
Dimorphic	5.39±0.62	
Megaloblastic	5.05±0.78	

Figure 17: Comparison of Types of Anemia with HbA1c



Similarly, comparison of types of anemia with HbA1c did not show statistical correlation with a p value of 0.1314. However, we observed a slightly higher value of HbA1c in iron deficiency anemia.

Table 18: Comparison of Severity of Anemia with HbA1c in Males

Male / Severity	HbA1c (%)		
	≤5.6	**5.7-6.4**	**≥6.5**
Mild	0	0	0
Moderate	14(66.67%)	7(33.33%)	0(0%)
Severe	20(71.43%)	7(25%)	1(3.57%)

In all our 100 patients, when gender was compared with severity of anemia as well as HbA1c, there was no significant correlation between severity, HbA1c and male gender (p value 0.846)

Table 19: Comparison of Severity of Anemia with HbA1c in Females

Female / Severity	HbA1c (%)		
	≤5.6	**5.7-6.4**	**≥6.5**
Mild	1(100%)	0(0%)	0(0%)
Moderate	15(62.5%)	7(29.17%)	2(8.33%)
Severe	19(73.08%)	3(11.54%)	4(15.38%)

Similarly, it was observed that there was no significant correlation between severity of anemia, HbA1c and the female gender (p value 0.508)

Table 20: Comparison of Iron Deficiency Anemia with HbA1c

Iron Deficiency / Severity	HbA1c (%)		
	≤5.6	**5.7-6.4**	**≥6.5**
Mild	1(100%)	0	0
Moderate	15(71.43%)	6(28.57%)	0(0%)
Severe	20(68.97%)	5(17.24%)	4(13.79%)

The above table depicts severity of iron deficiency anemia alone with HbA1c and was found to have no correlation (p value 0.399)

Table 21: Comparison of Dimorphic Anemia with HbA1c

Dimorphic / Severity	HbA1c (%)		
	≤5.6	**5.7-6.4**	**≥6.5**
Mild	0	0	0
Moderate	10 (62.5%)	4(25%)	2(12.5%)
Severe	13(86.67%)	1(6.67%)	1(6.67%)

Similarly, comparison of dimorphic anemia with HbA1c did not show any correlation (p value 0.400)

Table 22: Comparison of Megaloblastic Anemia with HbA1c

Megaloblastic / Severity	HbA1c (%)		
	≤5.6	**5.7-6.4**	**≥6.5**
Mild	0	0	0
Moderate	4 (50%)	4 (50%)	0 (0%)
Severe	6 (60%)	4 (40%)	0 (0%)

Similarly, megaloblastic anemia also did not show any correlation with HbA1c as depicted in the above table (p value 1)

DISCUSSION

In the present study of 100 patients, the effect of severity of anaemia on HbA1c was carried out. The same was compared with various factors like age, sex, severity of anemia, type of anemia and the following results were observed.

In our study, the age ranged from 18-86 years. The number of cases were more in the age groups of 31-40 years, 41-50 years and 61-70 years, that is 19 patients in each group (19%). 18 patients (18%) were in the age group of 18-30 years, 6 patients (6%) in the age group of 71-80 years and only 1 patient in the age group of more than 80 years. In a study by Parlapally RP et al.[70], in their series of 126 patients (63 in patient group and 63 in control group), the average age of the patients was 38.41±17.6 years while a study by Manjhavar SK et al.[74] found a mean age of 37.10± 12.39 years (number of patients studied were 500). In our study group of 100 patients, the mean age was 48.12±16.47 years.

We observed 51 patients were female (51%) in our study group and the remaining 49 patients (49%) were male patients with a M:F ratio of 0.96:1. A slight female preponderance was observed in our study. Study by Parlapally RP et al.[70] found in their study group female preponderance. Manjhavar SK et al.[74] also observed a female preponderance in their group of patients.

Patients presented with symptoms of anaemia in the form of generalised weakness (79 patients, 79%), easy fatigability (61 patients, 61%), dizziness (33 patients, 33%). Overlapping symptoms were observed in 62 patients (62%). Blood loss was observed in 9 patients (9%). Of the 9 patients, 5 were female patients with excessive per vaginal bleed (menorrhagia), while the remaining 4 patients (2 males

and 2 post-menopausal females) were with lower gastrointestinal bleed (hemorrhoids). 14 patients in our study were asymptomatic. A study by Manjhavar SK et al.[74] observed easy fatigability in all 500 patients of theirs (100%) followed by dyspnea on exertion in 375 patients (75%), giddiness in 101 patients (20.2%), melena in 113 patients (22.6%) and palpitation in 78 patients (15.6%). In a study by CH Manoj Kumar et al.[79], common symptom observed was generalized weakness in 82% patients.

In our study, the commonest physical sign was pallor (75 patients, 75%). Koilonychia was observed in 33 patients (33%), pale bald tongue in 32 patients (32%) and overlapping signs were seen in 47 patients (47%). No signs were observed in 23 patients (23%). Manjhavar SK et al.[74] observed in their study, all 500 patients had pallor (500 patients, 100%), bald tongue was observed in 134 patients (26.8%), koilonychia was observed in 136 patients (27.2%), hemic murmur was observed in 47 patients (9.4%) and pedal oedema was observed in 43 patients (8.6%) This is in sharp contrast to our study wherein we did not find hemic murmur or pedal oedema in patients. Study by Parlapally RP et al.[70] had physical signs in their patients that is pallor in 63 patients (100%), bald tongue in 20 patients (32%), koilonychia in 30 patients (48%), pedal oedema in 10 patients (16%) and hemic murmur in 32 patients (51%).

When an attempt was made to categorise our patients with type of anemia, it was found to have iron deficiency anemia in 51 patients (51%), dimorphic anemia in 31 patients (31%) and 18 patients (18%) had megaloblastic anemia. This is in sharp contrast to study by Maheshwari VD et al.[77] who have categorised their 30 patients as iron deficiency anemia and 30 patients as megalobastic anemia. Study by Gram-

Hansen P et al.[72] had 10 patients of iron deficiency anemia and 10 patients of megaloblastic anemia in their study group.

Further we made an attempt of categorising our patients based on WHO Criteria as mild, moderate and severe anemia and found to have 54 patients (54%) with severe anemia, 45 patients (45%) with moderate anemia and only 1 patient (1%) with mild anemia. This is in sharp contrast to study by CH Manoj Kumar et al.[79] who found moderate anemia in their majority patients (88%), followed by severe anemia (12%). None had mild anemia in their group. A study by Elhabbash M.[76] studied only female patients and categorised the patients into 2 groups. 50 patients with iron deficiency anemia and 50 patients without anemia. Further, an estimation of hemoglobin percentage of these 50 anemic patients found 25% patients with mild anemia, 15% patients with moderate anemia and 10% patients with severe anemia.

All our patients were subjected to hemoglobin estimation. Based on hemoglobin, we categorised them as mild, moderate and severe anemia and found to have 54 patients (54%) had hemoglobin below 8 g/dl (severe anemia), 45 patients (45%) had hemoglobin between 8-10.9 g/dl (moderate anemia) and only 1 patient had hemoglobin between 11-12.9 g/dl (mild anemia). Mean hemoglobin concentration in our population was 7.56±2.00 g/dl (for all types of anemia together), whereas studies by Parlapally RP et al.[70] and Rajagopal L et al.[56] have estimated mean hemoglobin percentage in only iron deficiency anemia in their study population (6.84±1.63 g/dl and 11.46±0.08 g/dl respectively). A study by Parlapally RP et al.[70] also observed the hemoglobin percentage ranged from 4.1-10.5 g/dl. In our study the range was 2.9-11 g/dl. Parlapally RP et al.[70] and Rajagopal L et al.[56] in their study have taken control groups with anemic patients and found to have significantly lower hemoglobin

percentage in the cases. However, in our study we did not compare our anemic patients with control groups.

In our study, further we attempted to categorise our 100 patients based on Peripheral smear finding and found to have microcyctic hypochromic picture in 51 patients (51%) (iron deficiency anemia), 31 patients (31%) constituted dimorphic blood picture and 18 patients (18%) had macrocyctic hypochromic blood picture (megaloblastic anemia). Most of the authors have not categorised their patients based on peripheral smear.

When fasting blood sugars were taken into consideration, we observed 93 patients (93%) had fasting sugars of 71-100 mg/dl. For the remaining 7 patients (7%), the range was from 101-109 mg/dl. None of our patients had fasting blood sugars of more than or equal to 110 mg/dl. The mean estimation of fasting blood sugar revealed 86.23±8.2 mg/dl. Study by Bae JC et al.[80] found to have fasting blood sugars mean of 94.1±8.7 mg/dl in their study group. Another study by Elhabbash M.[76] who has studied only female patients in her study found to have fasting blood sugars normal.

We subjected all our patients for post prandial blood sugar estimation also and found to have in 33 patients (33%), the post prandial sugars ranging from 131-139 mg/dl and for the remaining 67 patients (67%), it ranged from below 110 mg/dl to 130 mg/dl (Table No. 10). Most of the authors have not stated post prandial sugars in their cases, but one study by Parlapally RP et al.[70] have done both fasting and post prandial blood sugar levels and found to have normal ranges. Another study by Manjhavar SK et al.[74], observed in their study mean post prandial blood sugar of 112.65±10.50 mg/dl. This is in contrast to our study wherein we found mean post prandial blood sugars of 121.93±10.52 mg/dl.

HbA1c estimation revealed majority of our patients, 68 patients (68%) had HbA1c ≤ 5.6%, 26 patients (26%) had HbA1c between 5.7-6.4% and only 6 patients (6%) had HbA1c ≥ 6.5%. However these patients did not have abnormality of fasting or post prandial blood sugars. Most of the authors have compared HbA1c purely with iron deficiency anemia. A study by Rajagopal L et al.[56] in their series found mean HbA1c of 6.84±0.07% in non-diabetic iron deficiency anemia patients, whereas in our study we have taken the mean HbA1c for all types of anemia together. Hence, true reflection of correlation with HbA1c and type of anemia is difficult to state (we have included all types of anemia – iron deficiency anemia, dimorphic anemia and megaloblastic anemia). A study by Ford ES et al.[73], in their large series of 7478 patients of non-diabetics, found a mean HbA1c value was 5.3±<0.1%. This is almost similar to our study except for the sample size (our sample size is only 100).

An attempt was made to see if a correlation exists or not between age and severity of anemia and we found to have no correlation between age and severity of anemia (p value 0.4883). Study by Manjhavar SK et al.[74] found correlation between age and severity of anemia. They found in mild anemia 28.03%, in moderate anemia 31.1% and in severe anemia 37.1% in the age group of 31-40 years.

Similarly we tried comparison of age with HbA1c and did not find any correlation with the same (p value 0.4115). In one study by Bae JC et al.[80], they found a correlation between HbA1c and increasing age, with the p value being significant (p value < 0.001). With increasing age, an increase in HbA1c both in men as well as women was observed in their study. It is noted that HbA1c exists independent of glycemia in non-diabetic population. A probable mechanism for increasing HbA1c with age could be associated with RBC lifespan. RBCs will have reduced lifespan

because of diseases wherein, in these individuals the HbA1c is lower (reported by Masuch A et al.[81] in their study). Cohen RM et al.[82] demonstrated that the lifespan of RBC may vary in different individuals which may influence the HbA1c levels in diabetic and non-diabetic individuals. Recently, Beltran Del Rio M et al.[83] proposed that RBC turnover is governed by 2 major mechanisms; senescence mediated clearance of RBCs from circulation and random cell loss. It is possible that changes in erythropoiesis, RBC turnover or clearance could vary HbA1c levels with age which is independent of disturbed glycemic control. It is believed that aged people usually maintain hemopoiesis, but it may be affected by stressors like oxidative stress, which may increase RBC removal from circulation. It is possible that senescent RBCs are cleared from circulation by macrophages like Kupffer cells in the liver. It could also be because of impaired immune system with advancing age and reduced function of macrophages. Due to this impaired function of macrophages, the clearance mechanism of RBCs may be hampered which prolongs the exposure of blood glucose to RBCs, which would in turn increase the HbA1c levels which is independent of impaired glycemic control. Also, in patients with iron deficiency (iron deficiency anemia) and vitamin B_{12} deficiency (megaloblastic anemia) there is increase in HbA1c independent of glycemia. With increasing age, there is an increasing possibility of iron, B_{12} and folic acid deficiency. It is also possible that impaired splenic function may increase HbA1c levels, as splenic function reduces with age.

We tried to compare gender with severity of anemia and did not find any correlation as far as gender and severity of anemia was concerned (p value 0.6957). The reason for this could be reduced red blood cell mass and hemoglobin levels in female gender than in male gender, though there is no clear mechanism understood. In

one study by Bae JC et al.[80], the mean level of hemoglobin was 2.3 g/dl lower in females as compared to males.

We tried to compare sex with HbA1c levels and found there was a significant correlation of gender with HbA1c levels in our study. It was observed that it was slightly higher in females as compared to males (males had a mean HbA1c of 5.17±0.61% while females had mean HbA1c of 5.51±0.70%) and p value was significant (0.0106). This is contrary to study by Bae JC et al.[80] They found lower HbA1c levels in female gender compared to their counterpart. The possible mechanism for low levels of HbA1c with female gender could be because of menstruation, in spite of normal levels of hemoglobin. This is because menstruating women have more rapid erythrocyte turnover and increased reticulocyte production which may reflect on the lifespan of RBC, which may in turn reflect on low levels of HbA1c. It may also be due to sex hormones which may contribute to low levels of HbA1c.

Similarly, we compared types of anemia with severity of anemia and found to have no significant correlation between the types of anemia with severity of anemia (p value 0.7916) as shown in Table 16. Many studies have not compared the types of anemia with severity of anemia thus making it difficult to state whether correlation exists or not owing to a small sample size of 100 patients in our study.

When comparison of types of anemia with HbA1c was considered, there was no significant correlation of different types of anemia with HbA1c in our study (p value 0.1314) as shown in Table 17. Studies by Maheshwari VD et al.[77], Singh P et al.[84] and Rajagopal L et al.[56] have found a correlation of iron deficiency anemia and HbA1c levels in their study, with increased levels of HbA1c in iron deficiency

anemia. Some authors like Sinha N et al.[18] and Kalasker V et al.[85] have found correlation of iron deficiency with decreased levels of HbA1c, while one study by Ford ES et al.[73] has found no correlation between iron deficiency anemia with HbA1c levels. One study by Maheshwari VD et al.[77] also found statistically significant higher HbA1c levels in patients of megaloblastic anemia when compared to a group of healthy individuals. They have not compared HbA1c levels with dimorphic anemia. The reasons for elevated HbA1c levels in patients of iron deficiency anemia observed by these authors in their study could be due to:

- Change in the quaternary structure of hemoglobin moiety which increases the glycation of globin chains, or due to
- Decrease in hemoglobin concentration, resulting in an increased glycation fraction at a constant level of glucose. Measurement of HbA1c is as a percentage of total hemoglobin A, or it could because of
- Reduced red cell production which may reflect on higher life span of circulating erythrocytes and increase HbA1c levels

Some authors have found low levels of HbA1c with iron deficiency anemia probably because of lower socioeconomic strata wherein the cause for iron deficiency anemia could have been multifactorial (gastrointestinal bleed, malabsorption or nutritional deficiency) or any other unknown variables may have an effect on HbA1c results.

We further compared severity of anemia with HbA1c in males separately and found to have no significant correlation (p value 0.846) as shown in Table 18. Similarly, comparison of severity of anemia with HbA1c in females also did not show a correlation (p value 0.508) (Table 19). In our study we have compared severity of

all types of anemia (iron deficiency anemia, dimorphic anemia and megaloblastic anemia) with HbA1c and did not find correlation with either male or female gender, whereas study by Manjhavar SK et al.[74] have found a positive correlation of HbA1c in their study with pure iron deficiency anemia with male as well as female genders (p value < 0.0001). They have not compared with other types of anemia (dimorphic anemia and megaloblastic anemia).

We did not find comparison of HbA1c with severity of iron deficiency anemia in our study population to be significant (p value 0.399) whereas, some authors have found a correlation of HbA1c with iron deficiency in moderate to moderately severe iron deficiency anemia. A study by Rajagopal L et al.[56] has found with increasing severity of iron deficiency anemia, a positive correlation with HbA1c levels (p value < 0.05). They have found HbA1c levels increase as severity of anemia worsens.

Similarly, dimorphic anemia and HbA1c was compared and we found no correlation (p value 0.400). Most of the authors have compared HbA1c with iron deficiency anemia with severity, but have not compared dimorphic anemia or megaloblastic anemia separately.

Even we have not found comparison of megaloblastic anemia with HbA1c to be statistically significant (p value 1).-

In our present study of 100 non diabetic anemic patients, age ranged from 18-86 years. The number of female patients was slightly more. The commonest symptoms of patient presentation were generalized weakness, easy fatigability and 62 patients had overlapping symptoms. Most of our patients had physical signs of pallor as a presentation, while 23 patients did not have any signs. Majority of the patients

were in the iron deficiency group. Majority of our patients were in the group of moderate to moderately severe anemia (i.e. 99%). Only 1 patient was in the mild anemia group. Except 1 patient, 99 patients had a hemoglobin range of below 8 to 10.9 g/dl, constituting for moderate to severe anemia. Peripheral smear revealed microcyctic hypochromic anemia as the commonest type in our study followed by dimorphic anemia and megaloblastic anemia. Fasting blood sugar estimation revealed ranges from 71-109 mg/dl. None had fasting blood sugar more than 109 mg/dl. Similarly, post prandial blood sugar ranged from less than 110 to 139 mg/dl. No patient had post prandial sugars ≥ 140 mg/dl. Majority of our patients, the HbA1c was ≤ 5.6% and only 6 patients had HbA1c ≥ 6.5%. Age did not have a positive correlation with severity of anemia. Similarly age also did not influence HbA1c.

Sex did not have an influence on severity of anemia. We found a significant correlation of HbA1c with female gender in our study, which is in sharp contrast with other studies. Most of the studies have found lower levels of HbA1c with female gender. Earlier in our discussion, we have stated the cause for low levels of HbA1c in female gender. When we compared types of anemia with severity of anemia, types of anemia with HbA1c, severity of anemia with HbA1c in males separately and females separately, iron deficiency anemia with HbA1c, dimorphic anemia and megaloblastic anemia with HbA1c, we did not find any correlation in our present study.

We feel it is worth taking different variables like age, sex, types of anemia, severity of anemia and its influence on HbA1c levels with a large sample size. This is because many times we do come across blood sugars being normal (fasting blood sugars and post prandial blood sugars), but the HbA1c is more than cut off levels. With a large sample size, it would be possible to overcome these biases as stated

above. Apart from having a small sample size in our study, we did not follow up our patients after treatment of different types of anemia, and its effect on HbA1c. The effect of treatment of anemia on HbA1c levels has to be elucidated. The way known patients of diabetes mellitus could have a bearing on HbA1c associated with anemia has to be kept in mind, as many a times we try to manage diabetic patients based on HbA1c levels.

CONCLUSION

In our present study of 100 non diabetic anemic patients, we observed insignificant correlation with various factors. Prominent features of our study are mentioned as follows

- Among the patients who presented with anemia, majority were in the age groups of 31-40 years, 41-50 years and 61-70 years.
- Age did not have an influence on HbA1c.
- Females were more in number as compared to males.
- The common clinical presentations were generalised weakness, followed by easy fatigability and dizziness. 62 patients had overlapping symptoms.
- The most common physical signs noted were pallor, followed by koilonychia. 47 patients had overlapping signs.
- Majority of our patients were severely anemic, with most of them having iron deficiency anemia.
- We observed in 6 patients the HbA1c ≥ 6.5%. Of these 6 patients, 4 had iron deficiency anemia (the observation was statistically insignificant).
- Slight increase in HbA1c levels were observed in women in our study.
- Type of anemia or severity of anemia did not have an influence on the HbA1c levels.

HbA1c may be affected by variables such as age, sex, anemia, chronic illnesses or drugs. In our study, based on HbA1c cut off values, 6 patients had an HbA1c ≥ 6.5%, but none had fasting or post prandial blood sugar abnormalities. We feel that it is worthwhile to take a large sample size and compare these confounding

factors like age, sex, chronic illnesses, types of anemia and severity of anemia to see whether truly there is a relationship with these variables or not as often in clinical scenarios, we do come across situations when blood sugars are normal but HbA1c is abnormal.

SUMMARY

In the present study of 100 non-diabetic patients with anemia, admitted in the Department of General Medicine of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi with the study period extending from January 2018 to December 2018 was conducted to know whether or not HbA1c is influenced by various variables like age, sex, types of anemia or severity of anemia.

The results observed were all inconclusive except comparison of sex and HbA1c. So we feel, with a large sample size these issues have to be addressed, comparing HbA1c with different variables. We also feel that follow up with treatment of these anemic patients and its effect on HbA1c needs to be studied. We did not find any positive correlation with variables like age, types of anemia and severity of anemia. However, we found a slightly higher HbA1c in the female gender.

BIBLIOGRAPHY

1. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in hemoglobin concentration and prevalence of total and severe anemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob Health. 2013;1(1):e16-25.
2. Cappellini MD, Motta I. Anemia in Clinical Practice-Definition and Classification: Does Hemoglobin Change With Aging? Semin Hematol. 2015;52(4):261-9.
3. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009;12(4):444-54.
4. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123(5):615-24.
5. Ramachandran P, K. K. Prevalence of Anemia in India and Strategies for Achieving Sustainable Development Goals (SDG) Target. Proc Indian Natn Sci Acad 2018;84(4):899-912.
6. WHO Guidelines Approved by the Guidelines Review Committee. Use of Glycated Hemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization Copyright (c) World Health Organization; 2011.
7. Association. AD. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2013;36 Suppl 1:S67-74.

8. American Diabetes A. Standards of medical care in diabetes--2013. Diabetes care. 2013;36 Suppl 1(Suppl 1):S11-S66.
9. Use of Glycated Hemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization; 2011. 2, Glycated hemoglobin (HbA1c) for the diagnosis of diabetes. Available from: https://www.ncbi.nlm.nih.gov/books/NBK304271/.
10. Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. Diabetes Care. 2011;34 Suppl 2:S184-90.
11. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988-2006. Diabetes Care. 2010;33(3):562-8.
12. Welch TR. "Diuretics for diabetes insipidus". The Journal of Pediatrics 2015;167(3):503–50.
13. Dvorak MM, De Joussineau C, Carter DH, Pisitkun T, Knepper MA, Gamba G, et al. Thiazide diuretics directly induce osteoblast differentiation and mineralized nodule formation by interacting with a sodium chloride co-transporter in bone. Journal of the American Society of Nephrology. 2007;18(9):2509-16.
14. Hardikar PS, Joshi SM, Bhat DS, Raut DA, Katre PA, Lubree HG, et al. Spuriously high prevalence of prediabetes diagnosed by HbA(1c) in young indians partly explained by hematological factors and iron deficiency anemia. Diabetes Care. 2012;35(4):797-802.
15. Attard SM, Herring AH, Wang H, Howard AG, Thompson AL, Adair LS, et al. Implications of iron deficiency/anemia on the classification of diabetes using HbA1c. Nutrition &Amp; Diabetes. 2015;5:e166.

16. Ahmad J, Rafat D. HbA1c and iron deficiency: a review. Diabetes Metab Syndr. 2013;7(2):118-22.
17. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree. Effect of iron deficiency on glycation of hemoglobin in nondiabetics. J Clin Diagn Res. 2013;7(1):15-7.
18. Sinha N, Mishra TK, Singh T, Gupta N. Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med. 2012;32(1):17-22.
19. Koga M, Morita S, Saito H, Mukai M, Kasayama S. Association of erythrocyte indices with glycated hemoglobin in pre-menopausal women. Diabet Med. 2007;24(8):843-7.
20. Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull. 2008;29(2 Suppl):S20-34; discussion S5-7.
21. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anemia and abnormalities of erythrocyte indices on HbA 1c analysis: a systematic review. Diabetologia. 2015;58(7):1409-21.
22. Kim C, Bullard KM, Herman WH, Beckles GL. Association Between Iron Deficiency and A1C Levels Among Adults Without Diabetes in the National Health and Nutrition Examination Survey, 1999–2006. Diabetes Care. 2010;33(4):780-5.
23. WHO. Hemoglobin concentrations for the diagnosis of anemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/ MNM/11.1) (http://www.who.int/vmnis/indicators/hemoglobin.accessed [18-7-19]).
24. Benoist B, McLean E, Egli I, M. C, . Worldwide prevalence of anemia 1993-2005. Geneva, Switzerland:World Health Organization. 2008.

25. Tiwari S, Packer RK, Hu X, Sugimura Y, Verbalis JG, Ecelbarger CA. Increased renal alpha-ENaC and NCC abundance and elevated blood pressure are independent of hyperaldosteronism in vasopressin escape. Am J Physiol Renal Physiol. 2006;291(1):F49-57.
26. FAO I, UNICEF, WFP,, WHO. The state of food security and nutrition in the world 2017. Building resilience for peace and food security. FAO Rome; 2017.
27. Nations within a nation: variations in epidemiological transition across the states of India, 1990-2016 in the Global Burden of Disease Study. Lancet. 2017;390(10111):2437-60.
28. Sciences IIfP, MEASURE/DHS+ OM. India National Family Health Survey (NFHS-2), 1998-99: International Institute for Population Sciences, Mumbai, India; 2000.
29. Toteja GS, Singh P, Dhillon BS, Saxena BN, Ahmed FU, Singh RP, et al. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. Food Nutr Bull. 2006;27(4):311-5.
30. Gonmei Z, Toteja GS. Micronutrient status of Indian population. The Indian journal of medical research. 2018;148(5):511-21.
31. Patel A, Prakash AA, Das PK, Gupta S, Pusdekar YV, Hibberd PL. Maternal anemia and underweight as determinants of pregnancy outcomes: cohort study in eastern rural Maharashtra, India. BMJ Open. 2018;8(8):e021623.
32. Singh S, Geddam JJB, Reddy GB, Pallepogula DR, Pant HB, Neogi SB, et al. Folate, vitamin B12, ferritin and hemoglobin levels among women of childbearing age from a rural district in South India. BMC Nutrition. 2017;3(1):50.

33. Bentley ME, Griffiths PL. The burden of anemia among women in India. Eur J Clin Nutr. 2003;57(1):52-60.

34. Garg P, Dey B, Deshpande A, Bharti J, Nigam J. Clinico-hematological profile of dimorphic anemia. Journal of Applied Hematology. 2017;8(3):123-4.

35. Weatherall DJ, Clegg JB. Inherited hemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12.

36. De-Regil LM, Jefferds ME, Sylvetsky AC, Dowswell T. Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. Cochrane Database Syst Rev. 2011(12):Cd009085.

37. Bhutta ZA, Ahmed T, Black RE, Cousens S, Dewey K, Giugliani E, et al. What works? Interventions for maternal and child undernutrition and survival. Lancet. 2008;371(9610):417-40.

38. A Stevens G, M Finucane M, de Regil L, J Paciorek C, R Flaxman S, Branca F, et al. Global, regional, and national trends in hemoglobin concentration and prevalence of total and severe anemia in children and pregnant and non-pregnant women for 1995–2011: A systematic analysis of population-representative data. The lancet global health. 2013;1:e16-e25.

39. King CH, Dickman K, Tisch DJ. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet. 2005;365(9470):1561-9.

40. Smith JL, Brooker S. Impact of hookworm infection and deworming on anemia in non-pregnant populations: a systematic review. Trop Med Int Health. 2010;15(7):776-95.

41. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008;31(8):1473-8.
42. Benedetti M. Changing trends in the treatment of type 2 diabetes. Curr Med Res Opin. 2006;22(2):S5-13.
43. Nathan DM, Turgeon H, Regan S. Relationship between glycated hemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50(11):2239-44.
44. Gillett MJ. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes. Diabetes Care. 2009;32(7):1327-34.
45. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. Arch Intern Med. 2007;167(14):1545-51.
46. Ryden L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer MJ, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J. 2007;28(1):88-136.
47. Rawal G, Yadav S, Kumar R, Singh A. Glycosylated hemoglobin (HbA1C): A brief overview for clinicians2016. 33-6 p.
48. Executive summary: standards of medical care in diabetes--2009. Diabetes care. 2009;32 Suppl 1(Suppl 1):S6-S12.
49. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of Hyperglycemia in Type 2 Diabetes: A Patient-Centered Approach. Position Statement of the American Diabetes Association (ADA)

and the European Association for the Study of Diabetes (EASD). 2012;35(6):1364-79.

50. Virtue MA, Furne JK, Nuttall FQ, Levitt MD. Relationship between GHb concentration and erythrocyte survival determined from breath carbon monoxide concentration. Diabetes Care. 2004;27(4):931-5.
51. Hempe JM, Gomez R, McCarter RJ, Jr., Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications. 2002;16(5):313-20.
52. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab. 2008;93(7):2447-53.
53. Higgins T. HbA(1c)--an analyte of increasing importance. Clin Biochem. 2012;45(13-14):1038-45.
54. Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence. Clin Biochem Rev. 2013;34(2):75-83.
55. Abass AE, Musa IR, Rayis DA, Adam I, Gasim IG. Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. Clin Pract. 2017;7(4):999.
56. Rajagopal L, Ganapathy S, Arunachalam S, Raja V, B. R. Does iron deficiency anemia and its severity influence HbA1C level in non diabetics? An analysis of 150 cases. JCDR. 2017;11(2):EC13.
57. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, et al. Tests of Glycemia in Diabetes. Diabetes Care. 2004;27(7):1761-73.

58. Soliman AT, De Sanctis V, Yassin M, Soliman N. Iron deficiency anemia and glucose metabolism. Acta Biomed. 2017;88(1):112-8.

59. Naqash A, Ara R, Bader GN. Effectiveness and safety of ferric carboxymaltose compared to iron sucrose in women with iron deficiency anemia: phase IV clinical trials. BMC Womens Health. 2018;18(1):6.

60. Schindler C, Birkenfeld AL, Hanefeld M, Schatz U, Köhler C, Grüneberg M, et al. Intravenous Ferric Carboxymaltose in Patients with Type 2 Diabetes Mellitus and Iron Deficiency: CLEVER Trial Study Design and Protocol. Diabetes Therapy. 2018;9(1):37-47.

61. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-74.

62. Franco RS. The measurement and importance of red cell survival. Am J Hematol. 2009;84(2):109-14.

63. Christy AL, Manjrekar PA, Babu RP, Hegde A, Rukmini MS. Influence of iron deficiency anemia on hemoglobin A1c levels in diabetic individuals with controlled plasma glucose levels. Iran Biomed J. 2014;18(2):88-93.

64. Coban E, Ozdogan M, Timuragaoglu A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol. 2004;112(3):126-8.

65. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated hemoglobin A. Lancet. 1980;2(8186):141.

66. Sluiter WJ, van Essen LH, Reitsma WD, Doorenbos H. Glycosylated hemoglobin and iron deficiency. Lancet. 1980;2(8193):531-2.

67. Mudenha ET, Aarella VG, Chandrasekaram S, Fernando DJ. Rising HbA1c in the presence of optimal glycaemic control as assessed by self-monitoring - iron deficiency anemia. JRSM Open. 2016;7(2):2054270415619321.

68. Silva JF, Pimentel AL, Camargo JL. Effect of iron deficiency anemia on HbA1c levels is dependent on the degree of anemia. Clinical biochemistry. 2016;49(1-2):117-20.

69. Solomon A, Hussein M, Negash M, Ahmed A, Bekele F, Kahase D. Effect of iron deficiency anemia on HbA1c in diabetic patients at Tikur Anbessa specialized teaching hospital, Addis Ababa Ethiopia. BMC Hematology. 2019;19(1):2.

70. Parlapally RP, Kumari KR, T. S. Effect of Iron Deficiency Anemia on Glycation of Hemoglobin in Non-diabetics. Int J Sci Stud. 2016;4(5):192-6.

71. Periasamy S, Xavier AA, Gowtham R. Incidence of anemia in type 2 diabetic mellitus and its prognostic index. Int J Med Res Rev 2016;4(7):1239-42.

72. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L. Glycosylated hemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med. 1990;227(2):133-6.

73. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT. Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. J Diabetes. 2011;3(1):67-73.

74. Manjhvar SK, Singh B, AS. N. To study the effect of iron deficiency anemia on HbA1c in nondiabetic. J Evolution Med Dent Sci. 2017;6(94): 6863-7.

75. Takeuchi M, Kawakami K. Association between Hemoglobin and Hemoglobin A1c: A Data-Driven Analysis of Health Checkup Data in Japan. J Clin Med. 2018;7(12).

76. Elhabbash M. Relation of Iron Deficiency Anemia and the Level of Glycosylated Hemoglobin (HbA1c) in Non-Diabetic Patients. International Annals of Medicine. 2018;2(6).

77. Maheshwari VD, Capoor S, Chaturvedi S. Impact of Iron and Vitamin B12 Anemia at GlycosylatedHemoglobin Level: A Case Control Study. Journal of Dental and Medical Science. 2017;16(1):1-4.

78. Rajagopal L, Arunachalam S, Ganapathy S, Ramraj B, V. R. A comparison of effect of Iron Deficiency Anemia on HbA1c levels in controlled diabetics and non-diabetics:A cross sectional analysis of 300 cases. Annals of pathology and laboratory medicine. 2017;4(2):A212-A8.

79. CH Manoj Kumar, Geethika Nutakki. A prospective study of effect of iron deficiency anemia on HbA1c levels in non-diabetics. IAIM, 2017; 4(12): 137-146.

80. Bae JC, Suh S, Jin S-M, Kim SW, Hur KY, Kim JH, et al. Hemoglobin A1c values are affected by hemoglobin level and gender in non-anemic Koreans. Journal of Diabetes Investigation. 2013May;5(1):60–5.

81. Masuch A, Friedrich N, Roth J, Nauck M, Müller UA, Petersmann A. Preventing misdiagnosis of diabetes in the elderly: age-dependent HbA1c reference intervals derived from two population-based study cohorts. BMC Endocrine Disorders. 2019Dec;19(1).

82. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood. 2008;112:4284–91.

83. Beltran Del Rio M, Tiwari M, Amodu LI, Cagliani J, Rodriguez Rilo HL. Glycated hemoglobin, plasma glucose, and erythrocyte aging. J Diabetes Sci Technol. 2016;10:1303–7.

84. P S, S S, S.k V. The Effect of Iron Deficiency Anemia on Glycated Hemoglobin (HbA1c) in Non Diabetic Adults. IOSR Journal of Dental and Medical Sciences. 2017;16(2):26–31.

85. Kalasker V, Kodliwadmath MV, Bhat H. Effect of iron deficiency anaemia on glycosylated haemoglobin levels in non diabetic Indian adults. Int J Med Hlth Sci. 2014;3(1):40-43.

ANNEXURE I

CONSENT FOR PARTICIPATION IN RESEARCH

TITLE OF RESEARCH STUDY: THE EFFECT OF THE SEVERITY OF ANEMIA ON HbA1c IN NON DIABETIC PATIENTS - A ONE YEAR HOSPITAL BASED STUDY IN KLES PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE

Principal Investigator: **Guide:**

Introduction and Purpose:

The use of HbA1c for the diagnosis of diabetes is now widely advocated despite caveats to its use. Anemia is cited as a major confounder to this use. This anemia must be corrected before making any diagnostic or therapeutic decision based on HbA1c values. Thus an understanding of the correlation between the two is necessary.

You are being asked to enroll yourself in the above said research as you are eligible for participation in this study being conducted at J N Medical college, KLES Dr. Prabhakar Kore Charitable Hospital, Belagavi from January 2018 to December 2018 conducted by Dr. Omkar S Rudra, post graduate student in the Department of General Medicine under the guidance of Dr. Vijayakumar G. Somannavar.

Purpose of the study

It is a study designed to evaluate the effect of severity of anemia on HbA1c levels.

Eligibility

He/She is a non-diabetic anemic individual.

Procedure

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigations.

Risk and Benefits

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness (rarely happens) at the site from where the blood is drawn.

You may not be benefitted by these investigations but you will be part of this study which is going to be useful to others in the future.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study.

If you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in

this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research.

Financial incentives for participation

You will not be paid / offered any gifts / incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

In case of the queries during study or in future you may contact following persons,

1. **Dr. Roopa M. Bellad** Professor Department of Pediatrics JNMC, Belagavi 9448113403

CONSENT FORM

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read this consent form, or it has been read to me, and have had all the questions answered in a language of my understanding.

Signature / Left Thumb print of the Participant or legally authorized representative

Participant's name: _____

Signature / Left thumb impression: _____

Name of the legally authorized: _____

Representative / guardian

Signature / Left thumb impression: _____

Witness' name: _____

Signature / Left thumb impression: _____

Investigator's name and signature: _____

Date:

Place:

ANNEXURE-II

PROFORMA

CASE NO:

NAME: **AGE/SEX:**

IP NO.: **OCCUPATION:**

ADDRESS:

COMPLAINTS AT PRESENTATION:

H/o generalized weakness:

H/o easy fatigability:

H/o fever:

H/o cold intolerance:

H/o reduced appetite:

H/o blood loss:

H/o swelling of legs:

H/o weight loss:

PAST HISTORY:

H/o similar complaints in the past:

H/o Blood Transfusions:

H/o Diabetes Mellitus:

H/o Tuberculosis:

H/o Blood Disorders:

H/o Drug Use:

FAMILY HISTORY:

H/o Blood Disorders:

PERSONAL HISTORY:

Diet:

H/o Alcohol consumption:

H/o Smoking/chewing Tobacco:

Menstrual History:

PHYSICAL EXAMINATION:

GENERAL CONDITION:

PALLOR- YES / NO

LYMPHADENOPATHY- YES / NO

CYANOSIS- YES / NO

CLUBBING- YES / NO

EDEMA- YES / NO

VITALS:

TEMPERATURE:

PULSE:

RESPIRATORY RATE:

BLOOD PRESSURE:

SYSTEMIC EXAMINATION:

RESPIRATORY SYSTEM:

CARDIOVASCULAR SYSTEM:

PER ABDOMEN:

CENTRAL NERVOUS SYSTEM:

INVESTIGATIONS:

Hemoglobin:

Peripheral Smear:

Fasting Blood Sugars:

Post Prandial Blood Sugars:

HbA1c:

ANNEXURE-III- ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 46 Date: 22/11/2017

To,

REG NO. BG0117007

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled "THE EFFECT OF THE SEVERITY OF ANAEMIA ON HbA1c IN NON DIABETIC PATIENTS – A ONE YEAR HOSPITAL BASED STUDY IN KLE'S PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE", is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research.
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research.
J.N.Medical College, Belagavi.

30

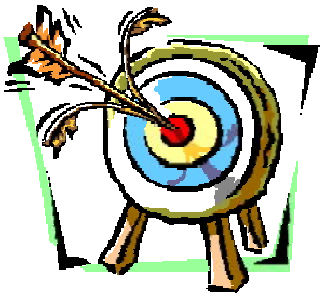
ANNEXURES IV - MASTER CHART

S. No.	IP Number	Name	Age (years)	Sex	Clinical Presentation				Clinical Signs			Type of Anemia	WHO Grading	Hemoglobin (g/dl)	Peripheral Smear	FBS (mg/dl)	PPBS (mg/dl)	HbA1c (%)
					Generalised Weakness	Easy Fatigability	Dizziness	Blood Loss	Pallor	Koilonychia	Bald Tongue							
1	854928	Yallappa B. Mugad	59	M	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	5.2	Microcyctic Hypochromic	90	110	5.6
2	856197	Deepa R. Tallur	18	F	Yes	Yes	Yes	No	Yes	Yes	Yes	Iron Deficiency	Severe	3.6	Microcyctic Hypochromic	86	112	5
3	856272	Parwathi B. Patil	65	F	No	No	Yes	No	No	No	No	Iron Deficiency	Moderate	9.9	Microcyctic Hypochromic	90	130	5.4
4	855297	Shailesh S. Joshi	40	M	Yes	No	No	No	Yes	No	No	Iron Deficiency	Severe	7.9	Microcyctic Hypochromic	92	126	4.6
5	856386	Rama S. Khot	55	M	Yes	Yes	Yes	No	Yes	Yes	Yes	Dimorphic	Severe	2.9	Dimorphic Anemia	76	92	4.3
6	857472	Vishwanath K. Magdum	49	M	Yes	No	No	No	Yes	No	Yes	Dimorphic	Severe	5.9	Dimorphic Anemia	100	129	5.5
7	859734	Shailaja S. Hanchinali	43	F	Yes	Yes	No	Yes	Yes	Yes	Yes	Iron Deficiency	Severe	3.5	Microcyctic Hypochromic	92	132	4.6
8	861942	Vartika R. Mohan	25	F	Yes	Yes	No	No	No	No	No	Iron Deficiency	Moderate	9.6	Microcyctic Hypochromic	82	101	6.3
9	862896	Sulochana C. Hundre	65	F	Yes	Yes	No	No	Yes	No	Yes	Dimorphic	Severe	6.8	Dimorphic Anemia	100	136	5.5
10	869335	Anand R. Redekar	48	M	Yes	Yes	No	No	Yes	Yes	Yes	Iron Deficiency	Severe	3.9	Microcyctic Hypochromic	87	118	5.6
11	867501	Mary K. Gosipathla	24	F	No	No	No	No	No	No	No	Dimorphic	Moderate	9.5	Dimorphic Anemia	87	118	4.7
12	935494	Vasant G. Biranagaddi	45	M	Yes	Yes	Yes	No	Yes	No	No	Megaloblastic	Severe	4	Macrocyctic Hypoochromic	74	110	4
13	872870	Mahadev U. Charlekar	62	M	No	No	No	No	Yes	Yes	No	Iron Deficiency	Severe	7.3	Microcyctic Hypochromic	86	117	6
14	874808	Ashok K. Pujari	52	M	No	No	No	No	No	No	No	Megaloblastic	Moderate	10.4	Macrocyctic Hypoochromic	90	120	4.3
15	875677	Ratna Y. Atadamani	39	F	Yes	Yes	Yes	No	Yes	Yes	No	Iron Deficiency	Severe	6.8	Microcyctic Hypochromic	84	126	5.5
16	877668	Reshma P. Patil	25	F	Yes	No	No	No	Yes	No	No	Iron Deficiency	Severe	7.9	Microcyctic Hypochromic	78	118	6.5
17	877553	Geeta P. Thakuria	37	F	No	No	Yes	No	Yes	No	Yes	Dimorphic	Moderate	8.1	Dimorphic Anemia	90	131	6.3
18	935378	Rahul M. Bogar	48	M	Yes	Yes	Yes	No	Yes	Yes	No	Iron Deficiency	Severe	5.3	Microcyctic Hypochromic	90	119	5.3
19	877929	Govindappa S. Halikatti	60	M	Yes	Yes	Yes	No	Yes	No	No	Megaloblastic	Severe	5.8	Macrocyctic Hypoochromic	86	101	4.5
20	881441	Mahadevi S. Kajagar	41	F	Yes	No	No	No	No	No	No	Dimorphic	Moderate	9	Dimorphic Anemia	96	130	5
21	881786	Holeppa I. Shekugol	40	M	No	Yes	No	No	No	No	No	Iron Deficiency	Moderate	9.4	Microcyctic Hypochromic	82	118	5.3
22	883623	Shanta M. Mali	60	F	No	No	No	No	No	No	No	Iron Deficiency	Mild	11	Microcyctic Hypochromic	90	120	5.6
23	884156	Sumitra V. Kallolimath	54	F	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Moderate	8.7	Microcyctic Hypochromic	74	100	4.2
24	885493	Shivagangavva B. Iti	60	F	Yes	No	Yes	No	Yes	Yes	Yes	Iron Deficiency	Severe	6.6	Microcyctic Hypochromic	90	111	6.5
25	938921	Malleshappa B. Aidnal	86	M	No	No	No	No	Yes	No	No	Dimorphic	Moderate	8.5	Dimorphic Anemia	92	110	5
26	886839	Sushila S. Vairagi	69	F	Yes	Yes	Yes	Yes	Yes	Yes	No	Dimorphic	Moderate	8.6	Dimorphic Anemia	86	110	6.6
27	887588	Neminatha D. Jinagond	56	M	Yes	No	No	No	Yes	No	No	Iron Deficiency	Moderate	9.6	Microcyctic Hypochromic	82	101	6.4
28	888540	Sabavva R. Koravar	70	F	Yes	No	No	No	No	No	No	Dimorphic	Moderate	10.9	Dimorphic Anemia	101	130	5.9

29	888569	Parvati K. Badiger	51	F	Yes	Yes	No	No	No	No	No	Dimorphic	Moderate	9.2	Dimorphic Anemia	80	110	4.7
30	889115	Sehera J. Mulla	35	F	Yes	Yes	Yes	Yes	Yes	No	No	Iron Deficiency	Moderate	8.1	Microcyctic Hypochromic	90	120	6.3
31	889535	Sangavva B. Kalyani	68	F	No	No	No	No	No	No	No	Iron Deficiency	Moderate	9.2	Microcyctic Hypochromic	84	111	5.4
32	889744	Vidya R. Honnali	48	F	Yes	Yes	No	No	Yes	No	No	Dimorphic	Moderate	8.9	Dimorphic Anemia	96	122	5.6
33	890551	Tukaram P. Patil	76	M	Yes	Yes	Yes	No	Yes	Yes	Yes	Iron Deficiency	Severe	5.8	Microcyctic Hypochromic	92	128	5.9
34	891355	Hanamant B. Jaggal	35	M	Yes	Yes	No	No	Yes	No	Yes	Iron Deficiency	Severe	7.6	Microcyctic Hypochromic	99	132	5
35	890642	Suresh S. Awate	55	M	No	No	No	No	No	No	No	Iron Deficiency	Moderate	9.3	Microcyctic Hypochromic	86	112	5.7
36	890738	Nagatevva G. Patil	36	F	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	5.5	Microcyctic Hypochromic	82	120	4.2
37	892912	Roopa S. Dhamnekar	33	F	Yes	No	No	No	No	No	No	Iron Deficiency	Moderate	9.4	Microcyctic Hypochromic	100	138	5.7
38	893182	Veerappa A. Kundarnad	21	M	No	Yes	No	No	Yes	No	No	Iron Deficiency	Moderate	9.6	Microcyctic Hypochromic	74	100	5.2
39	894686	Shattu B. Ajjani	60	M	No	No	No	No	Yes	No	No	Megaloblastic	Moderate	8.3	Macrocyctic Hypoochromic	90	110	5.6
40	894808	Mubina A. Shirur	25	F	Yes	Yes	Yes	No	Yes	No	No	Iron Deficiency	Moderate	8.6	Microcyctic Hypochromic	74	110	5.2
41	897388	Banu R. Bagawan	45	F	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Iron Deficiency	Severe	4.8	Microcyctic Hypochromic	82	116	5.4
42	898015	Veeranaik B. Naikar	67	M	Yes	No	No	No	No	No	No	Dimorphic	Moderate	9.1	Dimorphic Anemia	90	130	4.7
43	898901	Mahaveer B. Naganuri	41	M	Yes	No	No	No	Yes	No	Yes	Dimorphic	Severe	7.8	Dimorphic Anemia	74	120	5.7
44	899029	Neelavva P. Halkarnimath	79	F	Yes	Yes	Yes	No	Yes	Yes	No	Iron Deficiency	Severe	5.3	Microcyctic Hypochromic	89	112	5.7
45	898660	Geeta K. Karigar	20	F	Yes	No	No	No	No	No	No	Megaloblastic	Moderate	10.9	Macrocyctic Hypochromic	90	120	5
46	939356	Gangadhar F. Sakrevgol	75	M	Yes	Yes	No	No	Yes	Yes	Yes	Dimorphic	Severe	5.9	Dimorphic Anemia	87	112	6.1
47	900570	Ramesh B. Keri	53	M	Yes	Yes	Yes	Yes	Yes	No	Yes	Dimorphic	Severe	3.8	Dimorphic Anemia	82	118	6
48	903813	Chandrashekhar K. Shetty	41	M	Yes	No	No	No	Yes	No	No	Iron Deficiency	Moderate	8.6	Microcyctic Hypochromic	86	112	4.8
49	905855	Heena K. Mulla	39	F	Yes	Yes	No	No	Yes	No	No	Iron Deficiency	Severe	7.6	Microcyctic Hypochromic	74	118	5.6
50	906161	Kashamma R. Rayangoudar	38	F	Yes	Yes	Yes	No	Yes	Yes	No	Dimorphic	Severe	4.3	Dimorphic Anemia	90	130	4.7
51	907904	Shivagouda A. Patil	50	M	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	5.5	Microcyctic Hypochromic	90	110	5.2
52	908623	Yankappa M. Bhavi	54	M	Yes	No	Yes	No	Yes	No	No	Dimorphic	Severe	7.3	Dimorphic Anemia	82	119	4.7
53	908723	Murghendra B. Hiremath	50	M	Yes	Yes	No	No	Yes	No	Yes	Dimorphic	Severe	6.3	Dimorphic Anemia	94	132	6
54	5006588	Dinesh C. Patil	28	M	No	No	No	No	No	No	Yes	Dimorphic	Moderate	10.3	Dimorphic Anemia	92	136	5.6
55	909339	Appaso L. Kumbhar	35	M	Yes	Yes	No	No	No	No	Yes	Megaloblastic	Moderate	9.6	Macrocyctic Hypochromic	79	114	5.6
56	910256	Fatima N. Kotwal	63	F	Yes	Yes	No	No	Yes	No	No	Iron Deficiency	Moderate	8.9	Microcyctic Hypochromic	76	123	6.6
57	909865	Bhimappa B. Ammanagi	33	M	Yes	Yes	Yes	No	Yes	No	Yes	Megaloblastic	Severe	5.7	Macrocyctic Hypochromic	91	132	5.2
58	909978	Mala D. Naik	60	F	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	7.8	Microcyctic Hypochromic	108	136	5.2
59	912073	Gangadhar L. Kumbar	54	M	Yes	No	No	No	No	No	No	Iron Deficiency	Moderate	9.4	Microcyctic Hypochromic	72	121	5.6
60	912837	Rahul S. Desai	37	M	No	No	No	No	No	No	No	Megaloblastic	Moderate	10.1	Macrocyctic Hypochromic	74	127	5.8
61	913293	Santoshini V. Kamble	35	F	Yes	Yes	No	Yes	Yes	Yes	No	Iron Deficiency	Severe	6.6	Microcyctic Hypochromic	88	134	6
62	913553	Ashwini S. Vojjaramatti	30	F	Yes	Yes	Yes	No	Yes	No	Yes	Megaloblastic	Severe	7.3	Macrocyctic Hypochromic	91	130	3.9
63	943114	Subanna K. Rajagolkar	65	M	Yes	Yes	No	No	No	No	No	Megaloblastic	Moderate	10.3	Macrocyctic Hypochromic	101	123	5.5
64	915057	Basagouda B. Huchannavar	65	M	Yes	Yes	No	No	Yes	No	No	Iron Deficiency	Moderate	8.9	Microcyctic Hypochromic	99	131	4.8
65	915298	Bhimraya V. Hatti	70	M	Yes	Yes	Yes	No	Yes	Yes	Yes	Dimorphic	Severe	7.4	Dimorphic Anemia	99	136	4.9
66	915366	Sridevi D. Nesargi	24	F	Yes	Yes	No	Yes	Yes	No	Yes	Iron Deficiency	Severe	6.5	Microcyctic Hypochromic	86	111	5.5
67	914513	Snehlata V. Pawar	62	F	Yes	No	No	No	No	No	No	Dimorphic	Moderate	10.4	Dimorphic Anemia	82	138	5.8
68	915461	Mahantesh B. Chougale	20	M	No	No	No	No	No	No	No	Iron Deficiency	Moderate	10.6	Microcyctic Hypochromic	92	134	5.3

69	915516	Rajendra V. Potdar	54	M	No	No	Yes	Yes	Yes	No	Yes	Iron Deficiency	Moderate	8.2	Microcyctic Hypochromic	75	135	4.6
70	915942	Parsappa B. Nadavinamani	45	M	No	No	No	No	No	No	No	Iron Deficiency	Moderate	10.6	Microcyctic Hypochromic	73	136	4.6
71	915979	Sulochana P. Patil	18	F	Yes	Yes	Yes	No	Yes	Yes	Yes	Iron Deficiency	Severe	4.8	Microcyctic Hypochromic	81	129	5.2
72	916033	Bhimappa M. Atamatti	28	M	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	5.9	Microcyctic Hypochromic	88	117	4.6
73	916177	Irappa G. Timmapur	59	M	Yes	Yes	No	No	Yes	No	No	Megaloblastic	Severe	6.9	Macrocyctic Hypochromic	91	133	4.3
74	917242	Sanjay M. Patil	46	M	Yes	No	No	No	Yes	No	No	Megaloblastic	Moderate	8.9	Macrocyctic Hypochromic	81	134	4.1
75	917808	Ramajan I. Nadaf	30	M	Yes	Yes	Yes	No	Yes	No	No	Megaloblastic	Moderate	8.9	Macrocyctic Hypochromic	90	116	4.7
76	919848	Vijaya L. Khannukar	61	F	Yes	Yes	No	No	Yes	Yes	Yes	Dimorphic	Moderate	8.1	Dimorphic Anemia	101	122	4.4
77	919852	Rahul K. Chougale	25	M	Yes	Yes	No	No	Yes	Yes	Yes	Iron Deficiency	Severe	3.2	Microcyctic Hypochromic	74	118	4.5
78	920824	Shantabai P. Dagekar	65	F	Yes	No	No	No	Yes	No	No	Iron Deficiency	Severe	6.3	Microcyctic Hypochromic	92	131	6.1
79	935138	Kabeentaj T. Chopdar	32	F	No	No	No	No	No	No	No	Iron Deficiency	Moderate	9.3	Microcyctic Hypochromic	99	124	5
80	921890	Ramachandra N. Naik	38	M	No	No	Yes	No	Yes	No	No	Megaloblastic	Severe	7.6	Macrocyctic Hypochromic	84	123	5.4
81	922520	Dayanand S. Nadamani	45	M	Yes	Yes	No	No	Yes	No	No	Dimorphic	Severe	6.8	Dimorphic Anemia	91	132	5.9
82	923233	Savita P. Auradkar	49	F	Yes	Yes	Yes	No	Yes	No	Yes	Megaloblastic	Severe	4.8	Macrocyctic Hypochromic	76	111	6.9
83	923014	Gurushant B. Chitti	48	M	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	7.6	Microcyctic Hypochromic	80	132	3.9
84	924034	Shubhangi S. Khatavkar	66	F	Yes	Yes	Yes	No	Yes	No	No	Dimorphic	Severe	5.9	Dimorphic Anemia	77	118	6.3
85	934818	Renuka B. Hosamani	29	F	No	No	No	No	No	No	No	Dimorphic	Moderate	9.6	Dimorphic Anemia	74	108	5.6
86	924431	Vimal S. Dalavi	35	F	Yes	Yes	No	No	Yes	Yes	Yes	Dimorphic	Moderate	8.6	Dimorphic Anemia	90	127	5.2
87	924691	Umadevi M. Murgamath	68	F	Yes	Yes	No	No	Yes	No	No	Megaloblastic	Severe	7.9	Macrocyctic Hypochromic	73	112	5
88	924485	Manohar B. Chavan	50	M	Yes	Yes	No	No	Yes	No	No	Dimorphic	Severe	6.9	Dimorphic Anemia	82	134	5.1
89	925217	Prema A. Jodagunde	58	F	Yes	Yes	Yes	Yes	Yes	Yes	No	Dimorphic	Severe	6.1	Dimorphic Anemia	93	138	4.7
90	922749	Lagmavva S. Baduli	37	F	No	No	Yes	No	No	No	No	Iron Deficiency	Moderate	9.6	Microcyctic Hypochromic	78	113	5.8
91	925817	Mahantamma A. Meti	75	F	Yes	Yes	No	No	Yes	No	Yes	Dimorphic	Moderate	8.2	Dimorphic Anemia	81	126	5.1
92	923853	Shivappa B. Churchihal	65	M	Yes	Yes	No	No	Yes	Yes	No	Iron Deficiency	Severe	6.8	Microcyctic Hypochromic	84	118	5
93	928062	Sunanda M. Devalapur	36	F	Yes	Yes	Yes	No	Yes	Yes	Yes	Iron Deficiency	Severe	3.9	Microcyctic Hypochromic	93	127	5.4
94	929384	Shanta B. Kattimani	45	F	Yes	Yes	Yes	No	Yes	Yes	No	Iron Deficiency	Severe	5	Microcyctic Hypochromic	71	136	5.7
95	928934	Padmavati D. Jorapur	72	F	Yes	No	No	No	Yes	No	Yes	Megaloblastic	Severe	7.4	Macrocyctic Hypochromic	92	129	5.8
96	931157	Umashree R. Tate	30	F	Yes	No	No	No	Yes	No	No	Iron Deficiency	Moderate	8.9	Microcyctic Hypochromic	81	138	6.5
97	931464	Rama S. Chindi	69	M	Yes	Yes	Yes	No	Yes	No	No	Megaloblastic	Severe	6.5	Macrocyctic Hypochromic	92	122	5.3
98	931772	Pragati I. Patil	21	F	Yes	Yes	No	No	Yes	Yes	Yes	Iron Deficiency	Severe	7.6	Microcyctic Hypochromic	88	134	6.4
99	932244	Sugarabi I. Soudagar	62	F	Yes	Yes	Yes	No	Yes	Yes	Yes	Dimorphic	Severe	7.5	Dimorphic Anemia	75	123	5.7
100	932064	Kallappa M. Bewar	80	M	Yes	No	No	No	Yes	No	No	Dimorphic	Moderate	8.2	Dimorphic Anemia	83	133	5.8

Introduction



Objectives

Review of Literature

Methodology

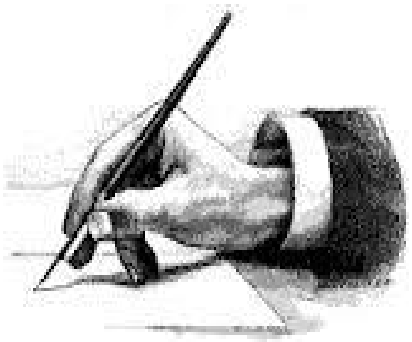
Results

Discussion

Conclusion

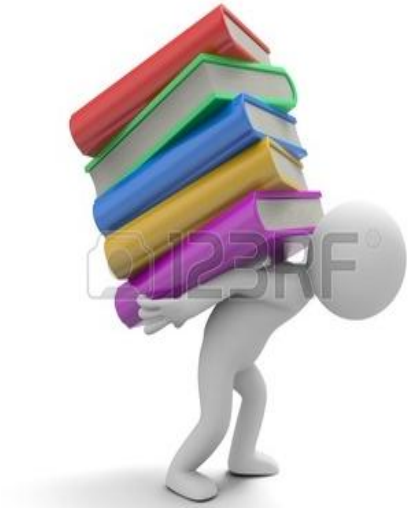
Summary

Bibliography



Annexure-I

Annexure-II



Annexure-III

Annexure-IV