A STUDY ON THE EFFECT OF MICROCYTIC ANAEMIA ON HbA1c LEVELS IN NON DIABETIC INDIVIDUALS IN GOVERNMENT ROYAPETTAH HOSPITAL, CHENNAI.

Dissertation submitted to

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI.

In partial fulfilment of regulations

For the award of the degree of

M.D. (GENERAL MEDICINE)

BRANCH-1

KILPAUK MEDICAL COLLEGE

CHENNAI 600010

APRIL 2016

BONAFIDE CERTIFICATE

This is to certify that the dissertation named **" A STUDY ON THE EFFECT OF MICROCYTIC ANAEMIA ON HbA1c LEVELS IN NON DIABETIC INDIVIDUALS**" is a bonafide work performed by Dr.S.Swetha, post graduate student, Department of Internal Medicine, Kilpauk Medical College, Chennai-10, under my guidance and supervision in fulfilment of regulations of the Tamil Nadu Dr. M.G.R Medical University, for the award of M.D. Degree Branch I (General Medicine) during the academic period from 2013 to 2016.

Prof. Dr.K.V.RAJALAKSHMI M .D.,
GUIDE FOR THE STUDY,
CHIEF UNIT-3,
DEPARTMENT OF MEDICINE,
GOVT.ROYAPETTAH HOPSITAL,
CHENNAI-600014.

Prof. Dr. S. USHA LAKSHMI M.D., FMMC,
THE HEAD OF THE DEPARTMENT,
DEPARTMENT OF MEDICINE,
GOVT KILPAUK MEDICAL COLLEGE,
CHENNAI - 600 010.

Prof. Dr. R. NARAYANA BABU M.D. DCH.,
THE DEAN,
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI -600 010.

DECLARATION

I solemnly declare that this dissertation **" A STUDY ON THE EFFECT OF MICROCYTIC ANAEMIA ON HbA1c LEVELS IN NON DIABETIC INDIVIDUALS**" was prepared by me at Government Kilpauk Medical College, Chennai, under the guidance and supervision of Dr.K.V.RAJALAKSHMI M.D., Professor, Department of Internal Medicine, Government Royapettah Hospital, Chennai.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the University regulations for the award of the degree of M.D. Branch I (General Medicine).

Place: Chennai

Date:

(DR. S.SWETHA)

ACKNOWLEDGEMENT

At the outset, I would like to thank my beloved Dean, Kilpauk Medical College Prof Dr.R. NARAYANA BABU M.D., DCH., for his kind permission to conduct the study in Kilpauk Medical College and also Dr.N.Nazeer Ahmed.M.S(ORTHO).,D.ORTHO., Superintendent of Government Royapettah Hospital for giving permission to investigate patients in the hospital. I would like to express my special thanks to Professor and Head, Department of General medicine Prof. Dr.S.USHALAKSHMI.M.D., FMMC, Govt. Royapettah Hospital for permitting me to conduct this study.

I would like to thank wholeheartedly, Prof. Dr.S.MAYILVHANAN.M.D., Professor of Medicine for his encouragement and guidance during the study.

I also express my thanks to Prof. Dr.K.V.RAJALAKSHMI.M.D. I am extremely thankful to Assistant Professors of Medicine, Dr.I.Rohini.M.D., Dr.N.JAYAPRAKASH.M.D and Dr.GOVINDARAJULU M.D., for their assistance and guidance.

I would always remember with extreme sense of thankfulness, the co-operation and constructive criticism shown by my fellow post graduate colleagues and friends.

Finally, I thank all my patients for their active co-operation in this study, without which this would not have become a reality.

CONTENTS

INTRODUCTION

Diabetes mellitus is a disorder of chronic hyperglycemia. It is caused by either deficient production or by the inefficient utilization of insulin, the major hormone responsible for lowering blood glucose levels in the body. Recently, more specific etiologies with molecular basis for specific types of diabetes are being put forth.

According to World Health Organization (WHO), Diabetes will be the 7th leading cause of mortality by 2030[1]. India has more people afflicted with diabetes than in any other country in the world, according to the details furnished by the International Diabetes Foundation. Thus, India is rightly named "the diabetes capital of the world" by Indian Heart Association[2]. Around 62 million Indians are currently affected by Diabetes, which comes to nearly 7.1% of India's adult Population. It is estimated that diabetes will affect 109 million individuals by 2035. Diabetes contributes to death in nearly 1 million Indians every year. Clinically evident diabetes becomes manifest around 42.5 years (i.e.) in the peak of productive years of life. All these data clearly expose the burden Diabetes imposes on the Indian economy. Adding to the misery, are the American Diabetes Association's reports that India is expected to see the greatest increase in newly diagnosed diabetic people by 2030[3]. The high incidence in Indians is ascribed to

the increased genetic susceptibility with growing middle class' high-calorie, low-activity lifestyle and the differences in body composition.

Investigations for the early detection and management of diabetes are an ever growing field of medicine. The history rolls from the simple tests of detecting the glucose in urine done in early twentieth century to estimations of glucose in the blood, to the study of various enzyme activities to the latest investigations of immunological and molecular markers done nowadays to identify specific disorders in glucose metabolism.HbA1c is one such entity which has secured a pivotal place in the diagnostic criteria for diabetes. It was introduced as a marker of the glycemic control status over the past 2-3 months and has come a long way in being used as a diagnostic modality for diabetes approved by the WHO and American Diabetes Association (ADA). The recent identification of various pitfalls and confounding factors in the measurement of HbA1c has necessitated the review of its role. It is advised to be used with caution in certain population groups such as children, pregnant women, patients with hemolytic anemia or hemoglobinopathies. One such confounding factor has been identified as anemia and the iron stores (ferritin) of the individual.[4] The exact role of anemia on Hba1c estimation is yet to be established as the studies conducted so far have given conflicting results. This study has been henceforth conducted to establish the effect of microcytic hypochromic anemia on HbA1c levels if any.

AIM OF THE STUDY

To analyze the effect of microcytic hypochromic anemia on HbA1c levels in non diabetic individuals.

REVIEW OF LITERATURE

OVERVIEW

DIABETES MELLITUS:

INTRODUCTION:

The term Diabetes Mellitus (DM) is defined as a metabolic disorder with heterogeneous etiologies characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism[5] . The defect may be at various levels. Insulin secretion, action or sometimes both are affected. Thus it is the pathogenic process leading to hyperglycemia which forms the basis for the classification of diabetes. The two broad categories are designated as type 1 and type 2. Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Other etiologies for DM include specific genetic and metabolic defects in insulin secretion or action, mitochondrial abnormalities and a host of conditions that impair glucose tolerance.

The classification proposed by American Diabetes Association in 2011 encompassing the various etiologies is as follows:

Table 1 : Classification of Diabetes Mellitus - ADA 2011[6]

I. Diabetes (β-cell destruction, usually leading to absolute insulin deficiency)
A. Immune mediated
B. Idiopathic
II. Diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types
A. Genetic defects of β-cell function
B. Genetic defects in insulin action
C. Diseases of the exocrine pancreas
D. Endocrinopathies
E. Drug- or chemical-induced
F. Infections
G. Uncommon forms of immune-mediated diabetes
H. Other genetic syndromes sometimes associated with diabetes
IV. Gestational diabetes mellitus (GDM)

HISTORY:

The history of diabetes dates back to ancient Egyptians describing clinical features very similar to Diabetes Mellitus 3000 years ago. The term "*diabetes*" meaning “siphon”, to explain the liquefaction of the flesh and bones into urine was first coined by Araetus of Cappadocia (81-133AD).[7] He used this term while describing a disease with symptoms of excessive thirst and urination

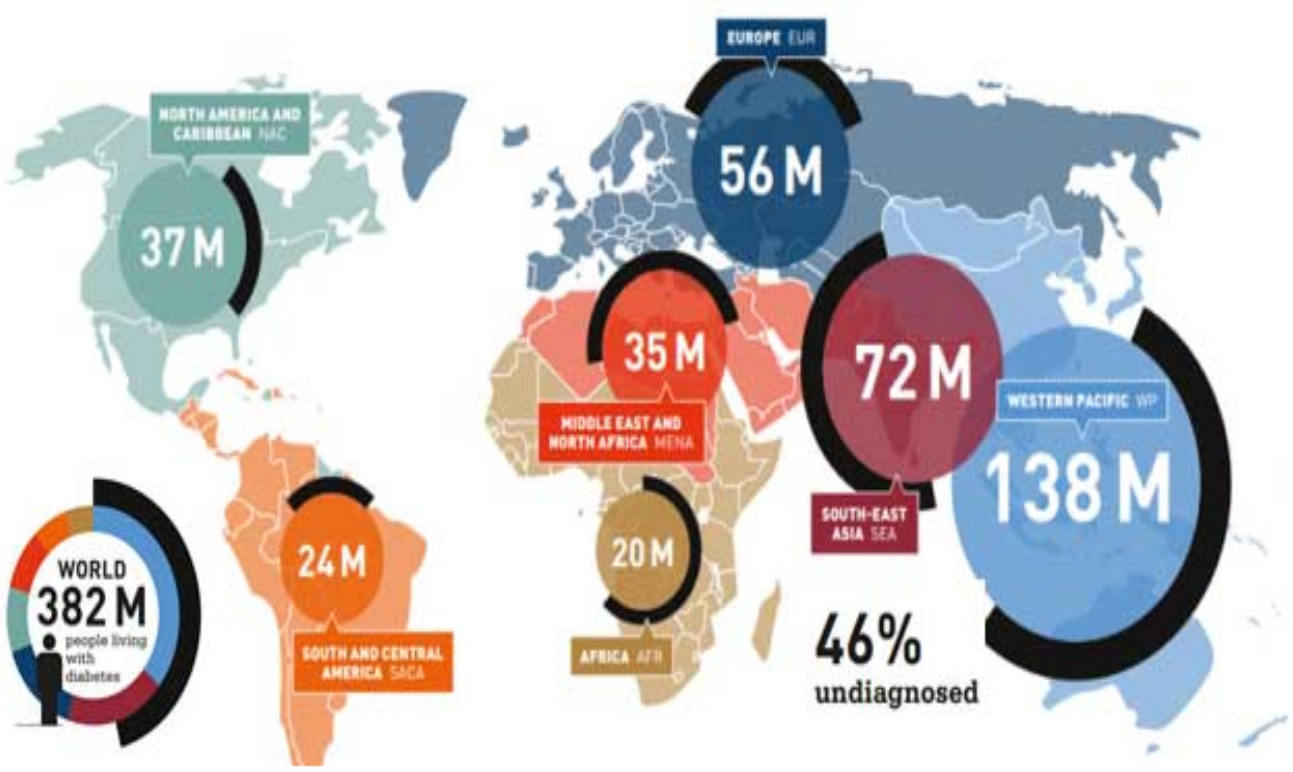
with weight loss. Ancient Indians have first observed that urine and blood of diabetic patients were sweet. Later, in 1675, Thomas Willis added the word "*mellitus"* meaning "sweet" after rediscovering the sweetness of blood and urine. This presence of excess sugar in urine and blood as a cause of their sweetness was confirmed in 1776 by Dobson. The identification of the role of liver in gluconeogenesis is an important accomplishment in the history of diabetes and this led to the conceptuation by Claude Bernard (France) in 1857 that excess glucose production causes diabetes. Extensive autopsy studies of diabetic patients concurrent with other developments led to the final identification of the etiological role of pancreas, especially the *islets of Langerhans.* Insulin principally designated as the isletin, as it was believed to be secreted by the islets was first discovered by a group of four people - John MacLeod, Fredrick Banting, Charles Best & J.B. Collip.[7] They could also extract insulin in sufficient amounts from beef & pork pancreas and use it for lowering blood sugar levels in depancreatized dogs. The evolution of process of purification, stabilization, production in adequate amounts from non human sources and recombinant insulin production spanned over 8 decades from 1920s. The long acting insulin was identified in 2001.

EPIDEMIOLOGY:

According to WHO data, 347 million people worldwide are suffering with diabetes[3]. In 2014, the estimated global prevalence of diabetes was 9% among

adults aged 18 years and above. It was a leading cause of death, that in 2012, an estimated 1.5 million deaths were directly caused by diabetes. The burden of diabetes is deeply rooted in the low and middle income countries that more than 80% of diabetes deaths occur in them. *Wild et al* [8] predicts the prevalence of diabetes to double globally from 171 million in 2000 to 366 million in 2030. The proposed increase is expected to be maximum in India with an estimated 79.4 million diabetics . Diabetes and its complications have a serious impact on the economy of a country as it predominantly affects people of the work force.

Fig: 1 WHO region wise distribution of Diabetes mellitus



INDIAN SCENARIO:

According to the model of four phases of health transition, manmade and degenerative diseases predominate while communicable diseases are in the decreasing trend in low and middle income countries such as India. A life expectancy bordering around 50-60 years and unhealthy lifestyles which promote lifestyle diseases like cardiovascular disease, diabetes and hypertension characterize this phase. There is an increase in the incidence and prevalence of non communicable diseases. The low education levels and poor awareness of diseases and their complications in the low income countries exaggerate the impact on health of the population.

In India, which has sadly earned the distinction of being the "diabetes capital of the world", diabetes is fastly reaching epidemic proportions with more than 62 million diabetic individuals. In 2000, India (31.7 million) topped the world with the highest number of diabetic population superseding China (20.8 million) and the United States (17.7 million). Most states in India have a prevalence rate of 10 %. Kerala tops the list having the highest incidence of 19.5% and Kashmir valley the lowest at 6% [9]. ICMR-INDIAB study which was conducted in three states and one Union Territory has estimated that India is likely to have 62.4 million diabetic patients. There is another 77.2 million people in the pre diabetic group. In a longitudinal cohort study done in Chennai, the incidence of diabetes was around

20.2 per 1000 person years among subjects who had normal glucose tolerance before. It was 64.8 per 1000 person years in those with pre-diabetes.

The prevalence of diabetes in urban areas varied between 10.9 % and 14.2%. The numbers were smaller in rural areas from 3.0% to 8.3%[10]. But, disturbingly, number of people detected with diabetes is increasing in rural areas recently. The likely explanation is the extension of urbanization and adoption of unhealthy lifestyles in the rural population.

WHY ARE THERE MORE DIABETICS IN INDIA?

Apart from the conventional risk factors for diabetes present globally like urbanization, industrialization, globalization, other factors also play a pivotal role in contributing to the increased prevalence in Indians. Obesity, regional distribution of adiposity, increased proportion of body fat for the same body weight, fetal programming and genetic factors proffer to higher risk. In adults , the classical variables associated were age, BMI, increased Waist Hip Ratio (WHR), sedentary life style , a family history of diabetes, , socio-economic status. The increased proportion of body fat due to visceral adiposity and a higher risk of diabetes for the same BMI as compared to the rest of the global population have made WHO recommending revised guidelines for Asian population. A BMI of

23–27.5 kg/m^2 is at more risk for type 2 diabetes. A BMI of 27.5 kg/ m^2 or higher is at high risk.

The other factors contributing to increased prevalence are:

1) Changing dietary habits from the traditionally consumed diet in urban and rural areas. People consume up to 32% of daily energy requirement as fat.

2) Widespread use of automobiles and decreased physical activity of the population that was once heavily dependent on farming and other manual labor.

3) Increasing "screen time" due to the increasing education and awareness about the disease is another aspect to be given due place in causing increased incidence of diabetes[11].

Apart from the modifiable risk factors as stated above, recent identification of new susceptibility locus at 2q21 by the Genome Wide Association Study (GWAS) is also being postulated to contribute. However, in a poly genetic disease like diabetes, no more than 10 % of incidence can be attributed to this genetic defect.

Fig: 2 Estimated numbers of diabetic subjects in India[9]

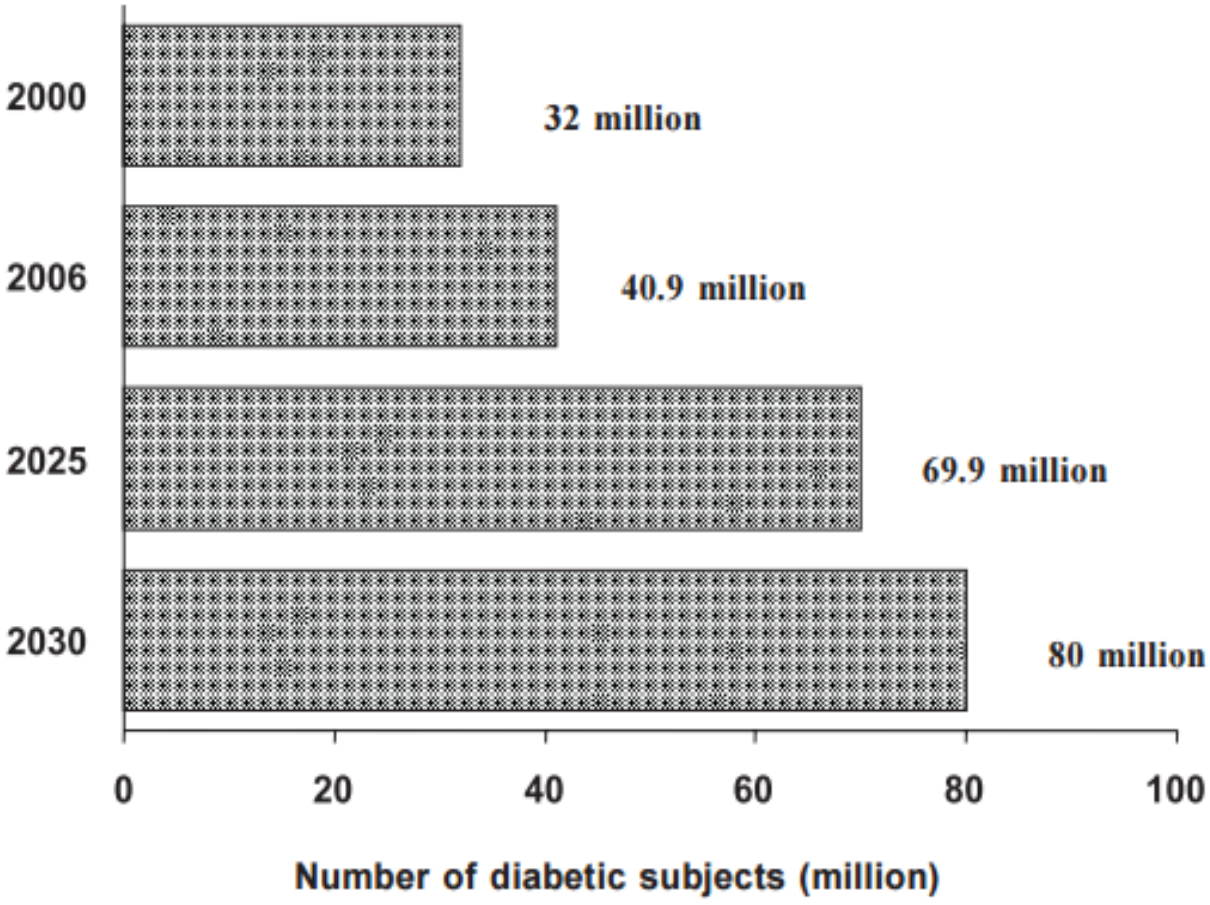


Fig.3 Prevalence of Diabetes in India: ICMR-INDIAB study[10]

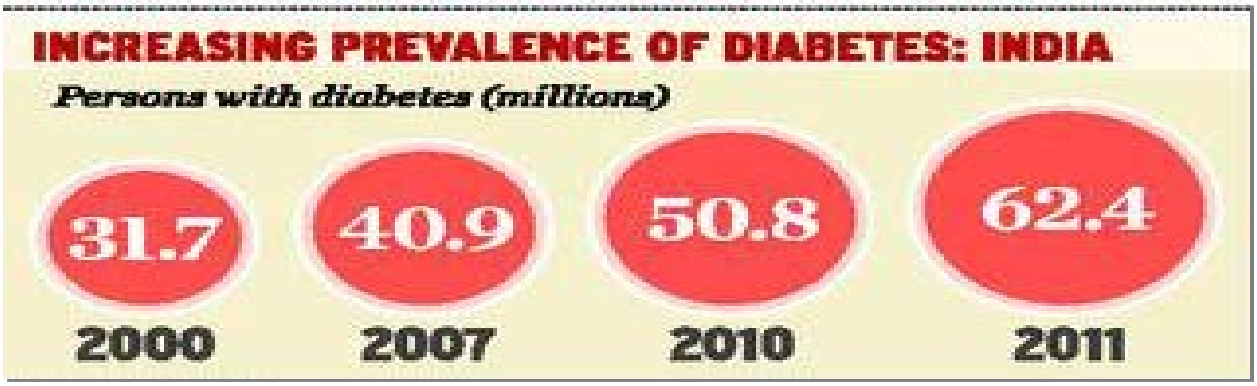
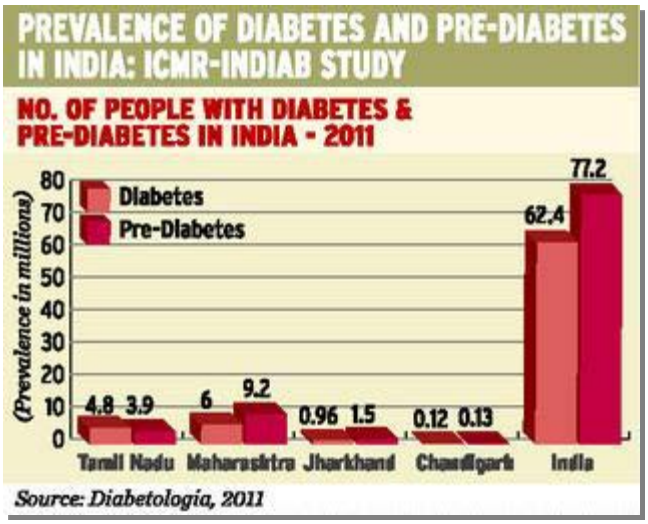


Fig.4 Prevalence of Diabetes & pre diabetes in India: ICMR-INDIAB study (contd.)[10]



Though type 2 diabetes is a disease of adults, according to new studies, risk factors start getting established even in childhood .The influences of early life including the intra-uterine period also contribute to the onset and progression of the disease. These factors are also contributing to the emergence of obesity and overweight as important public health problems in India. There is a reported prevalence of around 20-30% of metabolic abnormalities including dysglycemia (about 10%) and dyslipidaemia (25-40%) in urban socio-economically advantaged school going children who is apparently healthy but obese or overweight. An increase in obesity prevalence is also apparent from the National Family Health Survey (NFHS) data.

From 11% in NFHS- 2 (1998-1999) prevalence of obesity has gone up to 15% in NFHS-3 (2005-2006)[12].These people are at increased risk of developing diabetes and other obesity related problems in their future life. In the New Delhi Birth Cohort study, dysglycemia in later life was found to be inversely related to BMI and weight at 1 year of age. But, after 2 years of age, this relationship gets straight. An increase in BMI was associated with increased risk of diabetes. The study showed that the highest prevalence of diabetes and dysglycemia was in subjects who formed the lowest third of the group with respect to BMI at 2 years and highest at an age of 12 years[13]. Thus it has been established that low birth weight as seen in majority of the Indian new borns and accelerated weight gain after 48 months are predisposing to adult onset glucose intolerance.

ANATOMY OF PANCREAS:

Pancreas (meaning 'all flesh') is a glandular organ with both endocrine and exocrine functions. Situated behind the stomach, retroperitoneally, it secretes digestive enzymes through the exocrine part into the gut lumen. The endocrine part of pancreas, Islets of Langerhans lies embedded in the gland. It has three types of cells namely α, β and D cells and secretes insulin, glucagon, somatostatin, amylin into the blood stream and plays a vital role in glucose homeostasis.

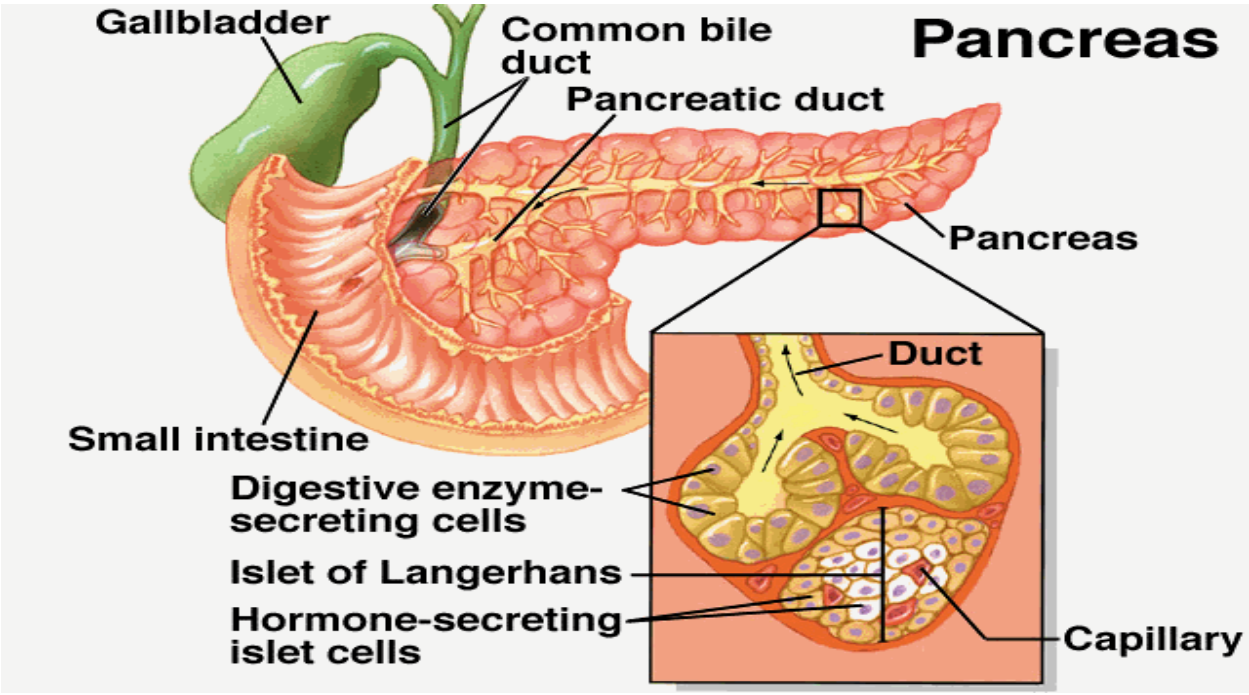


Fig: 4 Macro and micro anatomy of pancreas

INSULIN SYNTHESIS AND SECRETION:

Insulin is produced in the islet cells as a single chain preproinsulin with 86 amino acids. It is acted upon by proteolytic enzymes cleaving the signal molecule to pro insulin. Subsequent proteolysis cleaves C- peptide, α & β amino acid chains. α & β amino acid chains contain 21 & 30 amino acids respectively. Though insulin secretion is influenced by numerous factors like amino acids, ketones neurotransmitters and nutrients, glucose is the prime regulator. Entry of glucose into islet cells is mediated by facilitated transporter GLUT 2 . Glucose is utilized via the glycolytic pathways involving glucose-6-phosphate formation as the rate limiting step. ATP derived from glycolysis inhibits ATP dependent K+ channel causing depolarization of the cell. This depolarization causes opening of voltage

dependent Ca2+ channel causing calcium influx and insulin secretion. This occurs in a pulsatile manner occurring every 10 minutes with superimposed pulsations of higher amplitude occurring every 80-150 minutes. The ingested foods regulate the secretion of insulin through incretins secreted by neurotransmitter cells of the GI tract. GLP-1 analogues are the most potent incretins secreted by L type cells[5].

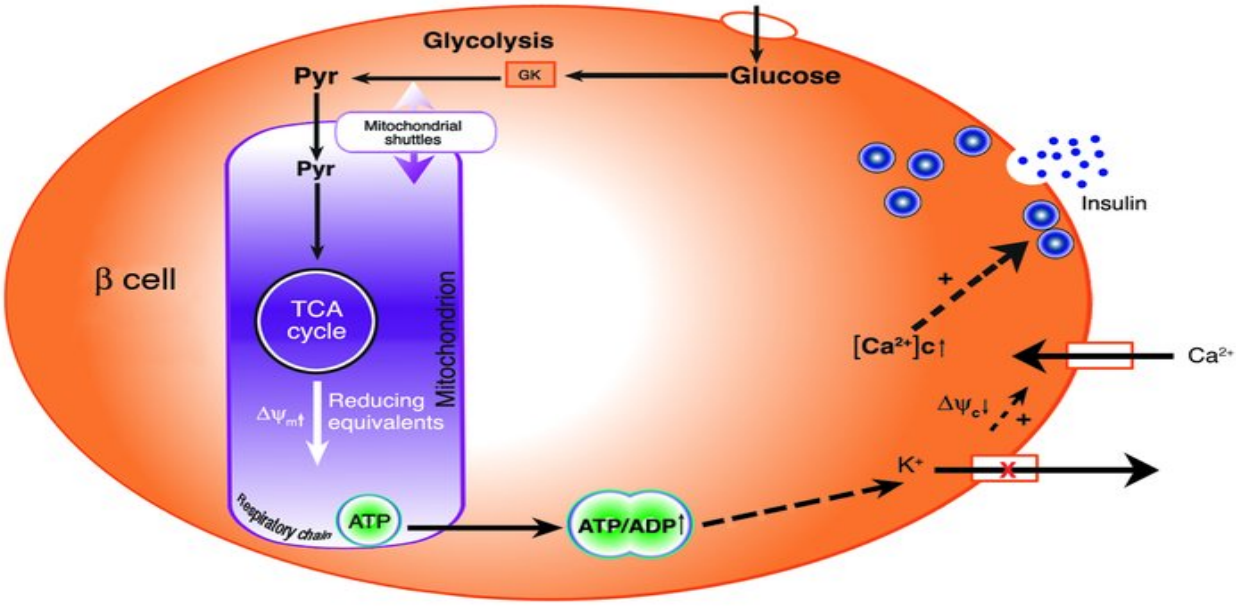


Fig: 6 Mechanism of glucose mediated insulin synthesis

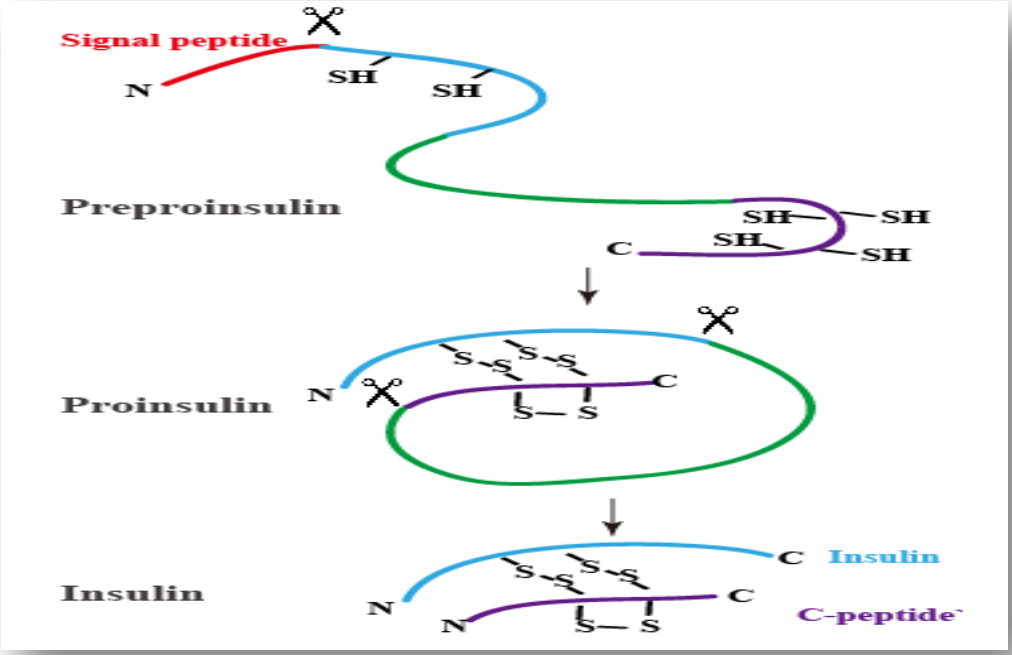


Fig: 7: Insulin synthesis and secretion

ACTION:

Once insulin is secreted into the portal system, approximately 50% is taken up and degraded by the liver. The rest of the insulin gaining access to the systemic circulation binds to insulin receptor which has intrinsic tyrosine kinase activity. Upon binding to insulin receptors, insulin causes stimulation of tyrosine kinase leading to auto phosphorylation of a subset of proteins called as Insulin Receptor Substrate (IRS) . They initiate a cascade of phosphorylation and dephosphorylation reactions accounting for the metabolic actions of insulin like increased uptake of glucose into skeletal muscles, liver and adipocytes.

In skeletal muscle, glucose is either used up or stored as glycogen . In liver, glucose is converted to the storage form of glycogen and proteins while in adipose tissue, glucose is converted into fat. Insulin is predominantly an anabolic hormone which not only stimulates uptake and utilization of glucose but also that of amino acids.

PATHOGENESIS:

TYPE 1 DIABETES MELLITUS:

Destruction of beta cells and absolute insulin deficiency are the pathognomonic features of type 1 DM. Various immunological, environmental and genetic factors interact and contribute to the destruction of beta cells and insulin deficiency. Auto antibodies are almost always detected in type 1 diabetic population though it may be absent in a few.

These people are born with normal population of beta cells which start to decline rapidly due to an auto immune insult secondary to an environmental or infectious organism. Having started, the process is sustained by beta cell specific molecule which is yet to be identified. Pathologically, it is this insulitis which characterizes type 1 DM. This process involves leucocytic infiltration of beta cells of islets causing destruction. Islet cell antibodies, activation of lymphocytes within islets, islet protein induced T cell proliferation, cytokine activation are the aberrancies noted in the humoral and cell mediated immunity After all beta cells

are destroyed, immunological process abates and islets become atrophic. The precise mechanism for islet cell death is yet to be known but involves apoptosis, formation of nitric oxide metabolites, CD8+ cell mediated cytotoxicity. The targets identified are insulin, Glutamic acid decarboxylase, and beta cell specific Zn transporter, ICA (Islet cell auto antibodies) -512 / IA-2. ICA assays are routinely being used to detect Type 1 DM. Testing positive for ICAs combined with impaired insulin secretion to parenteral glucose testing poses the individual at a risk of >50% for developing type 1DM in another five years. It is noteworthy to mention that these auto antibodies are positive in 5-10% of type 2 DM and in a minority in GDM. In addition to these, environmental factors, entero viruses, rubella, coxsackie and nitrosurea compounds are also implicated.

GENETIC FACTORS:

Genetic loci associated with increased susceptibility are linked to HLA 6 mapping to regions encoding the class II Major Histo compatibility class molecules. As these molecules are the antigen presenting molecules to helper T cells, the differences in the amino acid composition alter the specificity of immunological response. HLA DR3 &4 haplotypes are most commonly present in type 1 diabetics. The haplotype DQA1*0102, DQB1*0602 appear to protect people from type 1 DM (<1%) as it is extremely rare[5].

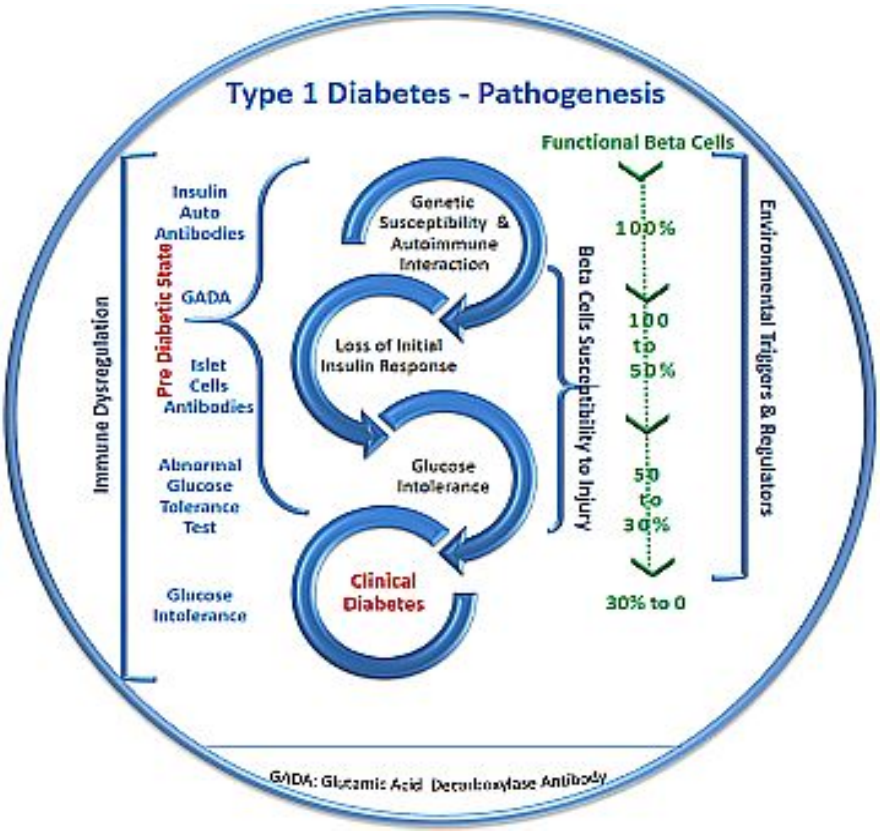


Fig: 8 Pathogenesis of type 1 Diabetes Mellitus

TYPE 2 DIABETES MELLITUS:

It encompasses an array of disorders all of which share a common phenotype of hyperglycemia. Variable degrees of insulin resistance and insulin secretory defects play a central role in the pathogenesis of type 2 diabetes. Now the consensus is that absolute insulin secretory deficiency is preceded in years by insulin resistance. Overt diabetes develops following inadequate insulin secretion.

GENETIC FACTORS:

Genetic factors play an important role in pathogenesis of type 2 DM than for Type 1 DM as shown by the concordance rate of 70-90% in monozygotic twins. The disease is polygenic and multifactorial . The genes implicated are inward rectifying potassium channel, calpain-10, Zinc transporter, IRS, transcription factor 7 like 2 gene . There is a 40 % risk of developing diabetes for an individual if both the parents are diabetic.

PATHOPHYSIOLOGY:

Impaired insulin secretion, insulin resistance, abnormalities in lipid metabolism, excessive hepatic gluconeogenesis are all central to the development of type 2 DM. Insulin resistance is the first to occur and is relative in nature as it is well compensated by supra normal levels of insulin . Insulin resistance occurs approximately ten to twenty years before clinical DM develops[5]. Once insulin resistance far exceeds hyperinsulinemia, impaired glucose tolerance develops as manifested by IGT. In due course, further decrements in insulin secretion is combined with excessive hepatic gluconeogenesis presenting as full blown diabetes with high fasting blood sugar levels. Further deterioration leads to beta cell failure and absolute insulin deficiency.

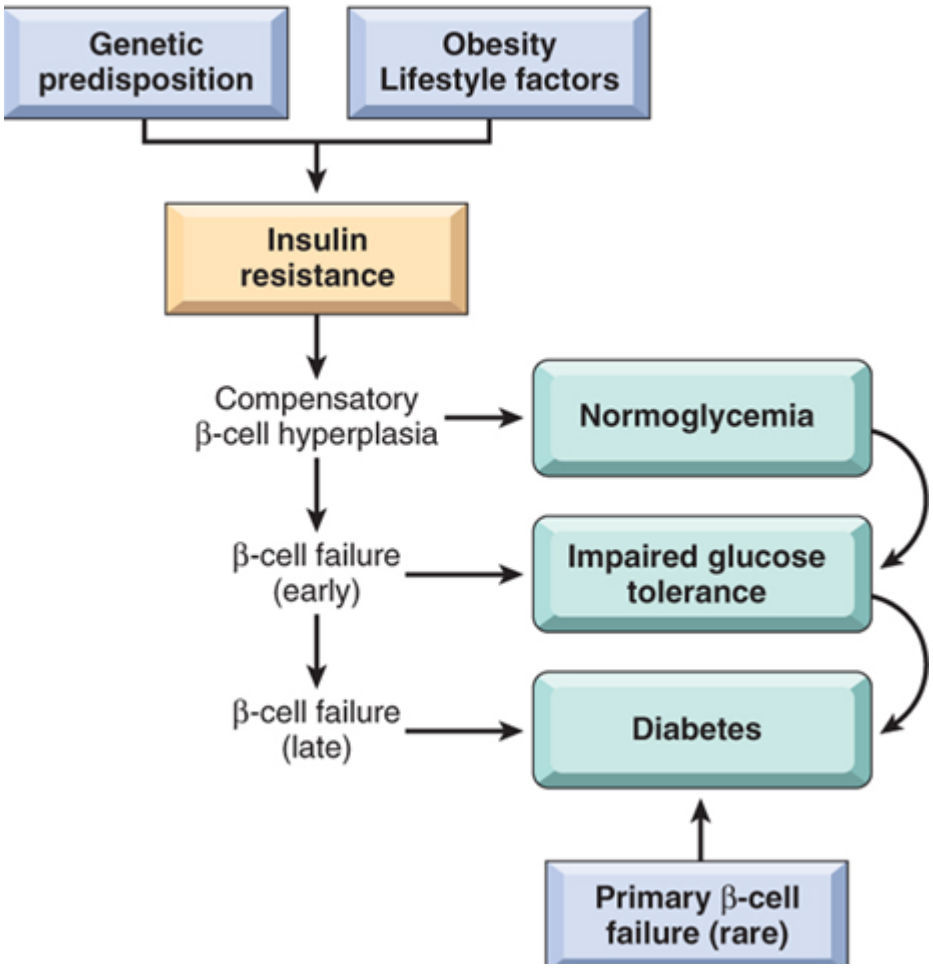


Fig 9: Pathogenesis of type 2 Diabetes mellitus

METABOLIC ABNORMALITIES:

ABNORMAL MUSCLE METABOLISM:

Skeletal muscle is the most important organ concerned with glucose homeostasis as it accounts for approximately complete glucose utilization following glucose loads either by oral or parenteral routes. Unlike in tissues like brain, placenta and cornea, this process is insulin dependent. Thus insulin

resistance alters skeletal muscle utilization of glucose significantly contributing to post prandial hyperglycemia. There is decreased activity of Insulin receptor Substrate -1 activity and inositol phosphatase -3 activity in skeletal muscle. There is also an up regulation of negative regulators of insulin activity like plasma cell differentiation factor-1and phosphotyrosine phosphatases which dephosphorylate insulin receptor causing decreased action of insulin.

ABNORMAL ADIPOSE TISSUE METABOLISM:

Adipose tissue mediated lipolysis is less effectively suppressed by insulin secondary to insulin resistance. This leads to increased circulation of free fatty acids. Adipokines are substances like resistin, adiponectin, leptin, TNF -α, retinol binding protein-4 that are secreted by adipocytes and regulate insulin sensitivity in an individual. These adipokines are reduced in obesity contributing to insulin resistance and diabetes[5].

ABNORMAL LIVER METABOLISM:

Increased hepatic gluconeogenesis is the process by which insulin resistance affects liver and contributes to hyperglycemia. In general, insulin suppresses hepatic glucose production by decreasing the flux of amino acids and free fatty acids and also helps in the conversion of glucose to glycogen. Insulin resistance makes this control lost contributing to reduced storage of glucose as glycogen and

increased fatty acid accumulation in the liver. Thus hepatic insulin resistance is an important factor contributing to both fasting and post prandial hyper glycemia.

ABNORMAL METABOLISM IN BRAIN:

Though glucose utilization in brain is an insulin independent process, some neurons in the hypothalamic area controlling satiety express insulin sensitive GLUT-4.Hypothalamic insulin resistance contributes to reduced satiety occurring both as a cause and consequence of obesity. Also experimental studies have shown that hypothalamic resistance contributes significantly to hepatic insulin resistance.

CLINICAL FEATURES:

The early symptoms of this disorder may be simple characteristic symptoms such as thirst, polyuria, weight loss, and polyphagia or rarely its most severe and potentially incurable forms as ketoacidosis or nonketotic hyperosmolarity. Often symptoms are not severe or may even be absent at the time of diagnosis though sufficient signs of end organ damage are evident clinically. Hyperglycemia sufficiently high enough to cause pathologic changes would have been present for a long time before the diagnosis is made. Consequently, diabetes often is discovered because of abnormal results from a routine blood or urine glucose test or because of the presence of a complication.

SCREENING:

The silent protracted course of diabetes necessitates the early and efficient diagnostic modalities for type 2 Diabetes and optimal screening of all high risk individuals. The ADA recommends screening all individuals >45 years for every 3 years and screening individuals at an earlier age if they are overweight [BMI >25 kg/m^2] and have one additional risk factor for diabetes which can be enlisted as a family history of diabetes, physical inactivity, race, ethnicity, positive history of hypertension , HDL cholesterol< 35 mg/dL or Triglycerides more than 250 mg/dL, gestational diabetes mellitus, polycystic ovarian syndrome, or being previously diagnosed with IFG, IGT or A1c levels of 5.7- 6.4 %[6]. This screening is valid for type 2 DM only as a long asymptomatic period of hyperglycemia is rare prior to the diagnosis of type 1 DM.

DIAGNOSIS:

EVOLUTION OF DIAGNOSIS OF DIABETES MELLITUS:

The first test used to identify and quantitate the glucose in urine was first deployed by Avicenna, an Arab physician in 1000 A.D[14]. Noteworthy in the history of diabetes are the tests for the detection of sugar in urine by Benedicts test and Self Monitored Blood Glucose (SMBG) instruments by glucose peroxidase strip methods. OGTT, oral glucose tolerance test was first described in 1923

by Jerome W. Conn[7]. In 1969, the first portable glucometer was developed. HbA1c was discovered in the late 1960s as an incidental finding.

An ideal glucose monitoring device will be a device which is non invasive or minimally invasive, monitors blood glucose levels continuously, warns the patient of hypo or hyperglycemia and calculates data about hourly, weekly and monthly sugar values. Such a device is yet to come into existence. However, the diagnostic modalities for diabetes have come a long way from crude methods of urine analysis. The following is a review of the history.

Before 1975, patients were routinely monitored by serial urine sugar measurements done at home with occasional blood sugar values obtained from the laboratory[14]. Though simple, routine analysis of urine for presence and quantification of sugar/glucose is limited by several factors:

1) Urine sugar estimation is affected by concentration, fluid intake, drugs ingested.

2) The varying threshold of renal glycosuria especially in long standing diabetics.

3) Absence of glucosuria does not equate to euglycemia as it is only a snapshot of blood sugar levels since the time of last void.

Though former tests use reduction of copper ions, formation of enediols, the latest ones apply glucose oxidase - peroxidase method for detection of glucose in urine.

Fig 10: Diastix urine glucose test strip & color chart

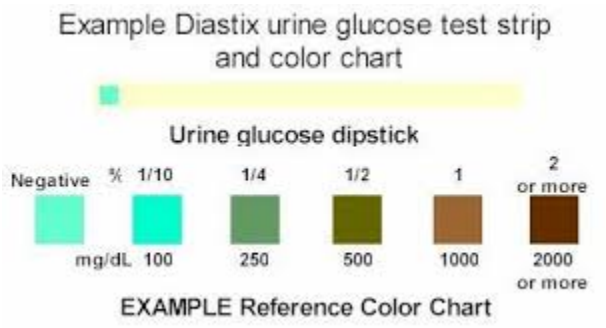


Fig11: semi quantitative urine analysis by Benedict's method

The results of DCCT trial[15] has led to wide consensus viz. strict control of blood glucose levels is associated with significant differences in the outcome for type 1 diabetes and revolutionized the treatment criteria for diabetes[15]. It necessitated the formulation of specific treatment goals. This led to the necessity and hence the invention of new tests to monitor blood glucose levels. Serum monitoring of blood glucose (SMBG) came into use and took over the field of diagnosis and continue to reign. All these instruments principally use glucose oxidase peroxidase method. This method involves formation of hydrogen peroxide which reacts with horse radish peroxidase to form nascent oxygen. This nascent oxygen oxidizes orthotoluidine to form purple or violet color which is read by colorimetry. It has expanded from laboratory based glucose estimations to self monitors used by the patients at home.

FASTING PLASMA GLUCOSE (FPG): Fasting is defined as no caloric intake for at least 8 hours after which plasma glucose levels are measured. In a patient

with symptoms suggestive of hyperglycemia such as thirst, polyuria, unexplained weight loss, the diagnosis can be easily established by the fasting hyperglycemia. An elevated FPG warrants a diagnosis of DM and no further testing with OGTT is necessary. If the patient is asymptomatic or has only minimal symptoms and fasting blood or plasma concentrations are not diagnostic, an OGTT is often required to establish or refute a diagnosis of DM.

ORAL GLUCOSE TOLERANCE TEST (OGTT):

This is the Gold standard in the diagnosis of Diabetes mellitus though it is cumbersome as it takes nearly four hours to complete the test. The test is administered in the morning after an overnight fasting of 10 -16 hours. The patient should have been on unrestricted diet for 3 days before this test and should have had normal activity prior to testing. Heavy exercises should be avoided prior to testing. Smoking and other drugs known to affect sugar levels are to be avoided during testing period. After drawing blood for fasting plasma glucose level determination, subject is made to drink 75 g of anhydrous glucose or equivalent. Plasma or blood samples are drawn 2 hours after the test load. This test is used in diagnosis of gestational diabetes mellitus in women with high risk and can ideally be used in all but seriously ill, post trauma or with recent infections.

Glucose tolerance normally varies from normal glucose homeostasis to impaired glucose homeostasis and diabetes mellitus, Using this blood glucose measurements, the spectrum of Glucose tolerance can be assessed using the fasting plasma glucose (FPG), the response to oral glucose challenge (OGTT).

NORMAL GLUCOSE TOLERANCE:

A FPG <5.6 mmol/L (100 mg/dL), a plasma glucose <140 mg/dL (11.1 mmol/L) following an oral glucose challenge, and an HbA1C <5.6% define normal glucose tolerance.

IMPAIRED GLUCOSE HOMEOSTASIS:

It can be either Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT). IFG includes individuals with fasting plasma glucose concentrations of 100 to 125 mg/dL (5.6 to <7.0 mmol/L). If an OGTT is performed consequently, some of these individuals will have IGT and some have diabetes. IGT cannot be defined on the basis of fasting glucose concentrations; an OGTT is needed to categorize such individuals. Although this group of individuals is at a higher risk of diabetes all will not develop.

DIABETES MELLITUS:

DM is defined as the level of glycemia at which diabetes-specific complications occur rather than being defined just on the basis of deviations from a population-based mean. The criteria are

1) Symptoms of hyperglycemia *plus* random blood glucose measurements > 200 mg/ dL.

2) Fasting plasma glucose levels > 126 mg/ dL or two hour post load plasma glucose levels > 200 mg/ dL during Oral Glucose Tolerance Test (OGTT).

3) HbA1 c > 6.5 %.

To summarize,

	Fasting plasma glucose level			
	Normal[a]	**Impaired[a]**	**Diabetes**	
2-Hour postload plasma glucose level	**<100 mg/dL (<5.5 mmol/L)**	**100–125 mg/dL (5.6–6.9 mol/L)**	**≥126 mg/dL (≥7.0 mmol/L)**	**Not done**
<140 mg/dL (<7.8 mmol/L)	Normal[b]	IFG	Diabetes	"Normal"
140–199 mg/dL (7.8–11.0 mmol/L)	IGT[b]	IFG/IGT[c]	Diabetes	IGT
≥200 mg/dL (≥11.1 mmol/L)	Diabetes[b]	Diabetes[b]	Diabetes	Diabetes
Not done	"Normal"	IFG	Diabetes	Unknown

HbA1c:

EVOLUTION:

While blood & urine sugar estimation yielded results about the day-to-day glucose levels in the blood, the need for the objective assessment of long term control of diabetes popped up. Then came the discovery that proteins like Hemoglobin (Hb) were non enzymatically glycated in vivo depending on the sugar values. Measuring these glycated proteins especially hemoglobin and serum proteins gave a new dimension in this field. The unique feature in the measurement of these proteins is that they can qualitatively assess the glycemic control over the past few weeks or months complementing the day-to-day testing.

Normal adult hemoglobin is predominantly comprised of HbA (α2β2) (97%) with HbA2 (α2δ2) (2.5%) and HbF (α2γ2) (0.5%) forming minor fractions. In 1958, Allen et al [14] discovered that Hb can be separated into at least three more minor Hb components more negative than Hb by cation exchange chromatography. The fractions were separated based on their electrophoretic properties. These fractions moved fast and were attributed to their glycated nature. Further works by Rabhar et al[14] showed that these minor hemoglobin fractions were elevated in diabetics and hence the link was established.

About 6% of total HbA is glycated in vivo as a function of circulating blood sugar. This is termed HbA1. This has fractions of HbA1a1, HbA1a2, HbA1b and HbA1c. These fractions are differ slightly from the major component HbA0 .They are defined to be the 'fast hemoglobins' as they elude fast in chromatography and migrate fast in electrophoresis. HbA1c makes the most of this fractions. HbA1c, varyingly called as the glucohaemoglobin, glycated hemoglobin has a stable adduct of glucose which is linked by covalent bonds to the N-terminal valine of the β chain.

The processes involved in the formation of HbA1c are as follows:

1) To the N-terminal of the β chain a glucose molecule binds. This glucose molecule is always in the open chain format, forming an aldimine (Schiff base)

2) A more stable ketoamine is formed when this Schiff base undergoes an Amadori rearrangement. It is a non- enzymatic process which happens continuously in vivo.

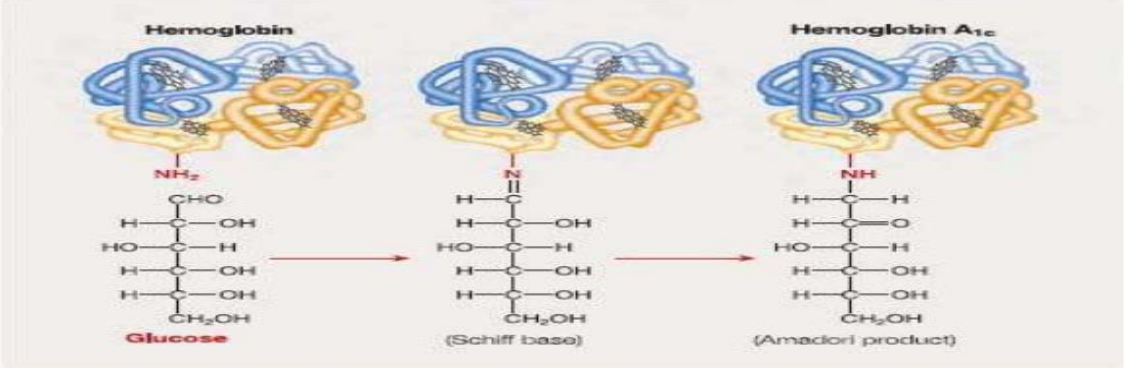


Fig: 12 Steps involved in the formation of HbA1c

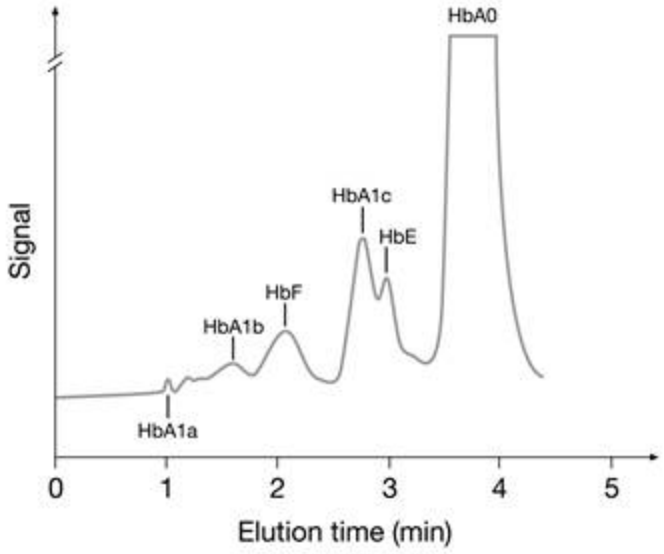


Fig: 13 hemoglobin fractionation in electrophoresis

Hemoglobin glycated at sites other than the N-terminus of the Beta chain can also be formed e.g., epsilon amino groups on lysine residues. These fractions also make up the total glycated hemoglobin.

The concentration of HbA1c depends not only on the concentration of glucose in the blood but also on the life span of the erythrocyte. Because life span of erythrocytes in the blood is approximately 120 days, HbA1c represents the overall picture of the glucose concentration over the preceding 8–12 weeks.

USE:

After the discovery of HbA1c and the process of glycation of proteins, a number of small studies were conducted correlating it to glucose measurements. All these studies established the fact that HbA1c, apart from being used as a

measure of glycemic control could also be used for diagnosis of diabetes. The A1C-Derived Average Glucose (ADAG) study which was conducted in 643 participants having a range of A1C levels is the first of its kind. It could provide a validated relationship between A1C and average glucose levels. This could be applied to a variety of diabetic types and patient populations[16]. HbA1c was recommended for clinical use in the 1980s and subsequently has become a diagnostic tool for DM.

HbA1c reflecting the average levels of plasma glucose over a period of past 3 months can be performed at any time of the day and fasting status of the patient has no bearing on the values measured. As it excludes the influence of day-to-day variability of glucose values, and avoids the need for the person to fast and to have preceding dietary preparations, HbA1c is now the preferred test for assessing glycemic control in people with diabetes. Also these advantages make HbA1c an ideal tool for early identification and treatment which is being strongly advocated in the recent years considering the potential complications and the socio - economic consequences of diabetes. Recognized as the gold standard parameter of diabetic survey now, it was successfully implemented in clinical practice in the 1970s and 1980s and internationally standardized in the 1990s and 2000s. WHO first made a mention of the promising utility of HbA1c in diabetes care in 1985 WHO report.

International Expert Committee has published in 2009 the role of HbA1c in the diagnosis of diabetes[17]. The recommendations are as follows:

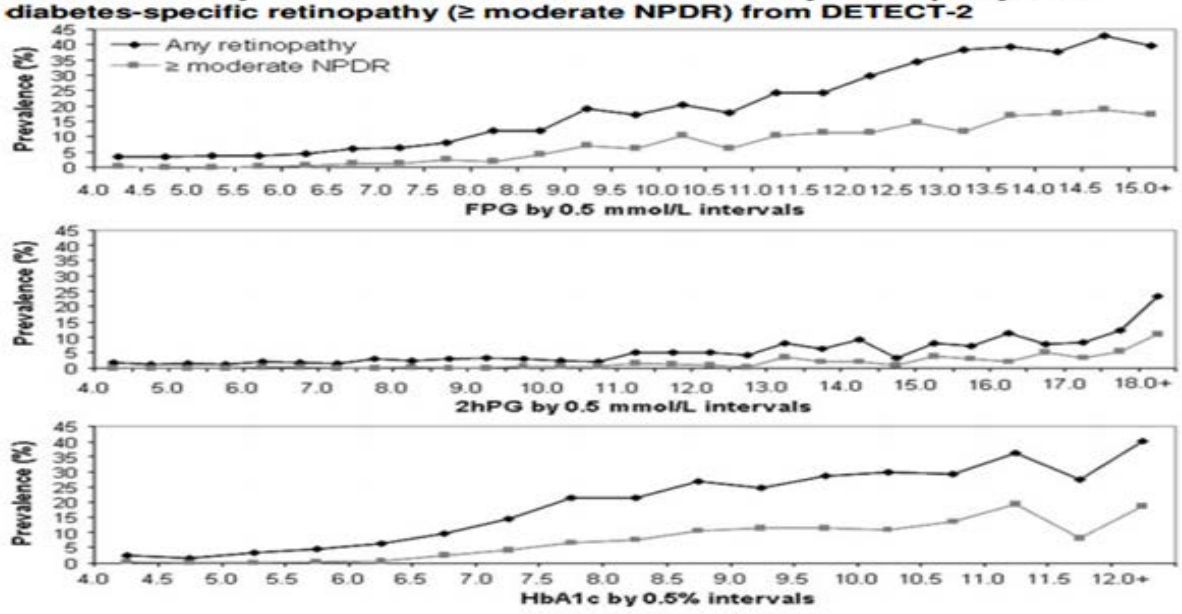
1) HbA1c to be used to diagnose diabetes at levels ≥6.5%.

2) If clinical symptoms and plasma glucose levels >11.1mmol/l (200 mg/dl) are not present, diagnosis should be confirmed with a repeat HbA1c test. A value of 5.7-6.4 % is suggested as the high risk range and levels just below 6.5% indicate the presence of intermediate hyperglycemia, the precise lower cut-off point is yet to be defined. Considering the continuum of risk captured by the HbA1c assay, the International Expert Committee recommended that HbA1c levels between 6.0 and 6.5% were at high risk for diabetes and might be considered for rigorous diabetes prevention interventions.

Larsen et al[18] in his study assigned type 1 diabetic patients into two groups as treatment and control groups. HbA1c testing was done in both groups at specified intervals. But results were informed only to the treatment group. Other than this both groups were essentially identical in their management. They showed that HbA1c was significantly reduced in the treatment group. The awareness of Hba1c alone has led to change in the behavior of the patients and better control. The importance of HbA1c cannot be overstated than this.

Though initially used as an indicator of past glycemic control it became a widely used measure of long standing complications of diabetes as close correlation could be drawn between HbA1c levels and fasting and post prandial glucose levels and micro vascular complication of diabetes. DETECT -2 was the first of the trials to establish such a relation. These findings were reinforced by numerous trials conducted later and it is now established that HbA1c can be used as a marker of micro vascular complication of diabetes. DCCT has also defined the quantitative relationship between HbA1c and sugar values. A 1 % increase in Hba1c is equivalent to 35 mg% increase in plasma glucose[15].

fig:14 **Prevalence of retinopathy by 0.5 mmol/L intervals for FPG and 2-h PG and by 0.5% intervals for HbA1c for any retinopathy and diabetes-specific retinopathy (≥ moderate NPDR) from DETECT-2**



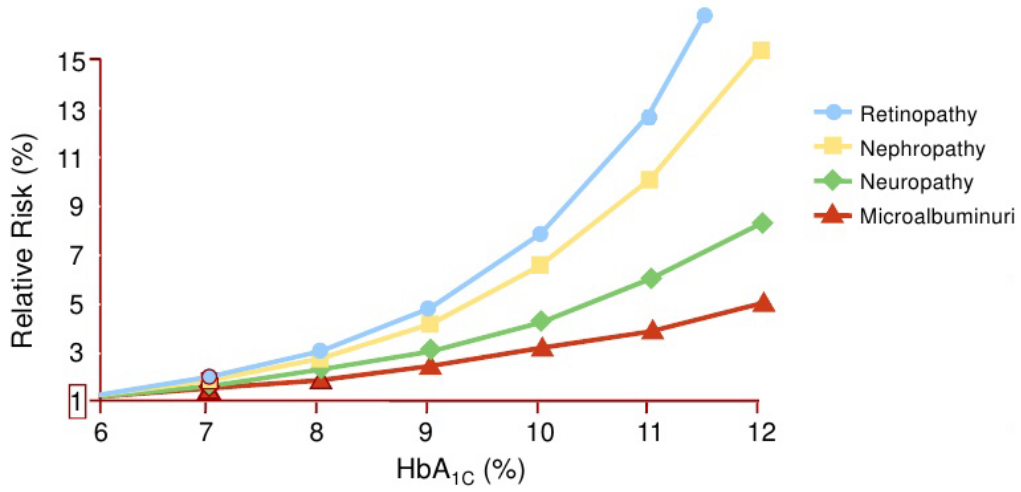


Fig 15: Relationship of HbA1c to risk of micro vascular complications - Diabetes Control Complications Trial (DCCT)[15]

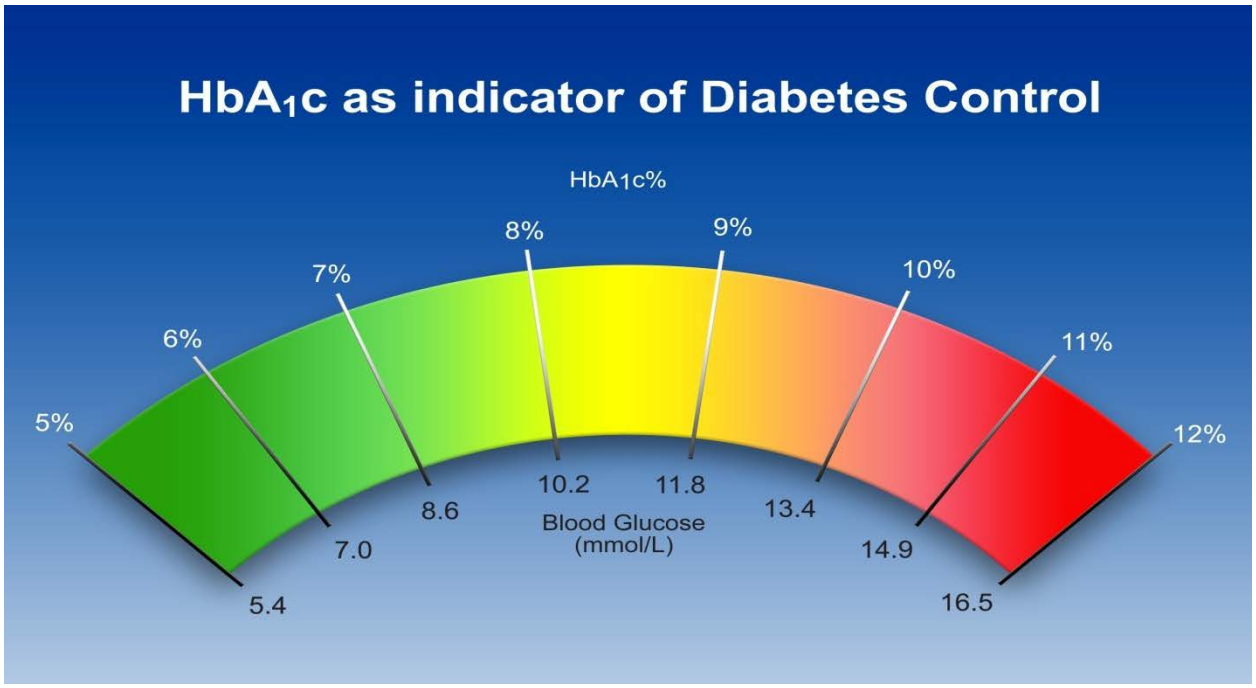


Fig: 16 Pictorial representation of correlation between HbA1c and blood glucose

DISADVANTAGES OF HbA1C:

HbA1c analysis has its own disadvantages. The high cost, non availability, sophisticated instruments used, need for skilled personnel, variability and lack of standardization procedures among different assays are some of them. Apart from the above said technical issues, patient factors, kinetics of HbA1c, genetic, hematologic and illness-related factors altering the values of HbA1c are also known to influence the results[19]. The most common and important factors prevalent worldwide affecting HbA1c levels are hemoglobinopathies (assay dependent), almost all types of anemia's and disorders associated with accelerated red cell turnover such as malaria and hemolytic conditions.

	Glucose	HbA1c
Patient preparation prior to collection of blood	Stringent requirements if measured for diagnostic purposes.	None.
Processing of blood	Stringent requirements for rapid processing, separation and storage of plasma or serum minimally at 4 °C.	Avoid conditions for more than 12hr at temperatures >23C. Otherwise keep at 4C (stability minimally 1 week).
Measurement	Widely available	Not readily available world-wide
Standardization	Standardized to reference method procedures.	Standardized to reference method procedures.
Routine calibration	Adequate.	Adequate.
Interferences: illness	Severe illness may increase glucose concentration.	Severe illness may shorten red-cell life and artifactually reduce HbA1c values.
Haemoglobinopathies	Little problem unless the patient is ill.	May interfere with measurement in some assays.
Haemoglobinopathy traits	No problems.	Most assays are not affected.
Affordability	Affordable in most low and middle income country settings.	Unaffordable in most low and middle-income country settings.

Fig: 17 Advantages and disadvantages of assays for glucose and HbA1c

FACTORS AFFECTING HbA1c ANALYSIS[19]:

In detail, some of the factors influencing HbA1c measurement are as follows:

1. Erythropoiesis

Increased HbA1c: Iron deficiency, Vitamin B12 deficiency, conditions associated with decreased erythropoiesis.

Decreased HbA1c: Erythropoietin administration, Iron and Vitamin B12 supplementation, reticulocytosis and chronic liver disease.

2. Altered Hemoglobin

Genetic and chemical alterations in hemoglobin structure: hemoglobinopathies, HbF, methaemoglobinemia,

3. Glycation

Increased HbA1c: Alcohol ingestion, deranged renal parameters, decreased intra erythrocyte pH.

Decreased HbA1c: Aspirin, vitamin supplementations mainly E and C, hemoglobinopathies, and increased intra-erythrocyte pH.

4. Erythrocyte destruction

Increased HbA1c: Splenectomy (causing prolonged RBC survival.)

Decreased A1c : hemoglobinopathies, splenomegaly, rheumatoid arthritis or antiretroviral drugs ribavirin and other drugs like dapsone causing decreased RBC survival.

5. Assays

Increased HbA1c: Jaundice, carbamylated haemoglobin, chronic opiate use.

Decreased HbA1c: hypertriglyceridemia.

RECENT ADVANCES:

There are a number of methods available for HbA1c assay. The most commonly used are cation exchange chromatography, immuno assay, affinity chromatography and capillary electrophoresis. The principle in all assays is the difference in charge between HbA0 and HbA1c and the differences between glycated and non glycated forms of hemoglobin. The laboratories should use an assay with inter assay coefficient of variation of <4% (ideally<3%) and it should be calibrated against DCCT standards. The DCCT standard is a high performance liquid chromatography.

COMPLICATIONS OF DIABETES MELLITUS:

Apart from the life threatening acute complications like Diabetic Kato Acidosis and Hyperosmolar non Ketotic coma, the most difficult part in the management of diabetes is the development of long-term complications and their consequences. Complications can be either vascular or non vascular. Vascular complications can be either disease specific micro vascular complications like retinopathy, nephropathy or neuropathy or less specific macro vascular complication of increased atherosclerosis.

There are episodic complications that can be treated only to re -occur numerous times (e.g., infections, ulcers and other skin diseases) or progressive with relentless course (e.g., nephropathy). They usually begin with mild severity but progress over time resulting in damage to the organ and irreversible loss of functionality. Other complications include dental disease, reduced resistance to infections such as influenza and pneumonia, macrosomia and other birth complications among pregnant women with diabetes. Both type 1 and 2 diabetic complications share similar complications but frequency or onset timing can vary and severity has no relation to types of diabetes.

MECHANISMS OF MICROVASCULAR COMPLICATIONS:

Sorbitol accumulation through aldose reductase mediated activation of polyol pathway and consequent osmotic stress is the major mechanism behind pathogenesis. Protein kinase C activation, formation of advanced glycosylation end (AGE) products through non enzymatic reaction, oxidative stress from accelerated free radical production and reactive oxygen species formation resulting in cellular injury are the postulated mechanisms responsible for formation of micro aneurysm, pericyte loss and thickened basement membrane . Another mechanism involved is the presence of excessive growth factors, including vascular endothelial growth factor (VEGF), transforming growth factor β and growth hormone probably secondary to hypoxia.

RETINOPATHY:

This is the most common micro vascular complication of diabetes. Milder form of this disease includes formation of micro aneurysms, hemorrhages with lipid accumulation appearing in the margin of hemorrhages collectively called background / Non Proliferative diabetic Retinopathy (NPDR). These lesions do not cause visual impairment unless when located near macula. Over time, micro vascular changes progresses to Proliferative Diabetic retinopathy (PDR). Capillary block causes retinal ischemia leading to new vessel formation. These vessels are

fragile which may bleed causing profound vision loss. The subsequent fibrotic tissue formation leads to retinal detachment. Duration of diabetes, uncontrolled hyperglycemic status, concomitant hypertension, and smoking are all linked to the development and progression of retinopathy[20]. Non proliferative retinopathy warrants yearly follow up while proliferative retinopathy needs photocoagulation as treatment.

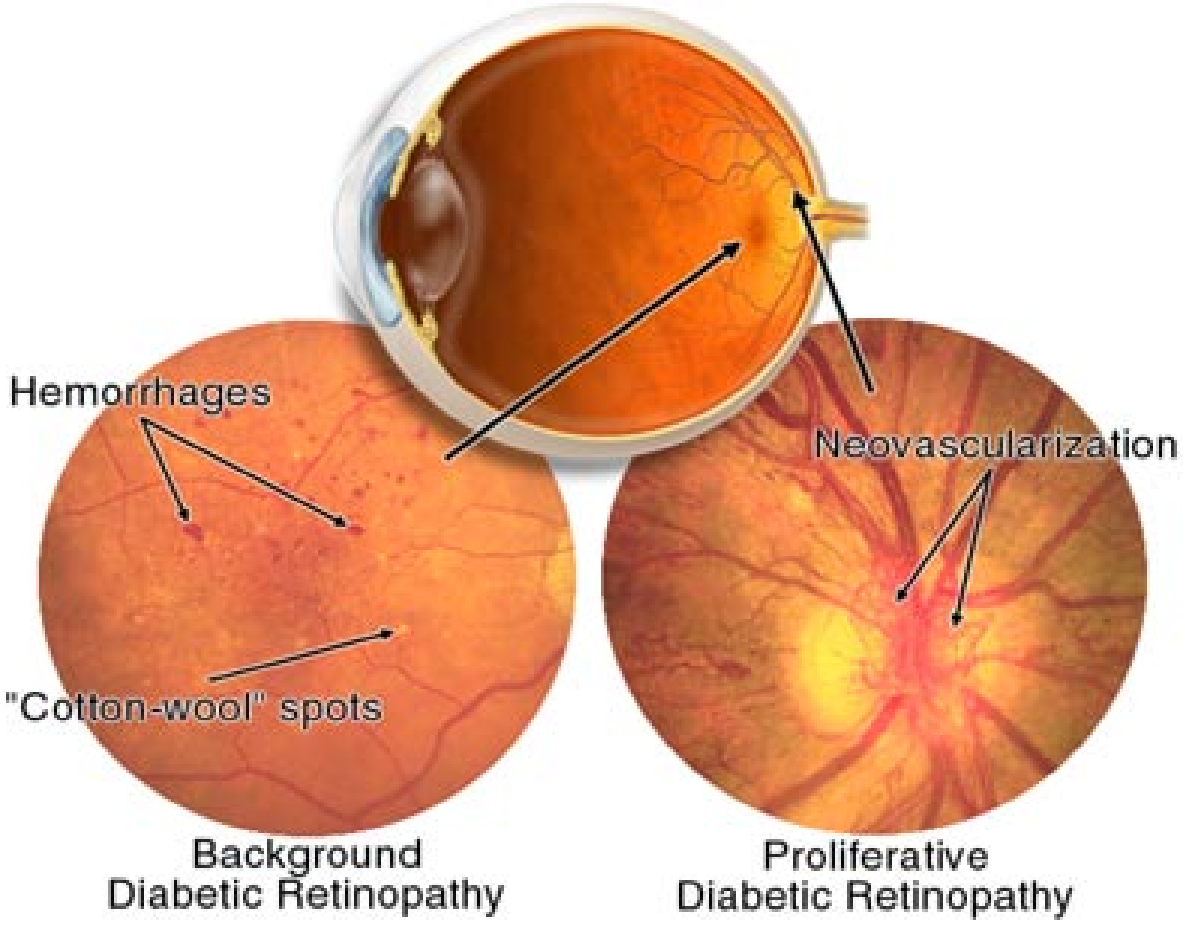


Fig: 18 Diabetic retinopathy- Proliferative and non proliferative

NEPHROPATHY:

This is the most common cause of End Stage Renal disease requiring dialysis in the western world. The hallmark of diabetic nephropathy, proteinuria predominantly albuminuria is a result of continuum of changes from normo albuminuria to microalbuminuria and macro albuminuria culminating in the development of End Stage Renal disease (ESRD)[21]. The pathological changes noted in the kidney are increased glomerular basement membrane thickness, formation of micro aneurysm, Kimmelsteil-Wilson bodies- mesangial nodule formation. The underlying pathophysiology is the same as that for retinopathy involving AGE products, polyol pathway activation, increased oxidative stress and protein kinase C activation. The prevalence of diabetes is directly proportional to the duration of diabetes with incidence around 10 % in the first years to 50 % after two decades. Uncontrolled hyperglycemias, duration of diabetes are important factors contributing to the development of nephropathy. Hypertension is another factor the effect of which as a cause or consequence remains to be settled. Male gender, smoking, increased protein intakes are the other factors postulated to play a role in the pathogenesis. Treatment with drugs blocking RAAS system like ACE inhibitors, Angiotensin Converting Enzymes, Direct Renin blockers have shown to delay the progression of diabetic nephropathy in Type 2 Diabetic Patients but not in type 1 DM.

NEUROPATHY:

Neuropathic complications of diabetes occur in approximately 50 % of the population with long standing diabetes. Any type of neuropathy like mononeuropathy, polyneuropathy, mononeuritis multiplex, autonomic polyneuropathy can occur. The pathogenesis of neuropathy is same as that for retinopathy and nephropathy. The symptoms can also mimic any other form of neuropathy. So it is always diagnosed after excluding other causes of neuropathy. Also the symptoms can be a spectrum ranging from no symptoms to mild paresthesias to grossly disabling.

A sensation of numbness, sharp pricking/burning pain, tingling sensation , that begins typically in the feet spreading proximally are the usual symptoms. Neuropathic pain is characteristically present at rest and gets worse at night. This pain may gradually subside and even eventually disappear when all the sensory neurons die. The sensory deficit usually persists with loss of ankle reflexes and abnormal position sense. Autonomic neuropathy manifests as anhydrosis, gastro paresis, diarrhea, erectile dysfunction, orthostatic hypotension, silent myocardial ischemia and even death. As with any other micro vascular complication, the duration of diabetes and hyperglycemia are the proposed mechanisms behind neuropathy.

Table 2: Diabetic Neuropathy classification[5]

General diabetic neuropathies
Symmetric polyneuropathies:
- Acute sensorimotor polyneuropathy[a]
  Chronic sensorimotor polyneuropathy[a]
  Autonomic polyneuropathy[a]

Mononeuropathies:
- Cranial nerves III, VI, VII (ischemic)
- Thoracoabdominal
  Focal limb (*ex*-femoral)
  Proximal motor (amyotrophy)
- Inflammatory demyelinating

Painful diabetic neuropathies
Acute painful neuropathies:
- Distal sensory[a]
- Thoracic radiculopathy (ischemic)
- Lumbar nerve root/plexus (ischemic)
- Insulin neuritis

Chronic painful neuropathies:
- Small fiber distal[a]
- Large fiber distal[a]
- Compressive mononeuropathies[a]
  Carpal tunnel
  Ulnar (cubital tunnel)
  Common peroneal nerve[a]
- Proximal inflammatory demyelinating

[a] Neuropathy most frequently presenting to foot & ankle providers.

Of the many types chronic sensory motor distal symmetrical poly neuropathy is the most common type. Least common is the sensory type associated with episodic hyperglycemia. No drug has been proved in curing neuropathy though drugs like anti epileptics, tricyclic anti depressants are used. Optimization of glycemic control delays the progression.

MACROVASCULAR COMPLICATIONS OF DIABETES:

The central mechanism of macro vascular complication of diabetes is the process of accelerated atherosclerosis as a result of increased oxidative stress, increased platelet adhesion , hypercoaggulabilty, reduced nitric oxide formation and altered calcium homeostasis.

CORONARY ARTERY DISEASE:

Diabetes is an independent risk factor for ischemic heart disease. Cardio vascular disease is the leading cause of mortality and morbidity in diabetics. The risk of myocardial infarction in a diabetic is equal to that risk in a non-diabetic individual who had MI so American Diabetes association has recommended considering diabetes as coronary artery disease risk equivalent than as a mere risk factor. This risk is high in women especially in post menopausal group when compared to men. Studies have shown that unlike micro vascular complications, stringent glycemic control did not alter macro vascular complications[20].

TREATMENT:

ADA has proposed stringent guidelines for treatment of diabetes. Glycemic Targets for No pregnant Adults with Diabetes are pre-prandial capillary PG 80-130 mg/dL; Peak postprandial capillary PG <180 mg/dL and HbA1c values <7.0%.Individualized treatment goals are adopted based on Age/life expectancy, Co morbidities and known cardiovascular diseases, duration of Diabetes, episodes of hypoglycemia if any.

LIFESTYLE MODIFICATIONS:

They form the principal modality of treatment irrespective of the stage and type of the disease. Abstinence from smoking and alcohol, healthy eating pattern, achieving and maintaining ideal body weight through medical nutrition therapy, aerobic exercises delays or even prevents the complications of diabetes. Also attainment of individualized lipid, BP goals help in delaying the onset of complications.

PHARMACOLOGICAL THERAPY FOR TYPE 1 DM:

Insulin remains the most important part of the treatment. Patients are ideally treated with 3-4 daily doses of basal and prandial insulin. Newer insulin analogues

prevent dangerous hypoglycemic episodes. The other agents that can be used are pramilinitide (an amylin analog). Metformin, incretins like GLP-1 analogues, DPP4 inhibitors, and SGLT-2 inhibitors are also being investigated to be used in type 1 DM.

PHARMACOLOGICAL THERAPY FOR TYPE 2 DM:

If not controlled by lifestyle modifications, metformin is the ideal initial agent of choice. Sulfonylureas, Thiazoledinidiones, GLP- 1 analogues are all added if metformin monotherapy fails to achieve desired glycemic target. Insulin is used if the patients are severely symptomatic at presentation or have very high values of blood sugar on detection. Also insulin remains the mode of treatment in times of acute stress like surgeries, severe infections, Diabetic keto Acidosis or Hyper Osmolol Non Ketotic Coma.

ANAEMIA:

Anemia is a condition in which either RBCs or their oxygen carrying capacity to meet the physiological needs is reduced. The needs may vary according to age, sex, growth phases, altitude above sea level, pregnancy status and thus varying cut offs are used to define anemia in various subgroups.

EPIDEMIOLOGY OF ANAEMIA:

Anemia is a major public health problem that affects populations in both rich and poor countries. It poses major consequences on human health as well as social and economic development. It is generally present in people in all stages of the life cycle, but is more prevalent in pregnant women and young children. Globally, iron deficiency remains the most significant contributor to the onset of anemia as to the extent that Iron Deficiency Anemia (IDA) and anemia are often used synonymously[23]. Even the prevalence of anemia has often been used as a proxy for IDA.

Though the proportion of cases of anemia contributed by iron deficiency is exactly estimated in only few countries, it is generally assumed that 50% of the cases are due to iron deficiency. The proportion varies among different population groups and in different areas with local conditions influencing it. The increased prevalence of IDA is due to a number of risk factors rampant in the economically under- privileged and even in economically well developed countries like low intake of iron, phytate rich diet causing reduced iron absorption, and period of life when iron requirements are especially high like pregnancy, early stages of growth, puberty not being given adequate iron supplementations. The presence of co-existent micronutrient deficiencies, including vitamins A and B12, folate,

riboflavin, and copper aggravates the problem. Numerous other factors can be enlisted to be contributing significantly to anemia:

1) Poor purchasing power, illiteracy, ignorance regarding nutritional value of available cheap food

2) Cultural taboos, superstition and parasitic infestations of hookworms, ascariasis, and schistosomiasis

3) Discrimination faced by women eating only the 'last food' or the remaining food, heavy blood loss during menstruation.

4) Acute infections like malaria and chronic infections including cancer, tuberculosis, and HIV can also lower blood Hb concentrations.

5) The presence of hemoglobinopathies in certain populations[23].

As the optimal values of Hb varies among people in different phases of life, WHO used various Hb thresholds to classify individuals living at sea level as anemic. The threshold and the prevalence of anemia are as follows:

Table 3: Hb threshold used to define anemia according to WHO[23]

Age or gender group	Haemoglobin threshold (g/l)
Children (0.50–4.99 yrs)	110
Children (5.00–11.99 yrs)	115
Children (12.00–14.99 yrs)	120
Non-pregnant women (≥15.00 yrs)	120
Pregnant women	110
Men (≥15.00 yrs)	130

Table 4: Global prevalence of anemia and number of population affected

Population group	Prevalence of anaemia		Population affected	
	Percent	95% CI	Number (million)	95% CI
Preschool-age children	47.4	45.7–49.1	293	283–303
School-age children	25.4	19.9–30.9	305	238–371
Pregnant women	41.8	39.9–43.8	56	54–59
Non-pregnant women	30.2	28.7–31.6	468	446–491
Men	12.7	8.6–16.9	260	175–345
Elderly	23.9	18.3–29.4	164	126–202
Total population	**24.8**	**22.9–26.7**	**1620**	**1500–1740**

Estimates from various countries were combined to calculate the estimates at the global level. This pooled data were also extended to the WHO region for women and preschool-age children by considering the weight given by these estimates to the population it represented. South East Asia and Africa top the WHO regions in anemia prevalence with values of 45.7% & 47.5% respectively.

INDIAN SCENARIO:

While WHO estimates provide a clue about the global picture, data from NFHS (National Family Health Survey -3) provide estimates about the burden IDA poses on the country. In India, anemia is more than twice prevalent in women than men. Half of these anemic women suffer from moderate to severe anemia. As expected, anemia is much more prevalent among pregnant women. In Adolescents and school- going children group, half of them are anemic. Though anemia is a common problem of both urban and rural areas, rural areas have a marginally higher percentage. But thanks to the various measures by the government and the spread of education, the prevalence of severe anemia is decreased. Iron deficiency remained the single most common cause of anemia.

Table 5: Prevalence of anemia according to severity in urban and rural population according to NFHS -3 data [12]

Anemia level	NFHS III			NFHS II		
	Urban	Rural	Total	Urban	Rural	Total
Mild (10.0 – 10.9 g/dl)	25.8	25.7	25.7	23.7	22.7	22.9
Moderate (7.0-9.9 g/dl)	42.0	51.7	49.4	42.0	47.1	45.9
Severe (< 7.0 g/dl)	4.4	3.5	3.7	5.1	5.5	5.4
Any anemia (< 11.0 g/dl)	72.2	80.9	78.9	70.8	75.3	74.3

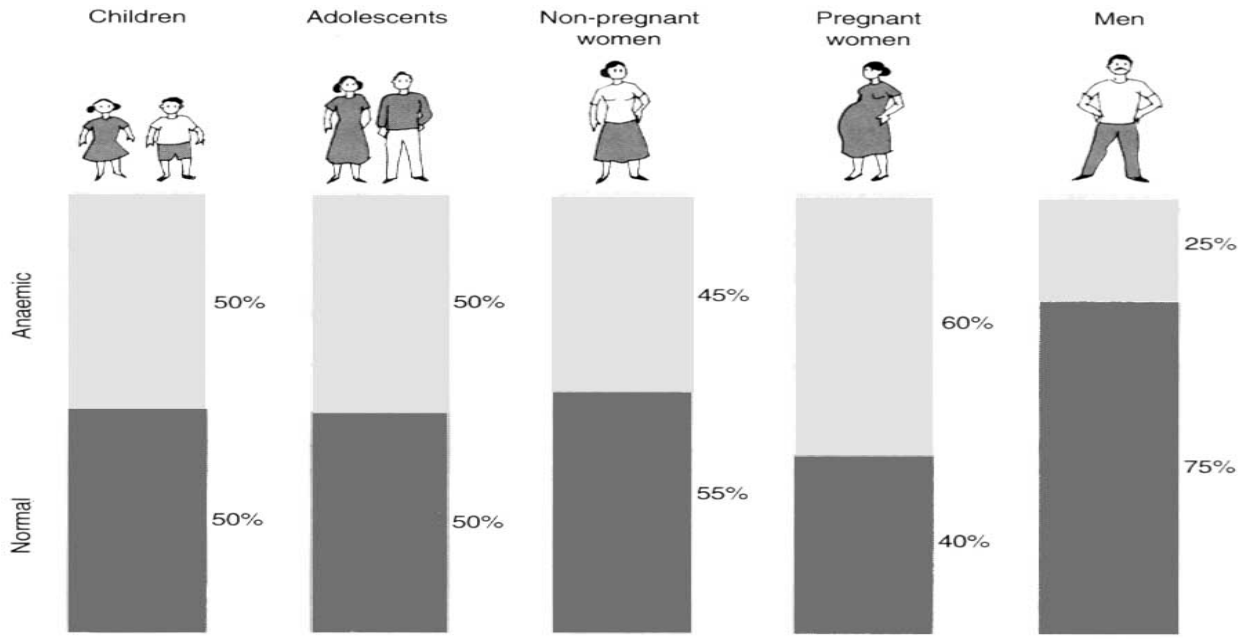


Fig 19: Pictorial representation of the prevalence of anemia according to NFHS -3 data[12]

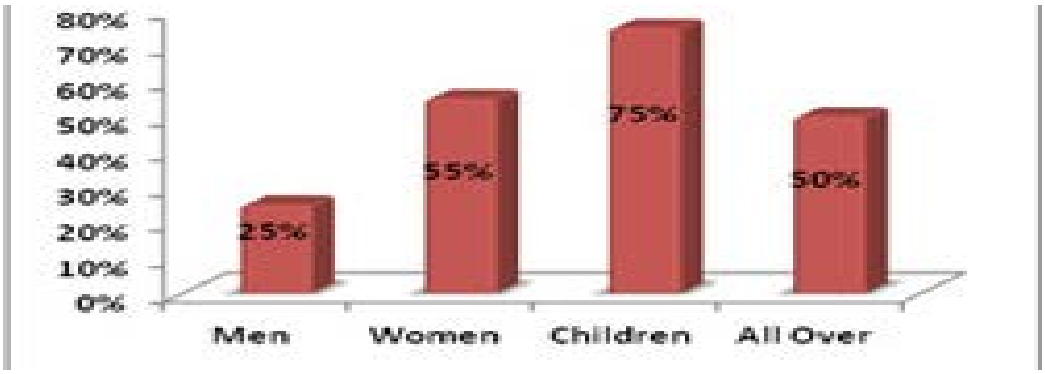


Fig 20: Prevalence of iron deficiency anemia according to NFHS 3[12]

CLINICAL MANIFESTATIONS:

The various symptoms of anemia results from hypoxia and the body's response to it. A variable presentation ranging from asymptomatic to mild symptoms of lassitude to severe symptoms of tachycardia with palpitations and pounding in the ears, headache and even classical pain of angina pectoris may all occur in patients who are severely anemic.

CLINICAL MANIFESTATIONS THAT MAY BE UNRELATED TO ANEMIA

- Decreased work performance
- Headache
- Paresthesias and other neurologic symptoms
- Oral And Nasopharyngeal Symptoms
- Dysphagia
- Menstrual bleeding
- Pica: Restless Legs
- Hair Loss

CLASSIFICATION OF ANAEMIA:

Anemia can result from increased RBC destruction (hemolysis), decreased production or blood loss. This serves as a basis for classification of anemia. Also the next important criteria for classification is the ability of the bone marrow to compensate for anemia quantified as Reticulocyte count which is usually <1 % Combining these two parameters, anemias are classified as follows:

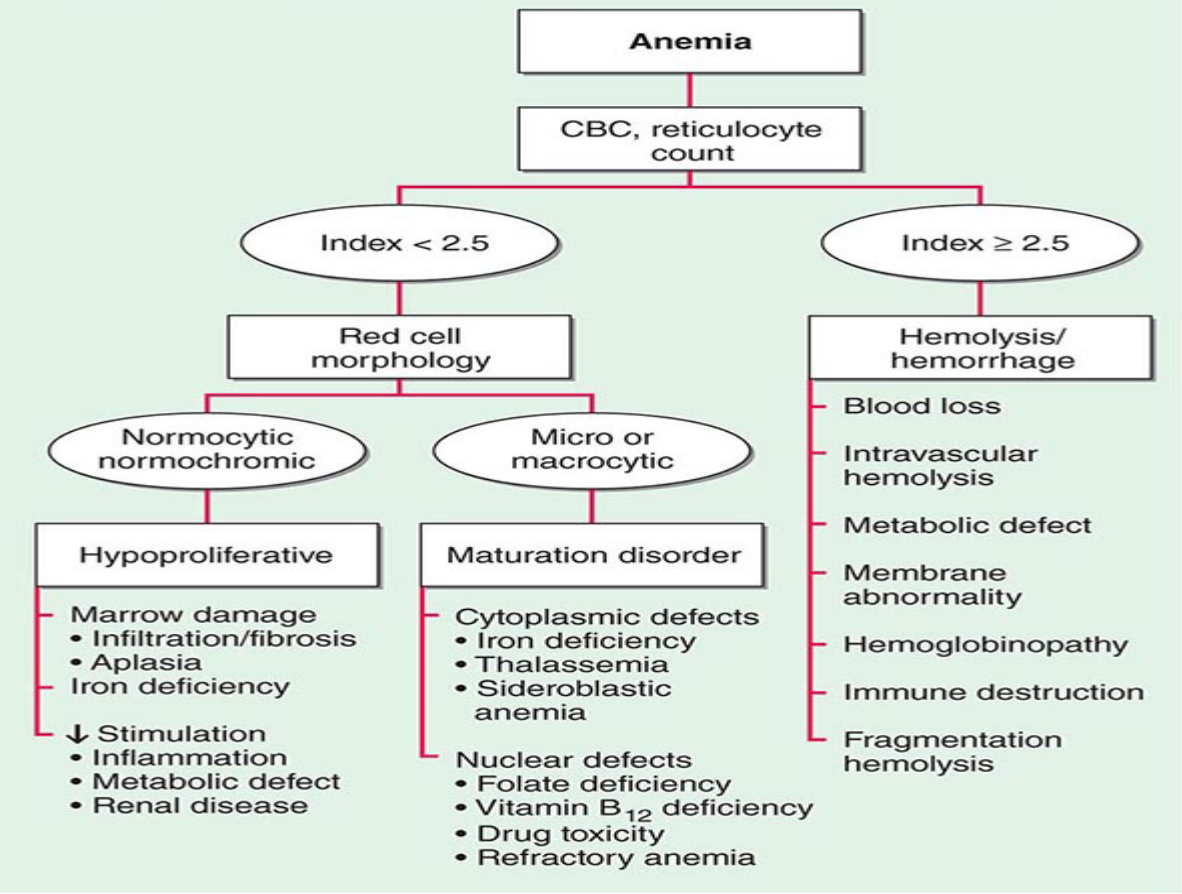


Fig: 21 Algorithm for the physiologic classification of anmeia[5]

The morphological classification of anemia as normocytic, microcytic, or macrocytic often correlates with the cause of anemia. The degree of hemoglobinisation reflected in the color of the cells (normochromic or hypochromic)-and the shape of the cells are the other indices used to classify anemia. Visual inspection of peripheral smears can be used as a subjective measure of classifying anemia while they are quantitatively expressed through the following RBC indices:

- *Mean cell volume* (MCV): average red cell volume, expressed in femto liters (fL).
- *Mean cell hemoglobin* (MCH): the average hemoglobin content (mass) per red cell, expressed in picograms (pg)
- *Mean cell hemoglobin concentration* (MCHC): the average hemoglobin concentration in a given volume of packed red cells, expressed in grams per deciliter (g/dL) A lower MCH and MCHC show hypochromia.
- *Red cell distribution width* (RDW): the coefficient of variation of red cell volume. Red cell volume distribution width (RDW) reflects the variation in the size of Red Blood Cells.

Table 6: Normal adult Red Blood Cell indices[25]

	Units	Men	Women
Hemoglobin (Hb)	g/dL	13.6-17.2	12-15
Hematocrit (Hct)	%	39-49	33-43
Red blood cells(RBC)	$*10^{6/}$ mm^3	4.3-5.9	3.5-5.0
Reticulocyte count			
Mean corpuscular volume(MCV)	fL	76-100	76-100
Mean corpuscular Hemoglobin (MCH)	Pg	27-33	27-33
Mean corpuscular Hemoglobin concentration (MCHC)	g/dL	33-37	33-37
Red cell distribution width (RDW)		11.5-14.5	11.5-14.5

A lower than normal MCV (<80) - *Microcytosis*

A higher MCV (>100) - *macrocytosis*.

A lower MCH and MCHC values indicate under hemoglobinoisation-*hypochromia*

Thus microcytic hypochromic anemia is defined as a condition where predominantly RBCs which are small and under hemoglobinized circulate in the blood. Most important cause is Iron Deficiency Anemia.

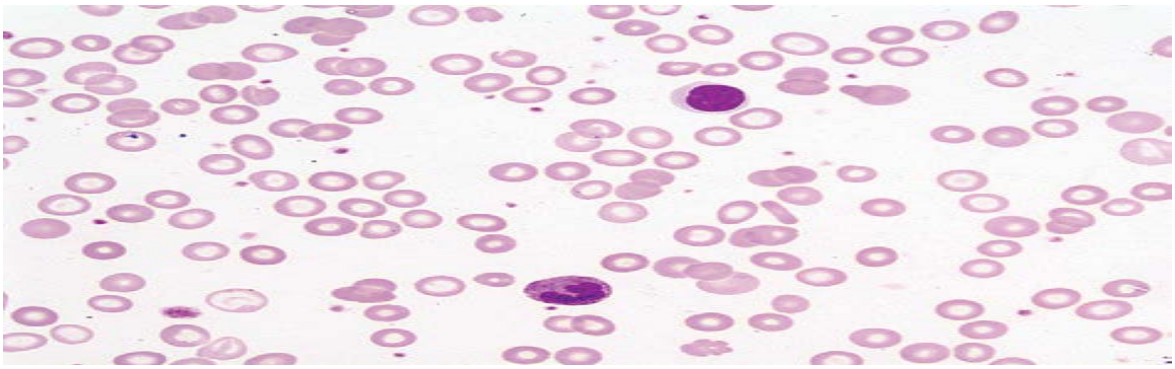


Fig: 22 Microcytic Hypochromic anemia

But there are also other causes which can cause microcytic hypochromic anemia.

Table: 7 List of conditions associated with microcytic anaemia[24]

Thalassemia & Hemoglobinopathies
B-Thalassemia Major
B-Thalassemia Minor
δB-Thalassemia Major
α -Thalassemia Major
HbE trait
Hb H disease
Homozygous Hb E disease
Blockade of heme synthesis caused by chemicals
Lead
Pyrazinamide
Isoniazid
Other disorders
Sideroblastic anemia
Hereditary sex linked
Idiopathic acquired
Anemia of chronic disease
Hb Lepore trait

BACK GROUND FOR THE STUDY

There are a number of studies analyzing the effect of microcytic anemia on HbA1c measurements. These studies have come up with conflicting results. Some have shown a positive correlation while some have shown a negative correlation.

Kim et al[26] conducted a study where the effect of iron deficiency on HbA1c distribution among non diabetic adults was investigated. This study spanned over a period of 7 years of the National Health and Nutrition Examination Survey (NHANES). A cut point of < 5.5% vs ≥5.5% was used to study the distribution of HbA1c values as the recommended cut point was <6.5% vs. ≥6.5%. Of the 6,666 participants, the prevalence of Iron Deficiency Anemia (IDA) was almost equal in the male and female populations at 33% and 30% respectively though iron deficiency was much more common in females at 13.6% when compared to 1.6% in males. The HbA1c values were 5.33% in iron deficient population and 5.27% in non-iron deficient population. The difference in these values were stastically significant (p=0.002) after adjusting for ethnicity and sex. Women belonging to reproductive age group had an increase in mean HbA1c from <5.5% to 5.5–6.0%. Thus the authors were of the conclusion that after adjusting for age and ethnicity, the HbA1c were elevated in iron-deficient individuals.

Ford et al[27] also evaluated 1999–2002 NHANES data. The Iron deficient anemic population and iron sufficient participants who were both diabetic and non diabetic were included in the study. Non-diabetics with low Hb but sufficient iron levels had reduced HbA1c values (5.16%) than the participants who had normal values of both Hb and iron (5.31%), $p<0.001$. Thus this study identified a positive correlation between low Hb and HbA1c. They could also identify an inverse correlation between iron levels and HbA1c. HbA1c values were raised in participants with normal Hb but deficient iron stores. This was stastically significant from HbA1c value in normal counterparts (5.39% [35 mmol/mol], $p=0.061$). All non diabetic participants with higher values of Hb had a higher HbA1c values. The mean HbA1c was 5.18% (33 mmol/mol) at Hb <100 g/l while it was 5.50% (37 mmol/mol) at Hb >170 g/l. The largest impact was seen in HbA1c <5.0% group. They inferred that caution should be exercised when using HbA1c values near diagnostic cut points in the setting of anemia. Hb values were expected to change HbA1c a value by approximately 0.2 % (2.2 mmol/mol).This change is more noticeable in especially at the two ends of the spectrum of Hb levels.

Hardikar et al[28] study is the first of its kind in India. Young adults belonging to Pune Children's Study cohort were the participants. This cohort follows children born between 1987 and 1989 in the King Edward Memorial

Hospital (KEMH), Pune. This study made a comparison between HbA1c and OGTT determined diabetes rates in this cohort and scrutinized the hematological, nutritional and other factors influencing HbA1c concentration. Approximately one third of the population was anemic .43.6% had microcytic anemia while 2.5% had macrocytic anemia. More number of people was diagnosed with diabetes or prediabetes when an HbA1c criterion was used than by the gold standard OGTT (25.9% vs. 10.4%). The iron deficiency was much more prevalent and ferritin levels low in the participants classified byHbA1c as prediabetic or diabetic when compared with the normal group. The hematological parameters that were relevant to higher HbA1c levels were erythrocyte microcytosis, low MCH, low MCHC, and high RDW. The author proposed that there was an increased potential for misdiagnosis of diabetes and prediabetes in iron-deficient populations when HbA1c values alone are used. Iron deficiency induced alterations in the quaternary structure of the hemoglobin molecule and increased glycation of the b-globin chain was the likely explanation offered yet the role of uninvestigated genetic and environmental factors, influencing erythrocyte dynamics could also have been a contributing factor.

Son and others [29]conducted a study in 329 non diabetic Korean participants. After performing OGTT, participants were categorized as normal, prediabetics or diabetics. HbA1c levels in anemic and non-anemic participants were then

compared. In the normoglycaemic group, HbA1c values were not different in anemic and non-anemic groups. Anemic participants of the prediabetes group had higher HbA1c values when compared with controls (p=0.05). There was a borderline significance in the diabetes group. The investigators could not hypothesize as to why decreased erythropoiesis and altered red blood cell life span affect glycosylation of hemoglobin. A drop in the specificity of HbA1c to 81.1% was noted in the anemic group than in the non anemic group. Though the sample size was small and anemia types were unclassified, the authors could still establish a variation in HbA1c.

Shanthi[30] and others studied the difference between HbA1c values in 50 individuals with Iron deficiency anemia and nonanemic healthy controls both of them not known to have diabetes previously. Though there was no stastically significant difference between the fasting and post prandial glucose values a difference could still be made out between the HbA1c values of IDA and non anemic healthy groups. HbA1c values were significantly higher in the IDA group (mean 7.6±0.5% [60±5.5 mmol/mol]) compared with the control group (5.5± 0.8% [37±8.7 mmol/mol]; p<0.001. The authors differed in their hypothesis for the increased HbA1c in Iron Deficiency Anemia from others leading to a reduced half-life of erythrocytes than elongations proposed by others though there was a

concurrence in the idea of Iron deficiency changing the quaternary structure of Hb molecule increasing glycation.

As the studies stated above have tried for establishing the difference in the HbA1c values in anemic and non anemic population, upcoming reviews are the reviews of the studies which after evaluating the difference in the levels of HbA1c proceeded for evaluating the effects of treatment/ iron supplementation to resolve anemia and aftermath of the supplementation on HbA1c.

Coban and others'[31] study comprised of 100 participants - 50 had IDA while the rest 50 were healthy controls chosen after matching for age and sex. Patients with IDA were given oral supplementation of ferrous sulphate 100 mg a day for 3 months. HbA1c values dropped significantly after iron treatment ($p<0.001$).This study suggests that iron deficiency must be corrected before using HbA1c for making a diagnostic or therapeutic decision

Sinha et al[32] came up with a study which contradicted the other results in that HbA1c and absolute HbA1c levels increased after correction of anemia. This study conducted in Lok Nayak hospital, New Delhi included administering iron supplementation to 50 participants with IDA and serial monitoring of the erythrocytic indices while the erythrocytic indices were measured only once in controls. A noteworthy attempt of estimation of absolute HbA1c was made in this study. Though the authors could not correct the anemia completely, mean HbA1c

after 2 months (5.9±0.6%) had significantly raised from the baseline ($p<0.01$).A significant increase was observed in the absolute HbA1c levels at 2 months after treatment (0.29g/dl vs. 0.73 g/dl, $p<0.01$).This study found decreased HbA1c levels at baseline and a rise in HbA1c with iron supplementation; these results were in complete contrast to the majority of other studies. The results were not explained. .

While the above mentioned studies analyzed the relation between (HbA_{1c}) and iron status in type 2 diabetes mellitus (DM) Tarim et al [35] investigated the relationship between hemoglobin A_{1C} (HbA_{1c}) and iron status in type 1 diabetes mellitus. This study also included HbA1c analysis *in diabetic patients who had insufficient iron stores.* All iron deficient patients were treated with iron at 6 mg/kg per day for a period of 3 months. The study's conclusions were that among type 1 DM patients with similar level of glycemia, iron deficiency anemia is associated with higher concentrations of HbA_{1c}. This study also established that iron replacement therapy leads to a drop in HbA1c *values in* both diabetic and non-diabetic patients and the consideration of the iron status before making any therapeutic decision based on HbA1c holds good in type 1 DM also.

Having conflicting results from previous studies, this study was principally conducted to establish the role of microcytic hypochromic anemia on Hba1c levels in non diabetic individuals if any.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was done at Government Royapettah Hospital, Chennai for a period of six months. The study was done after procuring informed written consent from all the participants involved. Clearance was obtained from the Ethical Committee of the Government Kilpauk Medical College & Hospital, Chennai.

STUDY DESIGN

The study design is a case - control study.

POPULATION

The study population included 100 people who attended the Dept. of Internal Medicine OPD in Govt. Royapettah Hospital and inpatients in the same hospital.

INCLUSION CRITERIA

50 non diabetic patients known to have microcytic hypochromic anemia are chosen as cases and 50 healthy age and sex matched individuals are chosen as controls. HbA1c levels in both the populations are compared.

Microcytic hypochromic anemia is defined as: Hb--< 13 g/dL in males; < 12g/dL in females; RBC count< 4,50,000 in males; < 3,50,000 in females; MCH< 27 pg & MCHC< 33g/dL ;MCV<76 fl having microcytic hypochromic anemia in peripheral smear study.

Healthy controls are defined as non anemic population with normal red cell indices and normal peripheral smear study.

EXCLUSION CRITERIA

- Confirmed cases of diabetes with one or more of the following: H/O diabetes or taking medications, H/o symptoms related to diabetes with FBS>126 mg/dL & PPBS >200 mg/dL.
- H/O acute blood loss, hemolytic anemia, hemoglobinopathies, recent surgery.
- H/O kidney disease, liver disease or any major systemic illness.
- H/O pregnancy and people with amenorrhea testing positive in urinary pregnancy test.
- H/O endocrinopathy with effects on glucose tolerance
- H/O blood transfusion in the past two months
- Patients with blood urea levels greater than 20 mg/dL or with serum creatinine levels greater than 1.2 mg/dL (male patients) or 0.9 mg/dL (female patients) were excluded.

METHODOLOGY:

50 non diabetic patients known to have microcytic hypochromic anemia were taken as cases. Age- matched healthy participants were taken as the controls. Both were chosen from the inpatient and outpatient departments of the hospital. A detailed history regarding age, sex, occupation, diet, menorrhagia and anthropometrical measurements made.

After obtaining their consent, blood was drawn and the following parameters done:

HEMOGLOBIN LEVELS:

MCV:

MCH:

MCHC:

RDW:

PLATELETS:

ERYTHROCYTES:

WBC:

SERUM FASTING AND POSTPRANDIAL GLUCOSE LEVELS:

SERUM CREATININE:

HBA1C:

ABSOLUTE HBA1C

Evaluation of the Hemogram was done using sysmax automated cytology analyzer. HbA1c was analyzed using turbidimetric immunoinhibition.

STASTICAL ANALYSIS

STASTICAL ANALYSIS

The results obtained were analyzed using SSPS software.

For all practical purposes, anemia defined in the analysis is to be considered as microcytic hypochromic anemia as defined above and healthy as healthy age and sex matched controls.

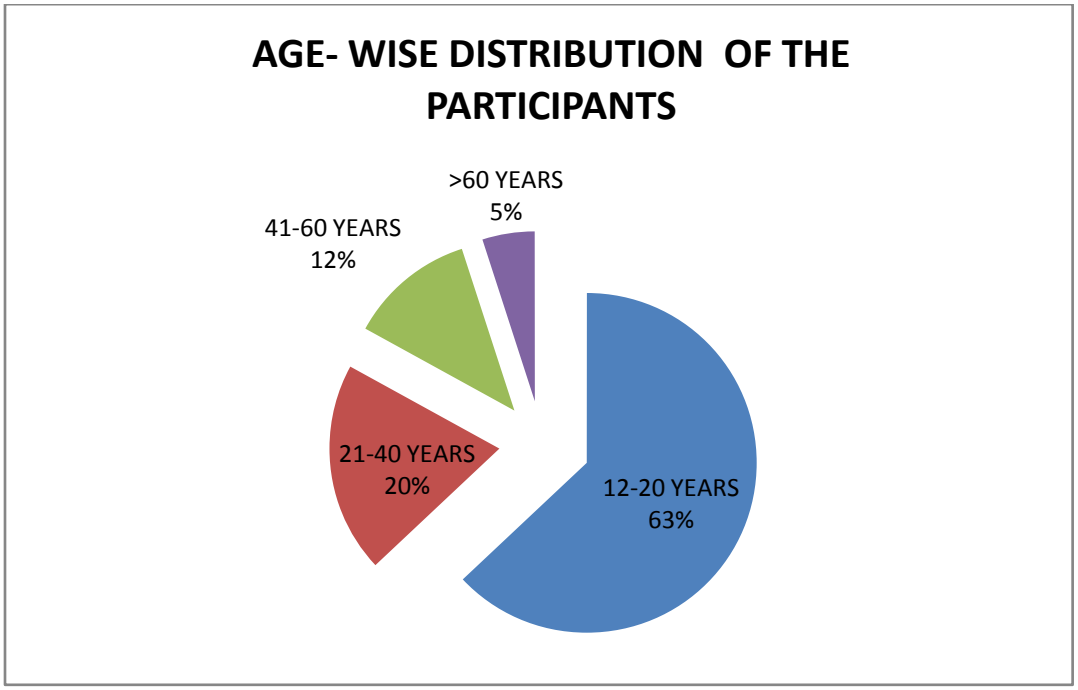
ANEMIA- DISTRIBUTION AND CHARACTERISTICS

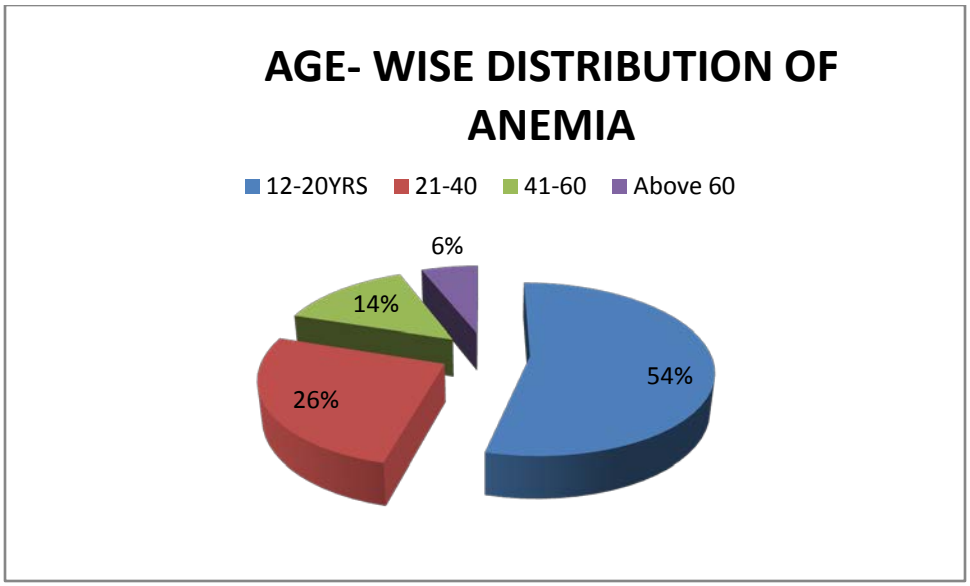
AGE DISTRIBUTION AND MICROCYTIC HYPOCHROMIC ANEMIA

AGE GROUP(years)	ANEMIA		TOTAL	P VALUE
	ANEMIC	HEALTHY		
12-20	27 (42.9%)	36 (57.1%)	63 (63%)	≥0.05
21-40	13 (65%)	7 (35%)	20 (20%)	
41-60	7 (58.3%)	5 (41.7%)	12 (12%)	
Above 60	3 (60%)	2 (40%)	5 (5%)	

The analysis shows:

- Of the 100 participants(50 cases and 50 controls) in the study, the highest percentage of participants 63% are in the age group of 12-20 years, 20% are in the age group of 21-40 years, 12% are in the 41-60 years age group and 5% are in the age group of above 60 years.
- There are 27 anemic patients in the 12-20 age group, 13patients in the 21-40 age group , 7 patients in the 41-60 years and 3 anemic patients in the age group of above 60 years.
- There are 36 healthy persons in the 12-20 age group, 7 healthy persons in the 21-40 age group, 5 healthy persons in the 41-60 years and 2 healthy persons in the age group of above 60 years.





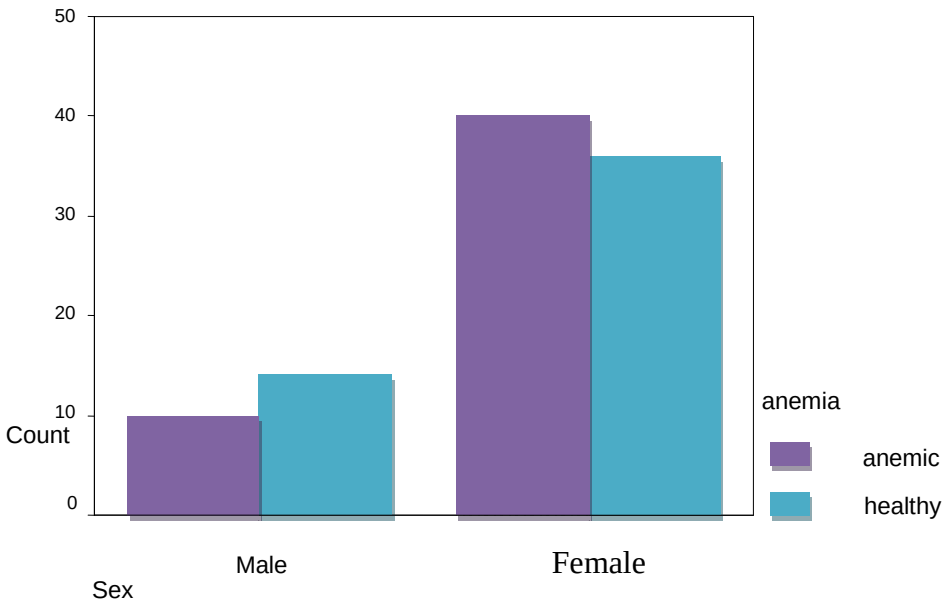
SEX DISTRIBUTION AND MICROCYTIC HYPOCHROMIC ANEMIA

.

SEX	ANEMIC	HEALTHY	TOTAL
Male	10 (41.7%)	14 (58.3%)	24 (24%)
Female	40 (52.6%)	36 (47.4%)	76 (76%)

- Out of the 100 participants in the study, 76 are females and 24 are males.
- Out of the 24 male participants, 10 (41.7%) are anemic and the rest 14(58.3%) are healthy controls
- Out of the 76 female participants, 40(52.6) are anemic and the rest 36(47.4%) are healthy controls.

- Male anemic patients -10 make up 20.0% of the total anemic population while 40 female anemic patients make up the rest.
- This analysis can be shown pictorially as



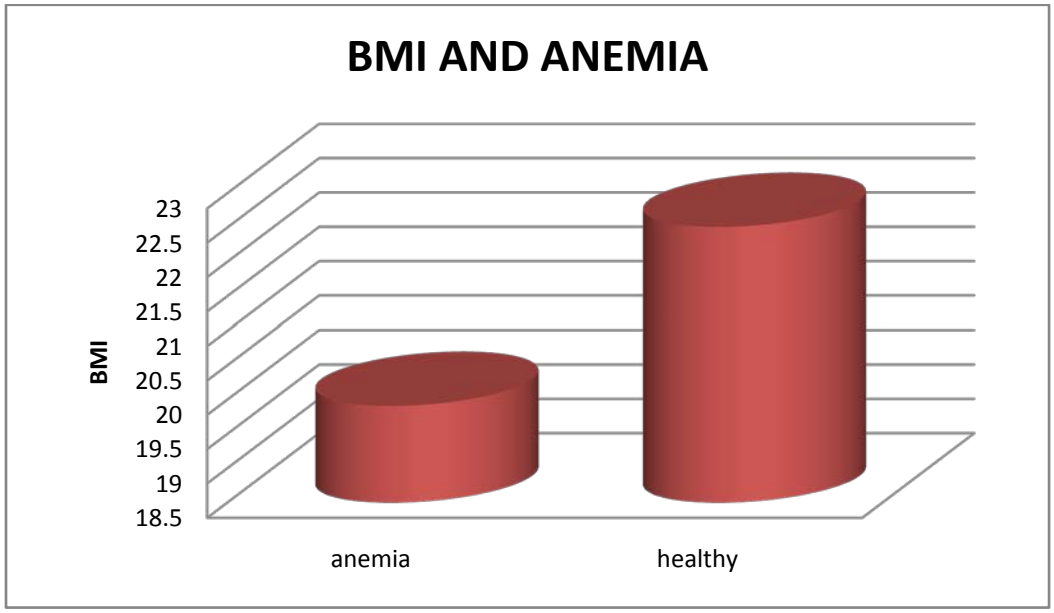
BMI AND ANEMIA

	ANEMIA	MEAN±S.D	P VALUE
BMI	Anemic	19.91±3.7	< 0.001
	Healthy	22.51±3.0	

From the analysis,

- The mean BMI in the anemic population is 19.91 with S.D .3.7
- The mean BMI in the healthy controls is 22.51 with S.D. 3.0

- The difference in the mean BMI between two groups is stastically significant (p <0.001)
- It is pictorially represented as



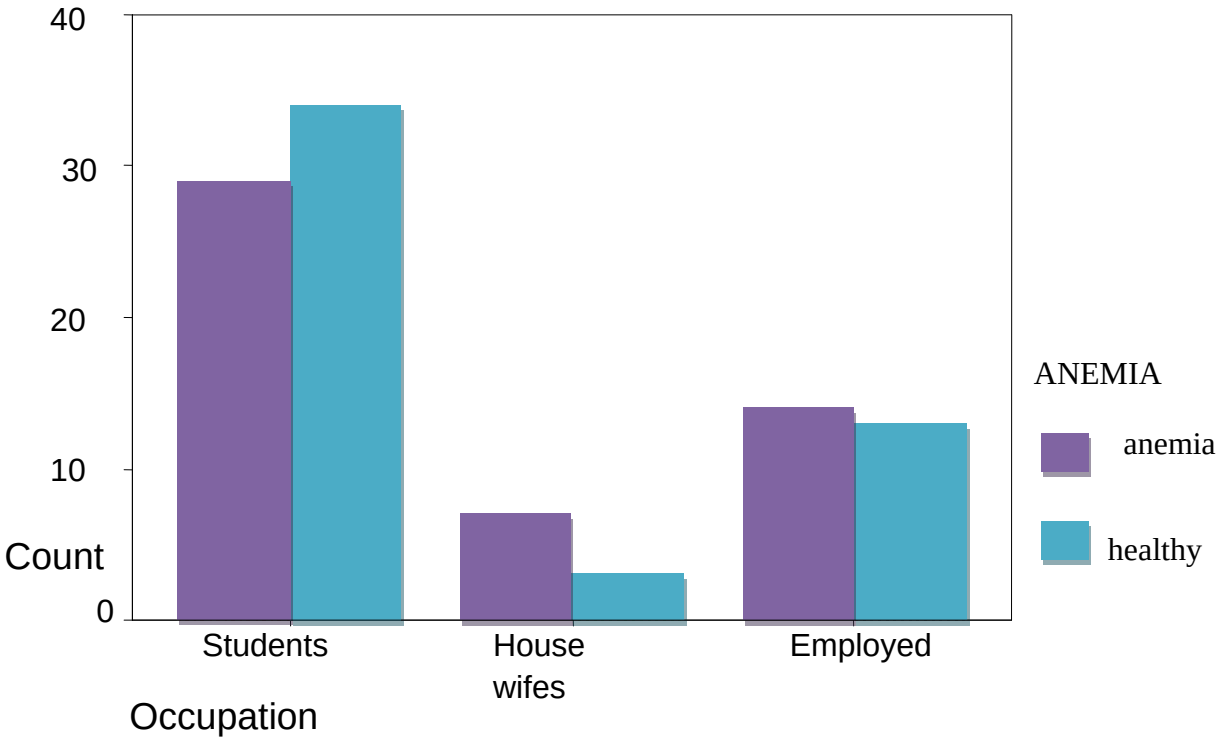
OCCUPATION AND MICROCYTIC HYPOCHROMIC ANEMIA

OCCUPATION		ANEMIA	
		ANEMIC	HEALTHY
Student	Count	29	34
	% within Occupation	46%	54.0%
	% within anemia	58.0%	68.0%
House wife	Count	7	3
	% within Occupation	70.0%	30.0%
	% within anemia	14.0%	6.0%
Employed	Count	14	13
	% within Occupation	51.9%	48.1%
	% within anemia	28.0%	26.0%

The analysis of results shows that

- Majority of the participants of the study are students (63%). Out of these 63 students, 29 (46%) have anemia.
- The next majority of the group involved is the employed people (27%) with 14 (51.9%) being anemic and the rest 13(48.1%) healthy.
- The anemic patients of these two groups constitute a total of 43 (86 %) cases of anemic patients included in the study.

THE DISTRIBUTION OF ANEMIA IN VARIOUS OCCUPATIONS:



MENORRHAGIA AND MICROCYTIC HYPOCHROMIC ANEMIA

MENORRHAGIA		ANEMIA	
		ANEMIC	HEALTHY
Yes	Count	25	22
	% within Menorrhagia	53.2%	46.8%
	% within anemia	62.5%	61.1%
No	Count	15	14
	% within Menorrhagia	51.7%	48.3%
	% within anemia	37.5%	38.9%

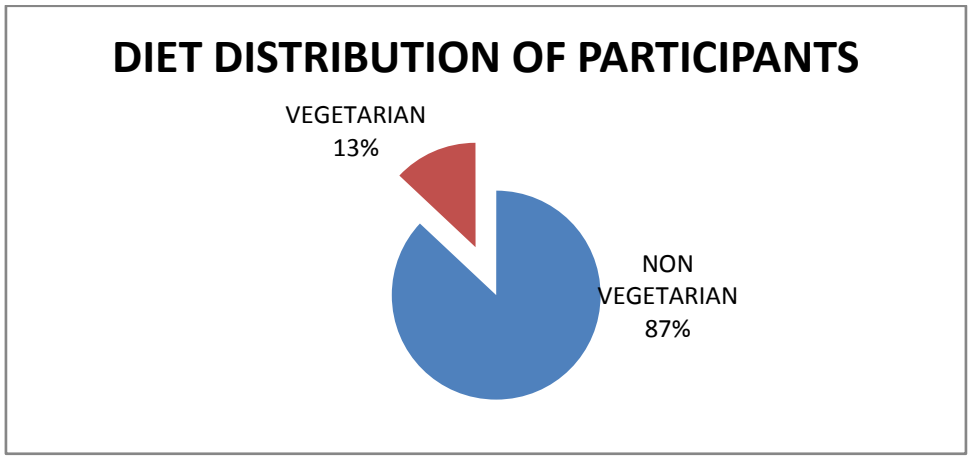
- The results on analysis show that out of the 76 female participants, 47 have menorrhagia
- Out of the 47 menorrhagic participants, only 25(53.2%) had anemia while the rest 22(46.8%) are healthy.
- But out of 29 participants who did not have menorrhagia, 15(51.7%) are anemic.
- The anemic menorrhagic participants 25 constitute 62.5 % of the total anemic patients.
- The association between menorrhagia and anemia is not stastically significant.

DIET AND ANEMIA

DIET	ANEMIA		TOTAL	P VALUE
	ANEMIC	HEALTHY		
Vegetarian	8 (61.5%)	5 (38.5%)	13	>0.05
Non Vegetarian	42 (48.3%)	45 (51.7%)	87	

The analysis of results shows the following:

- In the study, non vegetarian participants far outnumber the vegetarians.
- Out of the 87 non vegetarians, 42 (48.3%) are anemic while 45 (51.7%) are non anemic.
- Out of the 13 vegetarians, 8 (61.5%) are anemic.
- The results can be pictorially represented as



RED CELL PARAMETERS AND ANEMIA

SEX	ANEMIA			
	ANEMIC		HEALTHY	
	COUNT	MEAN (millions/cu. mm)	COUNT	MEAN (millions/cu. mm)
Male	9	3.01	15	4.75
Female	20	2.81	56	4.03

- The mean Red Blood Corpuscles (RBC) count in anemic population- male is 3.01 million/ mm^3
- The mean Red Blood Corpuscles (RBC) count in anemic population-female is 2.81 million/ mm^3
- The mean Red Blood Corpuscles (RBC) count in healthy male population is 4.75 million/ mm^3
- The mean Red Blood Corpuscles (RBC) count in healthy female population is 4.03 million/ $mm^{3.}$

SEX	Hb			
	ANEMIC		HEALTHY	
	COUNT	MEAN (g/dL)	COUNT	MEAN (g/dL)
Male	10	7.72	14	14.21
Female	40	7.26	36	13.51

The analysis of results shows that

- The mean value of Hb in anemic male population is 7.72 g/dL.
- The mean value of Hb in anemic female population is 7.26 g/dL.
- The mean value of Hb in healthy male population is 14.21 g/dL.
- The mean value of Hb in healthy female population is 13.51 g/dL.

		Mean±S.D	P VALUE
MCV(fL)	Anemic (n=50)	69.62±3.17	P<0.001
	Healthy(n=50)	91.20±3.67	
MCH(pg)	Anemic (n=50)	26.77±5.562	P<0.001
	Healthy (n=50)	30.50±2.71	
MCHC(g/dL)	Anemic (n=50)	38.50±7.93	P<0.001
	Healthy (n=50)	33.50±3.19	
RDW	Anemic (n=50)	19.65±2.88	P<0.001
	Healthy (n=50)	13.21±0.68	

- The mean value of Mean Corpuscular Volume (MCV) in anemic population is 69.92 fl & healthy population is 91.20 fl
- The mean value of Mean Corpuscular Hemoglobin (MCH) in anemic population is 26.7 pg & in healthy population is 30.5 pg.
- The mean value of Mean Corpuscular Hemoglobin concentration (MCHC) in anemic population is 33.5 g/dL & in healthy population is 38.5 g/dL.
- The mean value of Red Cell Distribution width (RDW) in anemic population is 19.65& in healthy population is 13.21.
- The difference in the parameters between the groups is significant

OTHER BLOOD CELL PARAMETERS AND ANEMIA

	Hb	MEAN ±S.D.	P VALUE
PLATELETS (lakhs/mm^3)	Anemic	3.22±0.83	P>0.05
	Healthy	2.95±0.94	
WBC (cells/mm^3)	Anemic	6950.00±1792.76	
	Healthy	6796.00±1752.08	

- The above table shows the mean platelets in anemic population is 3.22 lakhs/mm^3 which is not stastically significant from that of the mean platelets in healthy population which is 2.95 lakhs/mm^3.

- This table shows the mean White Blood Cell (WBC) count in anemic population 6,950cells/mm^3 not differing significantly from that of the healthy population's value of 6,796 cells / mm^3.

FASTING, POST PRANDIAL BLOOD SUGAR AND ANEMIA

	ANEMIA	N	MEAN	S.D	P VALUE
FASTING BLOOD SUGAR (FBS) (mg/dL)	Anemic	50	78.72	10.14	>0.05
	Healthy	50	81.14	7.81	
POSTPARANDIALBLOOD SUGAR (PPBS) (mg/dL)	Anemic	50	124.68	8.96	
	Healthy	50	126.32	8.43	

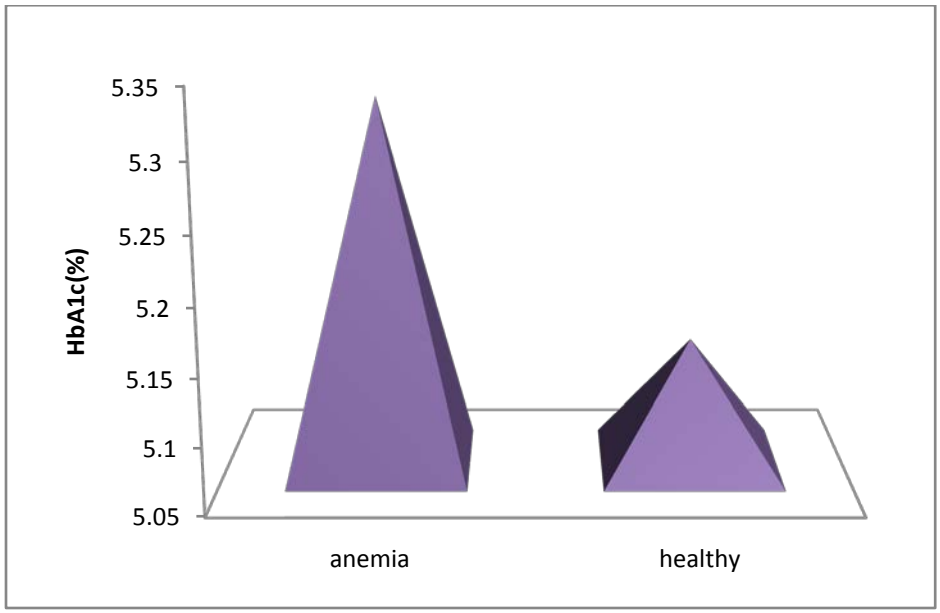
From the results, it can be inferred that

- The mean fasting blood sugar in the anemic population is 78.72 mg/dL and healthy population is 81.14 mg/dL.
- The mean post prandial blood sugar in the anemic population is 124.68 mg/dl and the healthy population is 126.32 mg/dl.
- It is to be noted that FBS and PPBS did not significantly vary between anemic and healthy groups.

ANEMIA AND HbA1c

	ANEMIA	N	MEAN	S.D	P VALUE
HBA1C(%)	Anemic	50	5.3248	.32798	0.043
	Healthy	50	5.1440	.53190	

- The above table shows the mean HbA1c values in anemic and healthy population.
- It shows that mean Hba1c in anemic population is 5.32%±0.32. The mean Hba1c in healthy non anemic population is 5.14%±0.53.
- The difference in two mean values is statistically significant. ($p<0.05$)



ANEMIA AND ABSOLUTE HbA1c

	ANEMIA	N	MEAN	S.D	P VALUE
Absolute HBA1C	Anemic	50	.6372	.09474	>0.05
	Healthy	50	.7042	.07299	

- It shows that mean absolute HbA1c in anemic population is0.63±0.09.The mean absolute HbA1c in healthy non anemic population is 0.70±0.07.
- The difference in two mean values are not statistically significant (p>0.05).

DISCUSSION

DISCUSSION

Demographic and clinical characteristics of our study population are that majority of the anemics (27 %) are in the 12- 20 years age group and least in the group above 60 years. This is in concordance with the study conducted by Ramesh Chellan et al[37].He has stated that severe anemia is highly prevalent in India and the prevalence is highest in adolescent girls. Majority of the individuals involved were females, with 76 participants being female. This is also in concordance with the studies conducted by Segun et al and Ramesh Chelan.

The predominant groups of anemic individuals involved were students- adolescent females. But no stratification of severity of anemia was attempted in this study. Hence the prevalence of severe anemia in this group of individuals could not be studied.

The study comprised of 76 female and 24 male participants. The increased number of female participants included in the study can be explained by the wide prevalence of anemia among women due to various reasons.

Menorrhagia was not a significant contributor to anemia in this study as only 25 anemic women had menorrhagia. The rest of the 22 women with menorrhagia were healthy. Similarly 15 anemic patients involved in this study had no menorrhagia. This is in contrary to the study by Chappal A, according to whom menorrhagia is a significant contributor to anemia in women of reproductive age.

The lower mean BMI in the anemic group can be explained as this study included patients with moderate to severe anemia (<10 g% Hb) and the cause and effect of anemia on BMI could be taken as the probable explanation.

The two groups - students and employed people having a higher percentage of anemia reflect the burden of anemia on the work force the society. The participants included in the study belonged to the out-patient and inpatient department of our hospital. They can be taken as a representative sample of the general population. Thus the results can be generalized to the population.

The mean Hb of anemia in male and female population is 7.72 g/dL and 7.26 g/dL respectively. These values were much lower than when compared to the mean Hb values calculated inthe studies of Shanthi et al , Segun et al, Hardikar et al. Of these studies comparison with Shanthi et al study is significant as it was conducted in the same city representing a similar population. Much lower values of Hb can be explained on the basis of involvement of more people from lower socio- economic status and the study population involving Inpatients who are probably severely anemic.

The mean MCV values were 69.6 fl and 91.2fl in anemic and healthy population respectively. These were almost similar to the values of 72.3 fl and 84.2 fl observed in Shanthi et al study. It was different from the mean value of MCV which was 85 fl and 87.8 fl in Hardikar et al study. However all the studies had involved population with microcytic hypochromic anemia.

The mean MCH in anemic and healthy population was 26.7 pg and 30.5 pg respectively. The mean MCH observed were 27.9, 22.6 and 28.6, 32.9pg in the above studies.

The difference in the mean HbA1c values between anemic and healthy population was stastically significant (P value 0.043) .This is in concordance with the observation by Shanthi et al, Hardikar et al, and Son et al. They had all observed a modest increase of HbA1c in anemic population. They postulated that iron deficiency causes increased glycation leading to spuriously high values of HbA1c. This is different from the observation by Nitin Sinha et al and Ford et al who have shown that mean HbA1c is decreased in anemic population.

Absolute HbA1c values were not significantly different between anemic and healthy participants. This is in concordance with the observation by Sinha et al . It was the first and only study which has correlated absolute HbA1c and anemia and that has been confirmed by the present study.

CONCLUSION

- There is no stastically significant association between age and anemia in this study.
- Anemia group has significantly lower mean BMI than healthy group.
- There is no stastically significant association between menorrhagia and anemia in the participants of the study.
- There is no significant association between dietary habits and anemia though the proportion of anemic patients among the vegetarian group is high.
- All the anemic patients included in the study were microcytic hypochromic anemic patients. The Hb, MCV, MCH, MCHC, RBC count values in anemic patients were low when compared to the healthy controls and the difference was stastically significant.
- RDW indicating poikilocytosis was higher in the anemic patients as compared to healthy controls.
- There was a significant difference in the mean values of HbA1c between anemic and healthy populations. Mean value of HbA1c in anemic population was significantlyhigher than the mean Hba1c value in healthy controls at p value 0.043 . This can cause diagnostic difficulties in detecting uncontrolled diabetes mellitus

in iron deficient diabetic population.

- Absolute HbA1c values corrected for anemia did not differ significantly in two groups.
- Fasting and post prandial blood sugar values did not differ significantly in both the groups. Thus the difference in HbA1c could be attributed to the presence of microcytic anemia.

LIMITATIONS OF THE STUDY

- The sample size was small.
- Majority of the individuals involved in the study was younger population. A study involving equal distribution of participants in various age groups would have thrown light upon other factors operating in the elderly individuals.
- Number of females outnumbered the males in this study.
- A confirmatory test for iron deficiency like serum ferritin was not applied in this study. A broad label of microcytic hypochromic anemia is used.
- The study was a case control study. No effort was made at correcting anemia and henceforth the changes in the trend were not observed.

  Further studies involving a larger population where the limitations of this study are duly taken into consideration are needed

BIBLIOGRAPHY

1. WHO/ facts and figures about diabetes; http://www.who.int/diabetes/facts/en/

2. INDIA- DIABETES CAPITAL OF THE WORLD; http://www.neeman-medical.com/sites/default/files/files/India%20-20Diabetic%20Capital%20of%20World.pdf

3. STASTICS ABOUT DIABETES; http://www.ndei.org/ADA-diabetes-management-guidelines-diagnosis-A1C-testing.aspx.

4. Hinzmann R, Schlaeger C, Tran CT. What Do We Need beyond Hemoglobin A1c to Get the Complete Picture of Glycemia in People with Diabetes?. International Journal of Medical Science 2012; 9(8) p 665-681.

5. Harrison's principles and practice of internal medicine -18th and 19th edition

6. DIAGNOSIS AND CLASSIFICATION OF DIABETES MELLITUS http://care.diabetesjournals.org/content/36/Supplement_1/S67

7. Joslin's Textbook of Diabetes mellitus- 14 th edition

8. Wild et al - GLOBAL PREVALENCE OF DIABETES Estimates for the year 2000 and projections for 2030 SARAH WILD, MB BCHIR , DIABETES CARE, VOLUME 27, NUMBER 5, MAY 2004

9. V. Mohan, S. Sandeep, R. Deepa EPIDEMIOLOGY OF TYPE 2 DIABETES: INDIAN SCENARIO, *Indian J Med Res 125, March 2007, pp 217-230*

10. The Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study;*Journal of diabetes science and technology* 07/2011; 5(4):906-14.

11. Mohan V Why are Indians more prone to diabetes? J Assoc Physicians India. 2004 Jun;52:468-74. .

12. NATIONAL FAMILY HEALTH SURVEY - 3; FACT SHEETS FOR KEY INDICATORS BASED ON FINAL DATA.

13. Adult Metabolic Syndrome and Impaired Glucose Tolerance Are Associated With Different Patterns of BMI Gain During Infancy.Data from the New Delhi birth cohort; *Diabetes Care* ;December 2008vol. 31 no. 12 p 2349-2356 .

14. David e. goldstein, MD; TESTS OF GLYCEMIA IN DIABETES; *diabetes care*, volume 27, number 7, july 2009.

15. DCCT and EDIC: The Diabetes Control and Complications Trial and Follow-up Study-1983-1993; http://www.niddk.nih.gov/

16. Richard Kahn, PHD - Translating the A1C Assay; *Diabetes Care August 2008 vol. 31 no. 8 1704-1707*

17. dnathan@partners.org- International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes; Diabetes Care. 2009 Jul;32(7):1327-34. doi: 10.2337/dc09-9033. Epub 2009 Jun 5.

18. Joseph Balatbat -Glycated (Glycosylated) Hemoglobin: HbA1c New directions to diagnose diabetes; *Continuing Education Topics & Issues*; 112 August 2010

19. elizabeta.topic@gmail.com; Guidelines and recommendations for testing in diagnosis of diabetes mellitus: The role of HbA1c. Biochemia *Medica 2014*;24(Suppl 1):S17-S20.

20. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study *BMJ 2000*; 321 doi:bmj.321.7258.405 Cite this as: BMJ 2000;321:405

21. David M. Nathan- Long-Term Complications of Diabetes Mellitus *N Engl J Med 1993*; 328:1676-1685 UK Prospective Diabetes Study Group; Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38*; BMJ. 1998 Sep 12; 317(7160): 703–713.*

22. PK Thomas - Classification, Differential Diagnosis, and Staging of Diabetic Peripheral Neuropathy; ADA - *Diabete*s September 1997vol. 46 no. Supplement 2

23. WHO GLOBAL DATABASE ON ANAEMIA- Worldwide prevalence of anaemia 1993–2005;

24. Williams HEMATOLOGY- SEVENTH EITION

25. Robbins Basic Pathology 8 th edition

26. Catherine Kim, M.D. M.P.H Association between iron deficiency and HbA1c levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999–2006; *Diabetes Care* January 12, 2010.

27. Earl S. FORD; Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US; *Journal of Diabetes* 3 (2011) 67–73 2011.

28. PALLAVI S. HARDIKAR, BMTECH ; Spuriously High Prevalence of Prediabetes Diagnosed by HbA1c in Young Indians Partly Explained by Hematological Factors and Iron Deficiency Anemia *; DIABETES CARE*, VOLUME 35, APRIL 2012

29. Jung Il Son; Hemoglobin A1c May Be an Inadequate Diagnostic Tool for Diabetes Mellitus in Anemic Subjects *Diabetes Metab J* 2013;37:343-348.

30. BALASUBRAMANIAN SHANTHI ; Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics; *Journal of Clinical and Diagnostic Research*. 2013 January, Vol-7(1): 15-17

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

32. Nitin Sinha, M.D. - Effect of Iron Deficiency Anemia on Hemoglobin A1c Levels ; Annals of Laboratory medicine 2012;32:17-22 ;

33. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L (1990)

Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. *Journal of Internal Mmedicine* 227:133–136.

34. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. Lancet.1980;2:141.

35. Tarim O, Küçükerdoğan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatr Int*. 1999 Aug;41(4):357–62.

36. Rafat D, Rabbani TK, Ahmad J, Ansari MA. Influence of iron metabolism indices on HbA1c in non-diabetic pregnant women with and without iron-deficiency anemia: effect of iron supplementation. *Diabetes Metab Syndr*. 2012 Apr-Jun;6(2):102–5.

37. DIABETES MANAGEMENT GUIDELINES- ADA (2015) *http://www.ndei.org/ADA-diabetes-management-guidelines-diagnosis-A1C-testing.aspx*

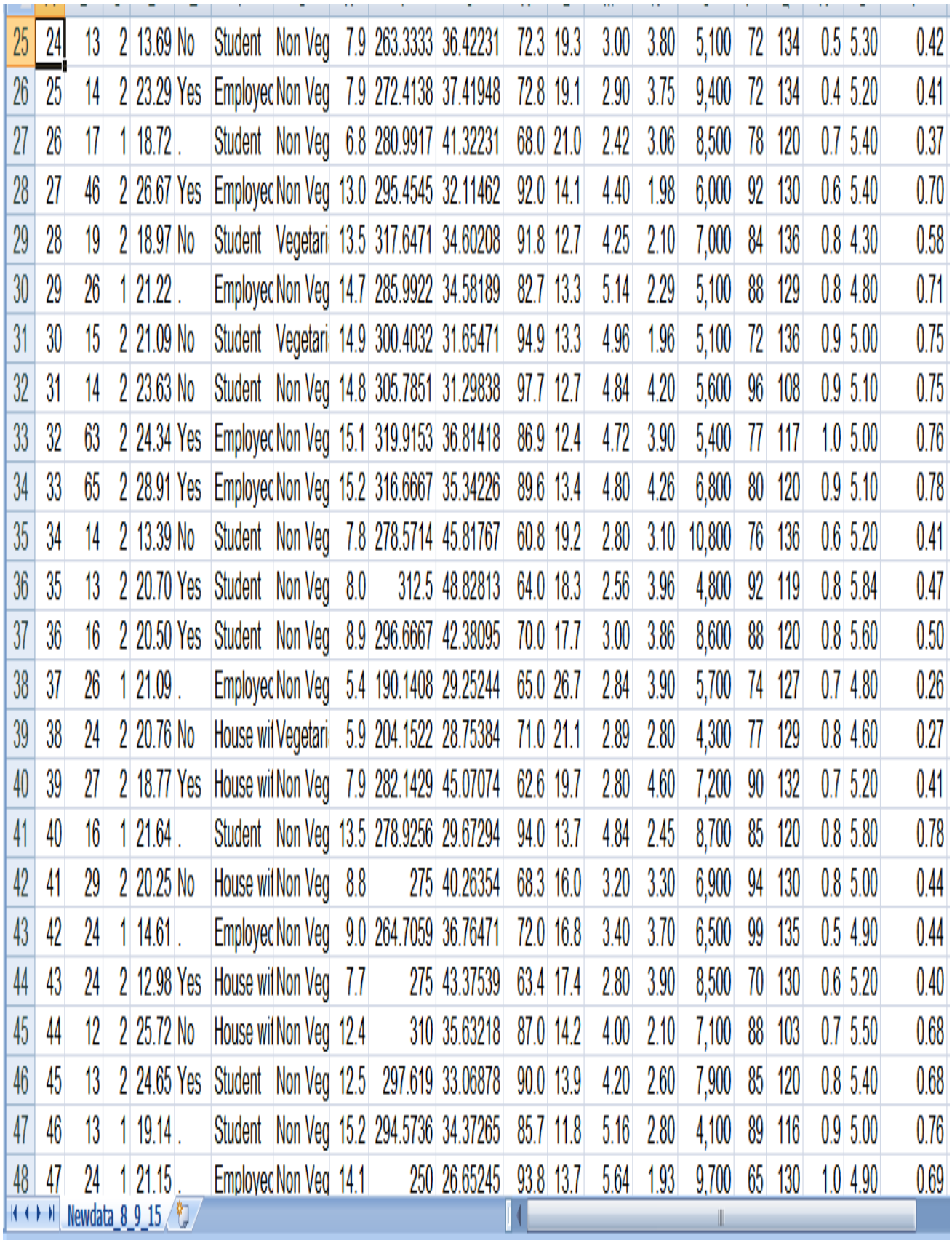
38. Segun et al - *Anemia and HbA1c level: is there a case for redefining reference range ; BJMP 2014 71 (A)706.*

39. Chapple A - Iron deficiency anaemia in women of South Asian descent: a qualitative study. National Primary Care Research and Development Centre, University of Manchester, UK.

ANNEXURES

MASTER CHART

S.N	AGE	SEX	BMI	MEN	OCC	DIET	HB	MCH	MCHC	MCV	RDW	RBC	PLT	WBC	FBS	PPBS	CREA	HBA1	ABS_HBA
1	15	2	18.67	Yes	Student	Non Veg	7.1	227.5641	33.17261	68.6	19.0	3.12	1.75	7,200	76	130	0.6	5.30	0.38
2	26	2	12.76	No	Student	Non Veg	8.6	252.9412	38.09355	66.4	21.1	3.40	4.12	8,400	79	114	0.6	5.20	0.43
3	62	2	18.85	No	House wif	Non Veg	9.7	251.9481	35.09026	71.8	18.5	3.85	3.40	6,200	97	123	0.5	5.40	0.52
4	28	1	19.78	.	Student	Vegetari	5.6	181.2298	26.53437	68.3	26.3	3.09	4.00	7,000	66	132	0.7	4.70	0.26
5	27	2	21.16	No	Student	Non Veg	5.8	193.3333	26.85185	72.0	25.9	3.00	2.40	5,400	93	123	0.8	4.60	0.27
6	46	2	18.58	Yes	Employec	Non Veg	9.5	263.8889	36.85599	71.6	16.0	3.60	4.10	5,200	77	128	0.5	5.40	0.51
7	12	2	13.08	No	Student	Non Veg	7.7	265.5172	36.87739	72.0	19.1	2.90	3.72	10,700	74	136	0.4	5.40	0.42
8	25	1	21.19	.	Employec	Non Veg	5.7	178.125	25.81522	69.0	21.9	3.20	3.80	5,100	81	125	0.7	4.60	0.26
9	45	2	19.02	Yes	Employec	Non Veg	9.4	261.1111	36.51904	71.5	17.0	3.60	2.80	4,800	94	114	0.7	5.60	0.53
10	43	2	21.80	No	Employec	Non Veg	13.9	278	29.38689	94.6	13.9	5.00	1.80	6,600	88	134	0.7	5.00	0.70
11	44	1	21.97	.	Employec	Non Veg	14.9	281.1321	31.83829	88.3	13.5	5.30	1.73	9,900	79	129	0.9	4.60	0.69
12	15	2	14.27	No	House wif	Non Veg	13.4	308.046	33.01672	93.3	12.9	4.35	2.23	6,500	88	127	0.6	4.40	0.59
13	16	2	18.05	Yes	Student	Non Veg	7.2	236.8421	33.45227	70.8	18.7	3.04	2.30	9,000	60	112	0.7	5.20	0.37
14	14	2	20.43	Yes	Student	Non Veg	7.4	255.1724	41.15684	62.0	19.5	2.90	3.88	4,100	77	134	0.6	5.50	0.41
15	15	2	20.28	Yes	Student	Non Veg	9.0	264.7059	36.76471	72.0	20.0	3.40	2.45	5,100	97	109	0.7	5.60	0.50
16	15	2	16.65	Yes	Student	Vegetari	7.0	257.3529	36.55582	70.4	19.4	2.72	2.51	10,200	90	130	0.6	5.30	0.37
17	24	2	21.23	Yes	Employec	Non Veg	5.5	200	29.85075	67.0	25.4	2.75	4.20	9,600	67	129	0.6	4.90	0.27
18	14	2	17.78	Yes	Student	Non Veg	6.9	218.3544	30.243	72.2	17.4	3.16	1.95	7,100	68	132	0.6	5.30	0.37
19	27	1	21.97	.	Employec	Non Veg	14.5	295.9184	34.24981	86.4	13.7	4.90	1.73	9,900	76	129	0.9	4.60	0.67
20	13	2	14.67	Yes	Student	Non Veg	7.0	227.2727	32.37503	70.2	19.3	3.08	2.26	4,400	66	127	0.5	5.30	0.37
21	40	2	17.86	Yes	Employec	Non Veg	8.8	234.6667	32.63792	71.9	17.6	3.75	3.38	6,800	98	121	0.9	5.50	0.48
22	12	2	16.88	Yes	Student	Non Veg	8.0	253.9683	36.22942	70.1	19.3	3.15	3.79	10,000	73	133	0.5	5.40	0.43
23	44	2	18.58	Yes	Employec	Non Veg	9.5	327.5862	45.75226	71.6	16.0	2.90	4.10	5,200	77	128	0.5	5.40	0.51

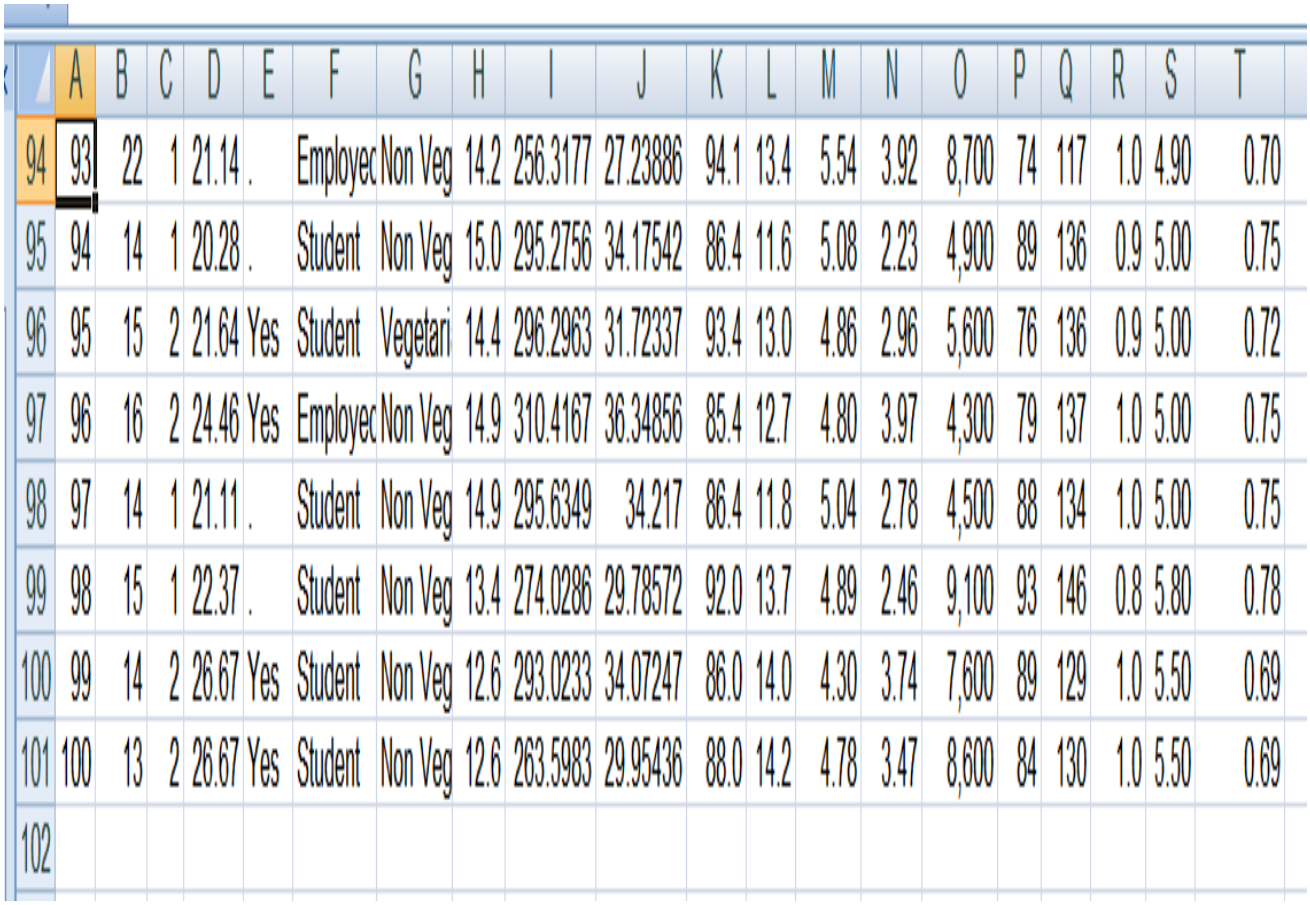


25	24	13	2	13.69	No	Student	Non Veg	7.9	263.3333	36.42231	72.3	19.3	3.00	3.80	5,100	72	134	0.5	5.30	0.42
26	25	14	2	23.29	Yes	Employec	Non Veg	7.9	272.4138	37.41948	72.8	19.1	2.90	3.75	9,400	72	134	0.4	5.20	0.41
27	26	17	1	18.72	.	Student	Non Veg	6.8	280.9917	41.32231	68.0	21.0	2.42	3.06	8,500	78	120	0.7	5.40	0.37
28	27	46	2	26.67	Yes	Employec	Non Veg	13.0	295.4545	32.11462	92.0	14.1	4.40	1.98	6,000	92	130	0.6	5.40	0.70
29	28	19	2	18.97	No	Student	Vegetari	13.5	317.6471	34.60208	91.8	12.7	4.25	2.10	7,000	84	136	0.8	4.30	0.58
30	29	26	1	21.22	.	Employec	Non Veg	14.7	285.9922	34.58189	82.7	13.3	5.14	2.29	5,100	88	129	0.8	4.80	0.71
31	30	15	2	21.09	No	Student	Vegetari	14.9	300.4032	31.65471	94.9	13.3	4.96	1.96	5,100	72	136	0.9	5.00	0.75
32	31	14	2	23.63	No	Student	Non Veg	14.8	305.7851	31.29838	97.7	12.7	4.84	4.20	5,600	96	108	0.9	5.10	0.75
33	32	63	2	24.34	Yes	Employec	Non Veg	15.1	319.9153	36.81418	86.9	12.4	4.72	3.90	5,400	77	117	1.0	5.00	0.76
34	33	65	2	28.91	Yes	Employec	Non Veg	15.2	316.6667	35.34226	89.6	13.4	4.80	4.26	6,800	80	120	0.9	5.10	0.78
35	34	14	2	13.39	No	Student	Non Veg	7.8	278.5714	45.81767	60.8	19.2	2.80	3.10	10,800	76	136	0.6	5.20	0.41
36	35	13	2	20.70	Yes	Student	Non Veg	8.0	312.5	48.82813	64.0	18.3	2.56	3.96	4,800	92	119	0.8	5.84	0.47
37	36	16	2	20.50	Yes	Student	Non Veg	8.9	296.6667	42.38095	70.0	17.7	3.00	3.86	8,600	88	120	0.8	5.60	0.50
38	37	26	1	21.09	.	Employec	Non Veg	5.4	190.1408	29.25244	65.0	26.7	2.84	3.90	5,700	74	127	0.7	4.80	0.26
39	38	24	2	20.76	No	House wit	Vegetari	5.9	204.1522	28.75384	71.0	21.1	2.89	2.80	4,300	77	129	0.8	4.60	0.27
40	39	27	2	18.77	Yes	House wit	Non Veg	7.9	282.1429	45.07074	62.6	19.7	2.80	4.60	7,200	90	132	0.7	5.20	0.41
41	40	16	1	21.64	.	Student	Non Veg	13.5	278.9256	29.67294	94.0	13.7	4.84	2.45	8,700	85	120	0.8	5.80	0.78
42	41	29	2	20.25	No	House wit	Non Veg	8.8	275	40.26354	68.3	16.0	3.20	3.30	6,900	94	130	0.8	5.00	0.44
43	42	24	1	14.61	.	Employec	Non Veg	9.0	264.7059	36.76471	72.0	16.8	3.40	3.70	6,500	99	135	0.5	4.90	0.44
44	43	24	2	12.98	Yes	House wit	Non Veg	7.7	275	43.37539	63.4	17.4	2.80	3.90	8,500	70	130	0.6	5.20	0.40
45	44	12	2	25.72	No	House wit	Non Veg	12.4	310	35.63218	87.0	14.2	4.00	2.10	7,100	88	103	0.7	5.50	0.68
46	45	13	2	24.65	Yes	Student	Non Veg	12.5	297.619	33.06878	90.0	13.9	4.20	2.60	7,900	85	120	0.8	5.40	0.68
47	46	13	1	19.14	.	Student	Non Veg	15.2	294.5736	34.37265	85.7	11.8	5.16	2.80	4,100	89	116	0.9	5.00	0.76
48	47	24	1	21.15	.	Employec	Non Veg	14.1	250	26.65245	93.8	13.7	5.64	1.93	9,700	65	130	1.0	4.90	0.69

Newdata_8_9_15

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
48	47	24	1	21.15	.	Employed	Non Veg	14.1	250	26.65245	93.8	13.7	5.64	1.93	9,700	65	130	1.0	4.90	0.69
49	48	28	2	22.14	Yes	Employed	Vegetari	15.0	277.7778	28.72573	96.7	11.8	5.40	1.68	7,900	89	127	0.9	4.60	0.69
50	49	15	2	18.55	No	Student	Non Veg	13.6	312.6437	34.09419	91.7	12.9	4.35	4.50	6,000	76	119	0.8	4.30	0.58
51	50	13	2	25.48	Yes	Employed	Non Veg	12.5	304.878	34.64523	88.0	13.9	4.10	2.25	6,800	79	118	0.8	5.40	0.68
52	51	14	2	20.03	Yes	Student	Vegetari	12.7	295.3488	33.56237	88.0	13.8	4.30	2.40	6,600	82	130	0.8	5.30	0.67
53	52	14	2	27.69	Yes	Student	Non Veg	12.6	300	33.89831	88.5	14.0	4.20	3.90	7,200	89	125	1.0	5.50	0.69
54	53	13	2	15.56	Yes	Student	Non Veg	13.2	306.9767	32.6571	94.0	12.8	4.30	2.00	6,000	76	128	0.6	4.40	0.58
55	54	14	2	17.31	Yes	Student	Non Veg	13.1	297.7273	31.67311	94.0	12.8	4.40	1.90	7,000	77	131	0.6	4.50	0.59
56	55	16	2	19.17	No	Student	Non Veg	13.4	301.1236	33.09051	91.0	12.7	4.45	1.80	8,000	72	126	0.6	4.40	0.59
57	56	13	2	13.69	No	Student	Non Veg	8.4	221.0526	29.75136	74.3	19.3	3.80	3.50	8,900	72	134	0.8	5.30	0.45
58	57	16	1	21.36	.	Student	Non Veg	13.7	330.9179	34.98075	94.6	13.1	4.14	2.61	5,400	80	120	0.8	5.80	0.79
59	58	18	2	21.64	Yes	Student	Non Veg	13.6	323.8095	33.00811	98.1	12.9	4.20	2.23	5,700	72	114	0.9	5.70	0.78
60	59	17	2	21.08	Yes	Student	Non Veg	13.4	335	34.78712	96.3	12.7	4.00	2.18	5,100	69	119	0.9	5.90	0.79
61	60	20	2	21.60	Yes	Student	Non Veg	13.9	323.2558	34.94657	92.5	13.3	4.30	4.00	4,900	89	129	0.7	5.60	0.78
62	61	21	1	21.83	.	Student	Non Veg	13.5	375	40.89422	91.7	13.5	3.60	3.96	5,500	91	128	0.7	5.70	0.77
63	62	17	2	21.08	Yes	Student	Non Veg	13.9	347.5	38.91377	89.3	12.7	4.00	2.18	5,100	69	119	0.9	5.90	0.82
64	63	14	2	22.67	Yes	Student	Non Veg	8.5	337.3016	47.24112	71.4	24.9	2.52	3.96	6,600	77	125	0.7	5.20	0.44
65	64	20	1	21.09	.	Student	Non Veg	7.9	232.3529	32.91118	70.6	19.2	3.40	3.10	8,000	76	112	0.8	5.10	0.40
66	65	19	2	20.85	Yes	Student	Non Veg	7.8	237.8049	34.97131	68.0	21.0	3.28	2.29	5,400	85	122	0.7	5.20	0.41
67	66	15	2	24.49	No	Student	Non Veg	13.5	300	32.89474	91.2	13.4	4.50	4.58	9,600	76	124	0.8	4.30	0.58
68	67	60	2	26.16	No	Employed	Non Veg	8.2	253.0864	36.52041	69.3	20.5	3.24	4.30	5,200	64	99	0.8	5.60	0.46
69	68	62	2	26.02	No	Employed	Non Veg	8.0	258.0645	38.92376	66.3	20.8	3.10	1.58	5,400	66	123	0.8	5.40	0.43
70	69	17	2	24.00	Yes	Student	Non Veg	13.7	318.6047	33.53733	95.0	12.4	4.30	2.80	5,400	69	149	0.9	5.90	0.81
71	70	22	2	21.31	Yes	House wif	Vegetari	8.0	222.2222	30.8642	72.0	18.3	3.60	2.24	6,900	88	114	0.8	5.00	0.40

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
71	70	22	2	21.31	Yes	House wif	Vegetari	8.0	222.2222	30.8642	72.0	18.3	3.60	2.24	6,900	88	114	0.8	5.00	0.40
72	71	21	1	21.83	.	Student	Non Veg	13.8	353.8462	39.14227	90.4	13.5	3.90	3.96	5,300	91	128	0.7	5.70	0.79
73	72	64	2	25.88	No	Employec	Vegetari	7.8	260	40.18547	64.7	21.4	3.00	2.64	6,200	70	115	0.7	5.40	0.42
74	73	18	2	21.88	No	Student	Non Veg	13.3	290.393	31.22506	93.0	12.9	4.58	3.47	8,800	74	125	0.6	4.40	0.59
75	74	58	2	26.78	No	Employec	Non Veg	8.4	247.0588	35.54803	69.5	19.9	3.40	3.65	6,600	62	113	0.4	5.50	0.46
76	75	15	2	24.49	No	Student	Non Veg	13.7	304.4444	33.56609	90.7	13.0	4.50	4.58	8,600	76	124	0.8	4.30	0.59
77	76	16	1	21.78	.	Student	Non Veg	13.6	295.6522	31.79056	93.0	13.5	4.60	2.15	9,900	85	126	0.8	5.80	0.79
78	77	56	1	27.39	.	Employec	Non Veg	8.6	252.9412	35.22858	71.8	19.7	3.40	4.23	5,200	85	125	0.6	5.50	0.47
79	78	15	1	22.01	.	Student	Non Veg	8.4	320.6107	44.96644	71.3	23.9	2.62	1.75	6,400	75	123	0.8	5.20	0.44
80	79	18	2	20.83	No	Student	Vegetari	10.8	415.3846	58.58739	70.9	15.7	2.60	4.06	7,600	73	137	0.6	5.80	0.63
81	80	14	2	29.33	Yes	Student	Non Veg	12.8	297.6744	34.6133	86.0	14.0	4.30	3.69	7,400	89	125	0.9	5.50	0.70
82	81	15	2	22.77	Yes	Student	Non Veg	8.7	266.055	37.5784	70.8	22.3	3.27	1.56	6,500	75	135	0.8	5.80	0.50
83	82	17	2	24.00	Yes	Student	Non Veg	13.6	328.5024	33.97129	96.7	12.4	4.14	2.80	5,100	69	119	0.9	5.90	0.80
84	83	16	2	22.64	Yes	Student	Non Veg	8.8	283.871	39.98183	71.0	22.5	3.10	2.29	6,400	74	134	0.9	5.80	0.51
85	84	13	1	23.01	.	Student	Vegetari	9.2	259.1549	35.21127	73.6	20.9	3.55	2.56	8,900	79	116	0.7	5.60	0.52
86	85	49	2	23.26	No	Employec	Non Veg	13.4	394.1176	43.59708	90.4	13.5	3.40	3.95	10,700	69	124	0.7	5.50	0.74
87	86	15	2	24.49	No	Student	Non Veg	13.4	297.7778	32.83107	90.7	13.0	4.50	4.45	6,600	79	126	0.8	4.30	0.58
88	87	48	2	23.39	No	House wif	Non Veg	12.5	347.2222	37.70057	92.1	14.0	3.60	4.00	5,800	80	134	0.8	5.50	0.69
89	88	13	2	26.67	Yes	Student	Non Veg	12.6	272.7273	28.40909	96.0	14.3	4.62	3.80	4,700	86	130	0.9	5.50	0.69
90	89	48	2	25.88	Yes	House wif	Non Veg	11.0	398.5507	56.1339	71.0	15.3	2.76	3.43	7,700	88	111	0.7	5.90	0.66
91	90	17	2	21.09	Yes	Student	Non Veg	10.7	382.1429	52.78216	72.4	15.9	2.80	2.74	9,400	77	114	0.7	5.60	0.60
92	91	19	1	20.66	.	Student	Non Veg	10.6	386.8613	55.74371	69.4	15.7	2.74	2.46	5,800	85	119	0.6	5.70	0.60
93	92	18	2	20.83	No	Student	Vegetari	10.5	403.8462	55.39728	72.9	15.8	2.60	3.96	6,600	73	137	0.6	5.80	0.61
94	93	22	1	21.14	.	Employec	Non Veg	14.2	256.3177	27.23886	94.1	13.4	5.54	3.92	8,700	74	117	1.0	4.90	0.70



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
94	93	22	1	21.14	.	Employec	Non Veg	14.2	256.3177	27.23886	94.1	13.4	5.54	3.92	8,700	74	117	1.0	4.90	0.70
95	94	14	1	20.28	.	Student	Non Veg	15.0	295.2756	34.17542	86.4	11.6	5.08	2.23	4,900	89	136	0.9	5.00	0.75
96	95	15	2	21.64	Yes	Student	Vegetari	14.4	296.2963	31.72337	93.4	13.0	4.86	2.96	5,600	76	136	0.9	5.00	0.72
97	96	16	2	24.46	Yes	Employec	Non Veg	14.9	310.4167	36.34856	85.4	12.7	4.80	3.97	4,300	79	137	1.0	5.00	0.75
98	97	14	1	21.11	.	Student	Non Veg	14.9	295.6349	34.217	86.4	11.8	5.04	2.78	4,500	88	134	1.0	5.00	0.75
99	98	15	1	22.37	.	Student	Non Veg	13.4	274.0286	29.78572	92.0	13.7	4.89	2.46	9,100	93	146	0.8	5.80	0.78
100	99	14	2	26.67	Yes	Student	Non Veg	12.6	293.0233	34.07247	86.0	14.0	4.30	3.74	7,600	89	129	1.0	5.50	0.69
101	100	13	2	26.67	Yes	Student	Non Veg	12.6	263.5983	29.95436	88.0	14.2	4.78	3.47	8,600	84	130	1.0	5.50	0.69
102																				

QUESTIONNAIRE

NAME:

AGE:

SEX:

HEIGHT:

WEIGHT:

BMI:

H/O MENORRHAGIA

OCCUPATION:

DIET

INVESTIGATIONS:

HAEMOGLOBIN LEVELS:

MCV:

MCH:

MCHC:

RDW:

PLATELETS:

ERYTHROCYTES:

WBC:

SERUM FASTING AND POSTPRANDIAL GLUCOSE LEVELS:

SERUM CREATININE

HBA1C:

ABSOLUTE HBA1C

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID No.12/01/2015 Dt 20 . 01.2015
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A study on the effect of Microcytic Ananemia on HbA1c Levels in Non Diabetic Individual". -For Project Work-submitted by Dr. S. Swetha, PG in General Medicine, KMC, Chennai- 10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

GOVT. KILPAUK MEDICAL COLLEGE * Kilpauk, Chennai-10. *
20 JAN 2015

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

19/1/2015

6 Comp> ME 1> Ethical Committee


Turnitin Document Viewer - Google Chrome
https://turnitin.com/dv?o=571275535&u=1042553853&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 30-..
Originality
GradeMark
PeerMark
A STUDY ON THE EFFECT OF MICROCYTIC ANAEMIA ON HbA1c LEVELS IN
BY SWETHA SATTANATHAN
turnitin
19%
SIMILAR
--
OUT OF 0
A STUDY ON THE EFFECT OF MICROCYTIC ANAEMIA ON HbA1c LEVELS IN NON DIABETIC INDIVIDUALS IN GOVT ROYAPETTAH HOSPITAL, CHENNAI
Dissertation submitted to
THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY
CHENNAI
In partial fulfillment of regulations
For award of the degree of
M.D. (GENERAL MEDICINE)
BRANCH-I
KILPAUK MEDICAL COLLEGE
Match Overview
1 www.diapedia.org Internet source 2%
2 www.diab.net.cn Internet source 1%
3 www.med.upenn.edu Internet source 1%
4 www.ncbi.nlm.nih.gov Internet source 1%
5 www.diabetologia-jour... Internet source 1%
6 Submitted to Universit... Student paper 1%
7 drugs-online-usa.com Internet source 1%
8 care.diabetesjournals.... Internet source 1%
PAGE: 1 OF 109
Text-Only Report
21:11
23-09-2015