A DISSERTATION

ON

A STUDY ON VITAMIN B12 DEFICIENCY IN CHRONIC METFORMIN THERAPY IN TYPE 2 DIABETES MELLITUS PATIENTS IN GMKMCH, SALEM

Submitted to

THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY,
CHENNAI

In partial fulfillment of the regulations
for the award of

M. D. DEGREE IN GENERAL MEDICINE
BRANCH I

GOVERNMENT MOHAN KUMARAMANGALAM
MEDICAL COLLEGE, SALEM
APRIL 2017

Government Mohan Kumaramangalam Medical College Hospital

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "**A STUDY ON VITAMIN B12 DEFICIENCY IN CHRONIC METFORMIN THERAPY IN TYPE 2 DIABETES MELLITUS PATIENTSIN GMKMCH, SALEM"** is a bonafide and genuine research work carried out by me under the guidance of ***DR. S.SURESH KANNA, M. D.,*** Professor, Department of General Medicine and under the co guidance of***DR. G.PRAKASH, M. D., D.Diab., Associate*** Professor and Head, Department of Diabetology, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamil Nadu, India.

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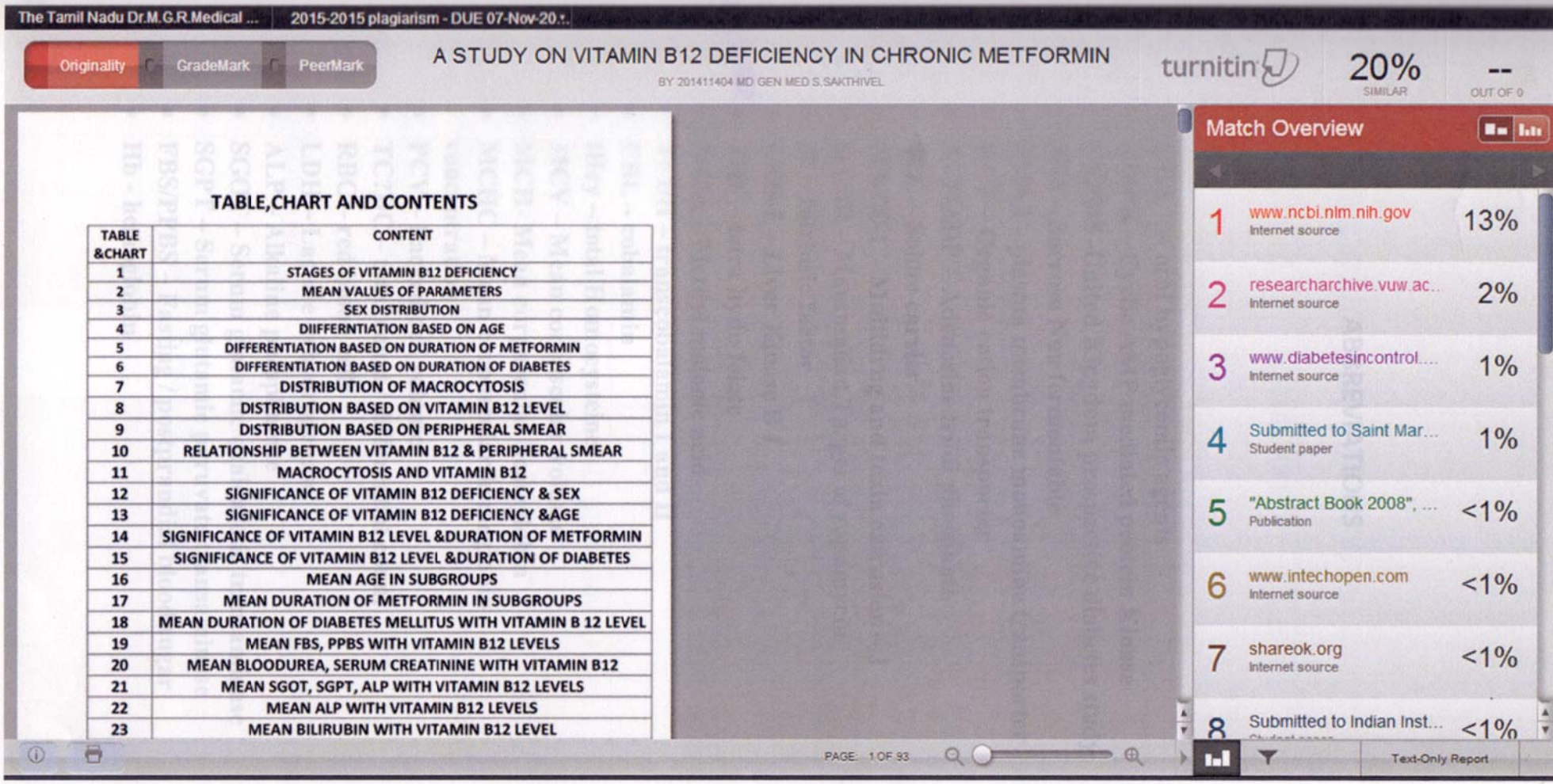
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Ethical Committee Meeting held on 18.06.2015 at 10.00 A.M in the Seminar Hall, IInd Floor, Medicine Block, Govt. Mohan Kumaramangalam Medical College Hospital, Salem 01.

The following Members were attended the Meeting.

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1.	Dr. S. Sakthivel, II Year, Post Graduate Student of MD (General Medicine), GMKMC, Salem-30.	"Prospective study on vitamin B12 deficiency in type 2 Diabetes mellitus patients who are on chronic metformin therapy-50 cases in GMKMCH".	Dr. S. Suresh Kanna, MD., Associate Professor of General Medicine and Dr. G. Prakash, MD., D.Diab., Associate Professor of Diabetology, GMKMC, Salem-30.	Approved

The Ethical Committee examined the studies in detail and is pleased to accord Ethical Committee approval for the above Post Graduate student of this College to carry out the studies with the following conditions.

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29/06/2015

DEAN

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ABBREVIATIONS

- **OHA - oral hypoglycemic agents**
- **AMPK – Cyclic AMP mediated protein Kinase**
- **UKPDS -United Kingdom prospective diabetes study**
- **SNF – Sucrose Non fermentable**
- **PMAT - plasma membrane monoamine transporter**
- **OCT – Organic cation transporter**
- **ATP/ADP – Adenosine tri/di phosphate**
- **SLC – Solute carrier**
- **MATE-1 - Multidrug and toxin extrusion – 1**
- **mTOR – Mammalian Target of rapamycin**
- **IF – Intrinsic factor**
- **LKB-1 - Liver Kinase B 1**
- **THF – tetra hydo folate**
- **MMA – Methyl malonic acid**
- **Tc I/II – transcobalamin I and II**
- **CBL – cobalamin**
- **tHcy – total Homocysteine**
- **MCV – Mean corpuscular volume**
- **MCH- Mean corpuscular hemoglobin**
- **MCHC – Mean corpuscular hemoglobin concentration**
- **PCV – Packed cell volume**
- **TC?DC – total count / differential count**
- **RBC – red blood cells**
- **LDH – Lactate dehydrogenase**
- **ALP – Alkaline phosphatase**
- **SGOT – Serum glutamic oxaloacetic transaminase**
- **SGPT – Serum glutamic pyruvate transaminase**
- **FBS/PPBS – Fasting / postprandial blood sugar**
- **Hb - hemoglobin**

ABSTRACT

BACKGROUND AND OBJECTIVE

The prevalence of vitamin B12 deficiency varies from 5.8 % to 30% among patients undergoing long-term treatment with metformin.

DESIGN

Cross sectional study

METHODS

Patients with Type 2 diabetes were selected based on inclusion and exclusion criteria. Basic biochemical investigation with peripheral smear done. Serum B12 assay were done. Vitamin B12 deficiency is defined as values <180pg/ml. Association between vitamin B12 deficiency with duration of metformin therapy, duration of diabetes, with age, sex were done.

RESULTS

The mean vitamin B12 level is low as the duration of metformin treatment increases. The sex, age relation with development of vitamin B12 deficiency was not significant. Mean MCV is higher in the Vitamin

B12 deficient group. Macrocytosis is mainly noted in the vitamin B12 deficient group.

CONCLUSION

Vitamin B12 deficiency occurs in type 2 diabetes mellitus patients treated with long-term metformin. The duration of metformin therapy significantly affects the development of vitamin B12 deficiency. Macrocytosis occurs in vitamin B12 deficient diabetic patients treated with metformin.

CONTENTS

INTRODUCTION

Metformin isa commonly used and first line oral hypoglycemic agent in treatment of type 2 diabetes mellitus. It is a known fact that age and duration of diabetes can affect the vitamin B12 status of a patient. Vitamin B12 deficiency is assessed by estimating the Vitamin B12 levels (2)Low vitamin B12 level is said to occur in diabetics due to metformin therapy also. Many cross sectional studies showed chronic metformin therapy reduced vitamin B12 levels. Often the initial clinical signs are subtle in low vitamin B12. Vitamin B12 deficiency can present with anemia, peripheral neuropathy, and altered cognition.

Most of the time vitamin B 12 deficiency was not thought in such clinical situation. There is no universal recommendation to supplement vitamin B12, especially in high risk populations. Studying the biochemical profile to detect vitamin B12 deficiency in these populations will provide useful data to support the need for supplementation.

AIMS AND OBJECTIVES:

1. To assess the Vitamin B12 level in chronic metformin treated type 2 diabetic patients.
2. To assess the relation between metformin therapy duration & development of vitamin B12 deficiency.

REVIEW OF LITERATURE

Sterne discovered metformin first in the year of 1957 and was named Glucophage. It came to United Kingdom market in 1958. The drug discovered based on the herb Galego officinalis, which was used in the ancient days for reduction of blood sugar based on the component name galegine. Muller first applied this principle agent clinically in the year of 1927. They noted the significant glucose lowering effect in all group of patients studied. Further refining studies on this compound was done by Leclerc and they did well in increasing the safety level and making it compatible for clinical use.

Later in the year of 1957, in France various studies used Metformin in multiple studies and they showed significant decrease in blood sugar levels. In the same period, Phenformin was introduced from America. Later due to increased documented cases of lactic acidosis, Phenformin was withdrawn from the market.

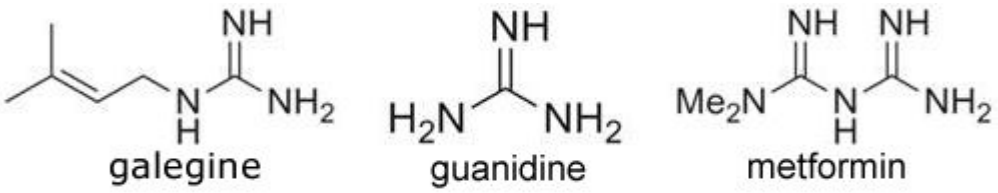


Fig 1

During 1970, the relation between treatment with metformin and reduced vitamin B12 status was established by Stowers and Smith, and estimated prevalence was stated around 30%. Since the prevalence varies between different studies, the ranges of incidence in such population vary by a large amount. In each patient, there are varying factors influencing the risk of developing deficiency of vitamin B12, it becomes mandatory to locate the key factor to reduce the compounding phenomena.

Metformin is one the most commonly used antidiabetic agent for the past few decades in the treatment of type 2 diabetes mellitus. It is an biguanide class of drug. It is an euglycemic agent. The proposed mechanism of action of biguanides is reduce the glucose output through the stimulation of cyclic AMP mediated protein kinase. Other mechanisms postulated are interference with renal gluconeogenesis, decreased rate of absorption of glucose from intestine, reduced plasma glucagon level, accelerated conversion of glucose to lactate in intestinal epithelial cells, enhancement of glycolysis in peripheral tissues. Metformin improves the lipid profile of the treated patient, by decreasing the adipose tissue mobilization from the periphery, decreasing the free fatty level in the blood, also lowers the VLDL. But

the precise molecular mechanism for metformin action is not known. Recent studies show some evidence towards the action of metformin in respiratory chain complex. This interfere with the mitochondrial respiratory cycle.

In the UKPDS study, Metformin showed to decrease the death due to diabetes such as stroke and myocardial infarction. Among the best known effects of metformin are the increased sensitivity of insulin by increasing the peripheral glucose usage and decreased hepatic glucose output and improved tyrosine kinase activity(12). Metformin prevents the cardiovascular mortality and morbidity in type 2 diabetic patients(11). Also it is weight neutral and associated with very low hypoglycemic episodes. Hence it is being considered first in treating the type 2 diabetic patients and one of the most prescribed drug too(3).

Targeting of AMPK

Cyclic AMP mediated protein kinase is a vital member of AMPK/SNF1 family. This kinase family is seen in eukaryotes, including plants, mammals, yeasts, Drosophila. It is a heterotrimeric subunit with α, β, ϒ subunits. The α subunit is the principal subunit.

The action of enzyme depends on the α subunit turnover. The β/ϒ subunit regulates the turnover and action of enzymes. The enzyme is easily activated by AMP and inhibited in abundance of ATP. This ADP to ATP cycle acts as a metabolic switch between synthesis and utilization of ATP. Creatine phosphate is a negative allosteric inhibitor of the kinase. Thus the AMPK activating effect of the Metformin is measured by alteration in the cellular adenine nucleotide pool, which manifest as decreased cellular respiration.

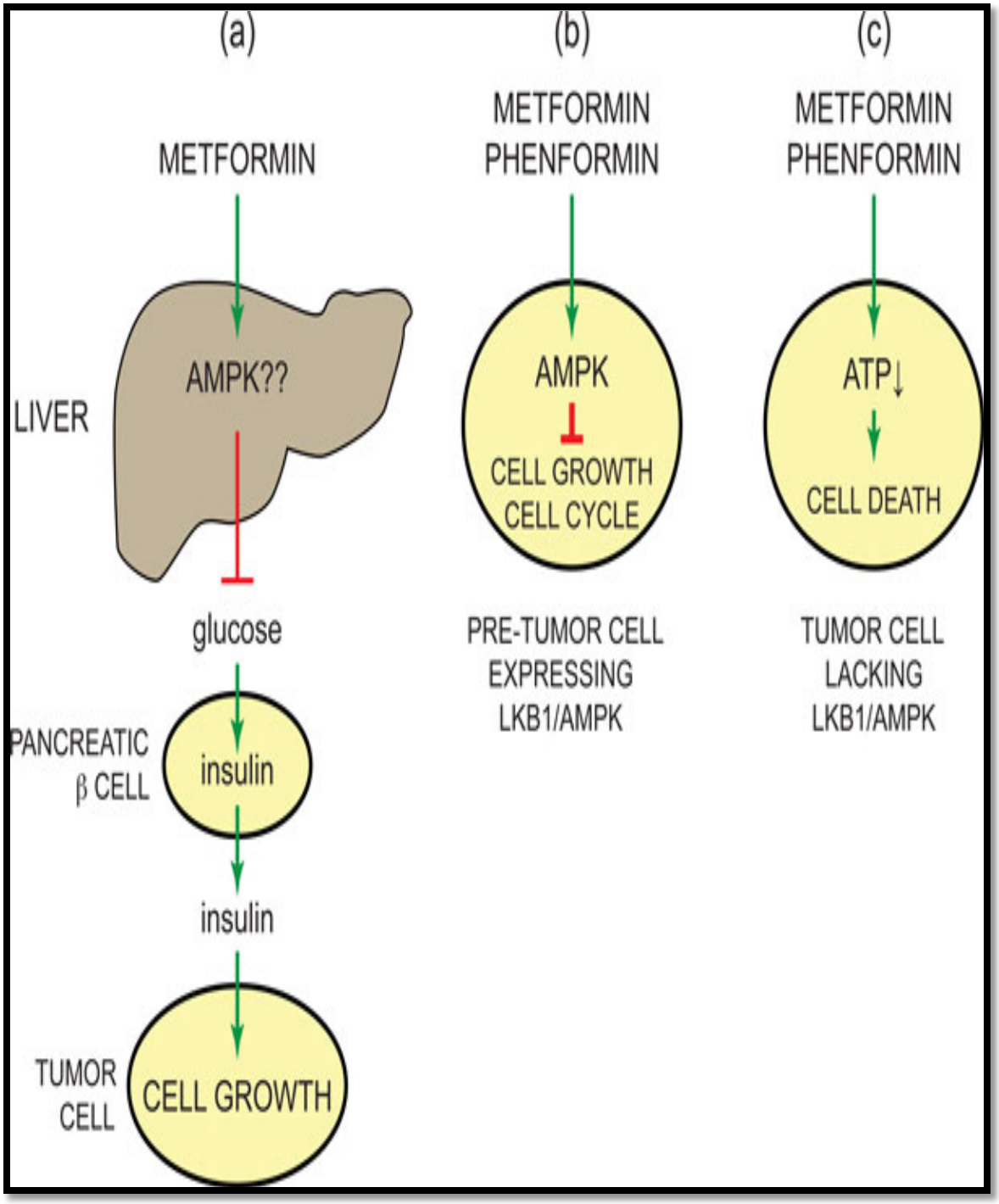

(a)
(b)
(c)
METFORMIN
METFORMIN
PHENFORMIN
METFORMIN
PHENFORMIN
LIVER
AMPK??
AMPK
CELL GROWTH
CELL CYCLE
ATP↓
CELL DEATH
glucose
PRE-TUMOR CELL
EXPRESSING
LKB1/AMPK
TUMOR CELL
LACKING
LKB1/AMPK
PANCREATIC
β CELL
insulin
insulin
TUMOR
CELL
CELL GROWTH

Fig 2

PHARMACOKINETICS

The main mode of elimination of metformin is via kidney. Tubular excretion is predominant in renal system. Less evidence is there for liver mediated removal in humans. Intestinal uptake of metformin is mediated by plasma membrane monoamine transporter(PMAT/SLC29A4) and OCT3(Organic cation transporter)

(Fig 2). Transfer of metformin from enterocytes to interstitial fluid is done by OCT1(SLC22A1). OCT1 and OCT3 plays the similar role in liver in uptake of metformin, where it inhibits gluconeogenesis and decreases glucose output. In kidney, transfer of metformin from circulation into tubular cells is mediated by OCT2(SLC22A2) (fig 3). Elimination from cell into the tubule is done by MATE1 (Multidrug and toxin extrusion – 1) (SLC47A1) and MATE2-K(SLC47A2). Liver doesn’t take part in excreting metformin(fig 3).Hence drug interactions can occur due to polymorphisms in drug transporters. For example PPIs(Proton pump inhibitors) inhibits Organic cation transporters.

PHARMACOKINETICS

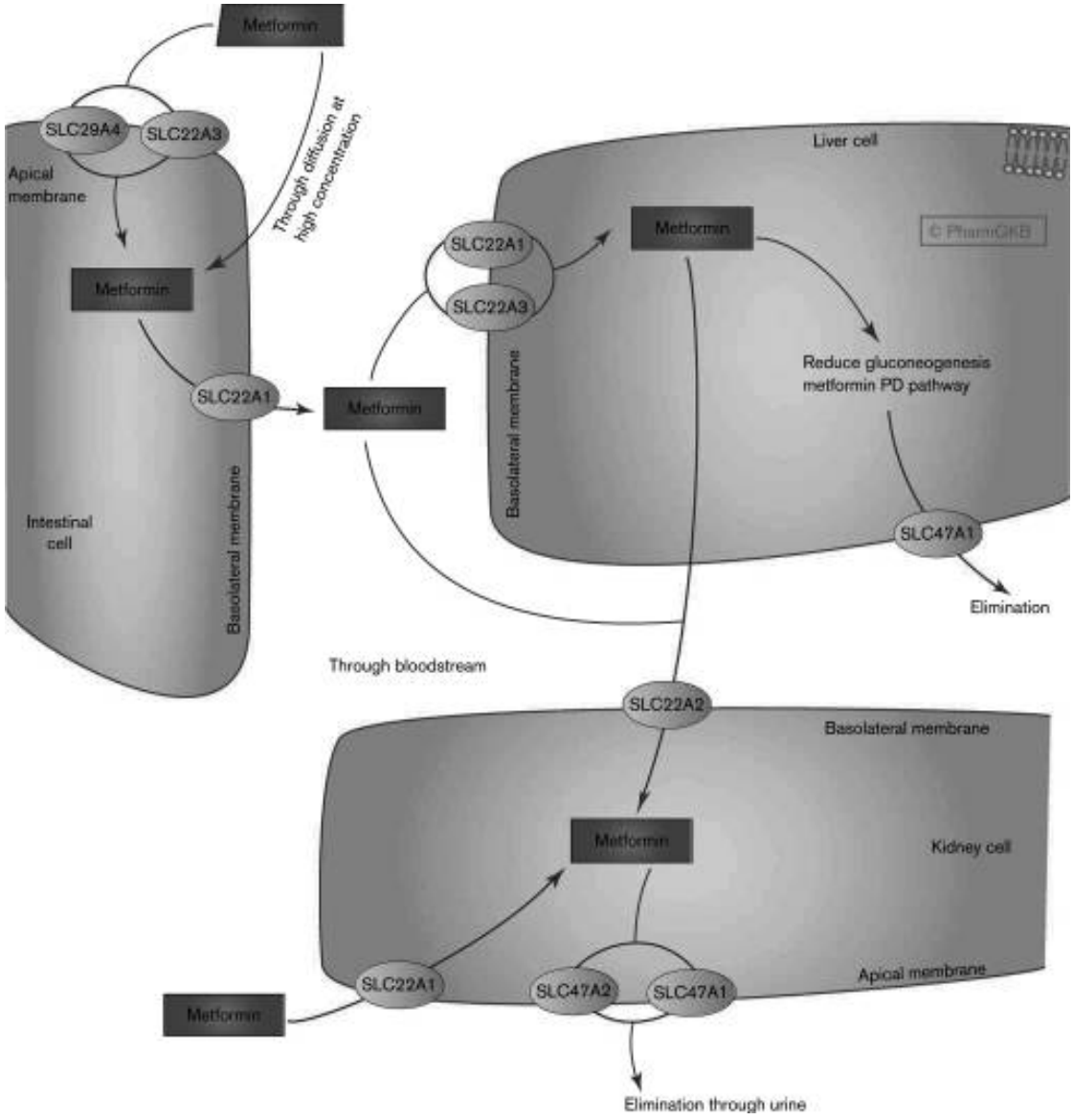
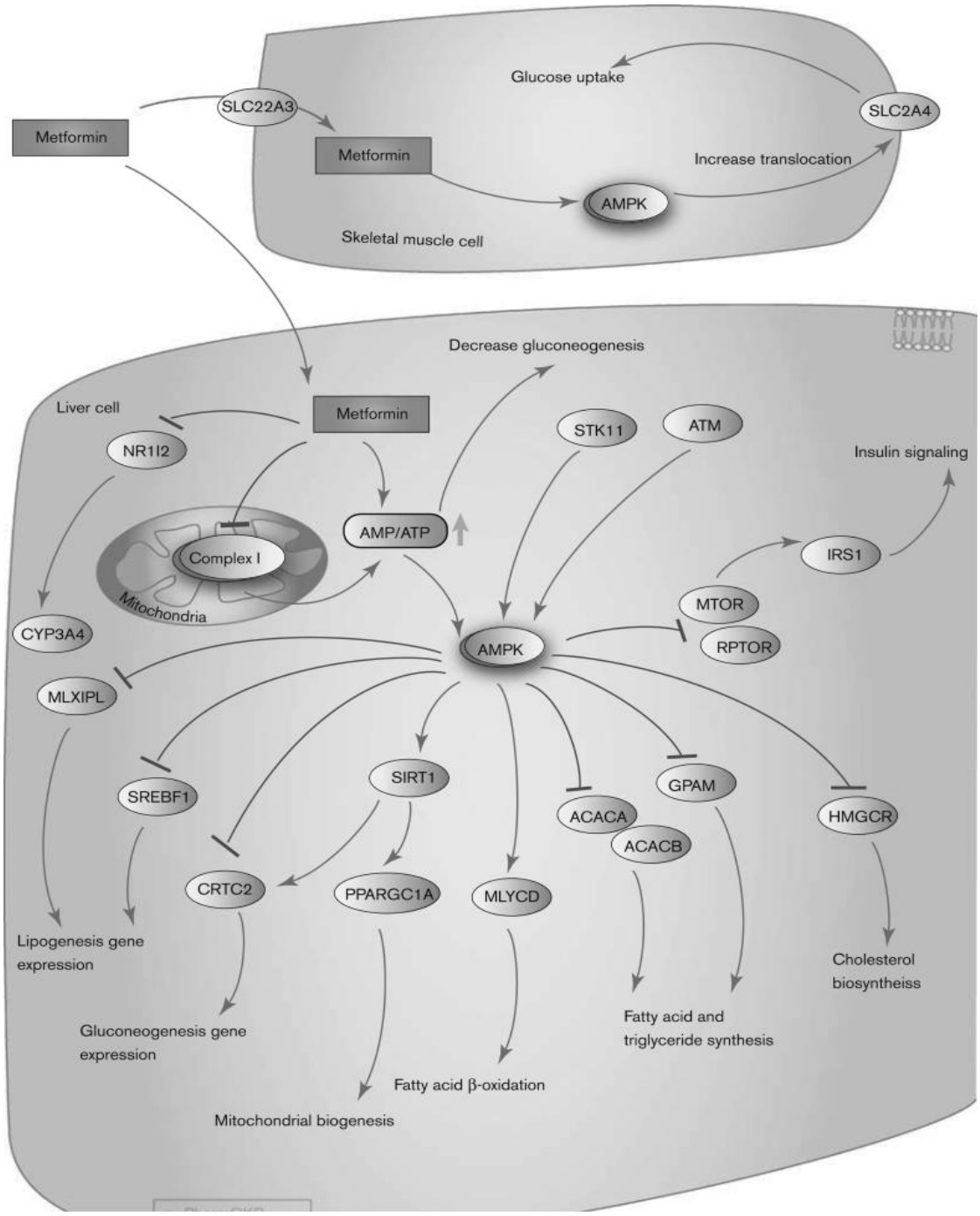


Fig 3

PHARMACODYNAMICS

Metformin exerts its effect on fasting and prandial sugar level by various mechanisms discussed before. It acts by inhibiting gluconeogenesis,increasing fatty acid oxidation and decreased lipid synthesis. So it favours both the glycemic and lipid profile of the patient. The postulated mode of action of metformin as discussed before is through the AMPK pathway(fig4). The compiled effect of AMPK stimulation in the liver includes the activation of fatty acid oxidation with inhibition of cholesterol and triglyceride synthesis. Peripheral effects include the stimulation of fatty acid oxidation and glucose uptake in skeletal muscle as well as a systemic increase in insulin sensitivity(fig 4). However, the role of metformin in insulin-mediated glucose uptake has been debated.



PHARMACODYNAMICS

Fig 4

SIDE EFFECTS

Nausea, vomiting, stomach upset, diarrhoea, weakness, or a metallic taste in the mouth may occur.Also there are some other disadvantages too. It can cause gastrointestinal problems, like discomfort and diarrhoea which may cause the patient to discontinue the drug. Other important treatable clinical situations are anemia, peripheral neuropathy in the setting of low vitamin B12 caused by chronic metformin usage in these patients(3).

Lactic acidosis occurs in drug overdose, liver, renal failure, any type of shock, dehydration, alcohol intoxication. Not noted in regular patients. Hypoglycemia due to metformin perse is not documented well. But it can occur when it is combined with other OHA(Oral hypoglycemic agents). This drug is found to be one of the cause of vitamin B12 deficiency. Among all the patients undergoing therapy with metformin around 10% - 30% is developing biochemical deficiency. This can be asymptomatic or with biochemical deficiency alone or symptomatic deficiency. Another study showed a 22% prevalence of B12 deficiency in type 2 DM on metformin therapy(13).

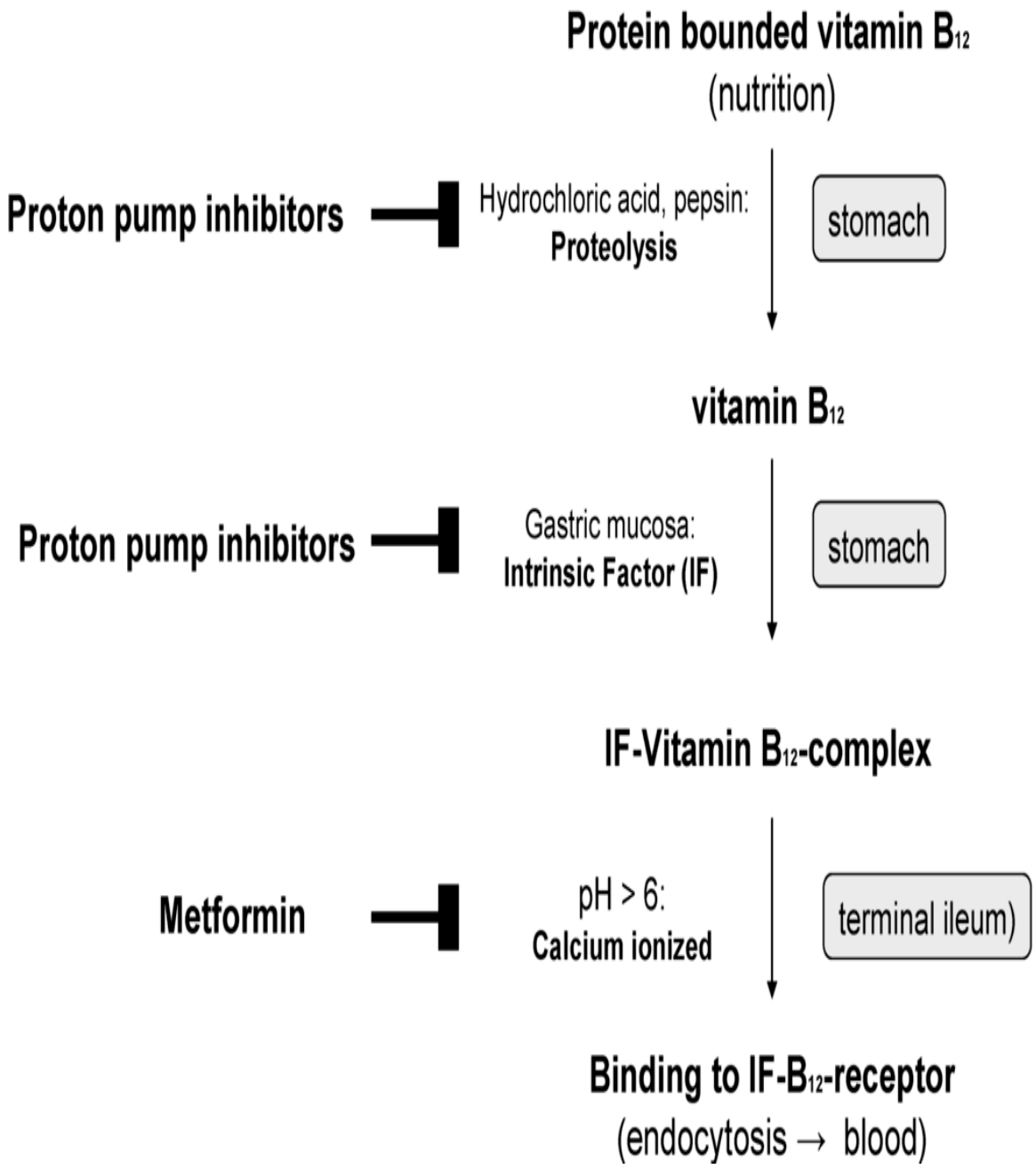
The mechanism for vitamin B12 deficiency induced by metformin therapy is not yet precisely defined. Studies during 1970s by Stovers and smith showed that the deficiency is not only due to prolonged

duration of therapy with metformin but also due to various other factors like diet, duration of diabetes, overall dosage used in each patient. Yet metformin being used as a first line drug in all type 2 diabetics it is essential to screen all the patients annually for their deficiency. Recent evidence related to this cause is due to interaction of metformin with the calcium channel and impairment of its activity. Soon after stopping metformin a detectable reduction in vitamin B12 can be noted, which can occur within 5 months. Yet, most of the patients are asymptomatic. For a patient to become symptomatic it may take atleast 5 to 10 years. For otherwise it is easy to miss a case of macrocytic anemia with or without signs of subacute combined regeneration of cord. So it is prudent to annually screen all patients who are on metformin therapy.The mechanisms causing the deficiency of vitamin B12 are controversial and were subjected to various studies(15). The postulated mechanisms are competitive inactivation and inhibition of cobalamin, individual variation in intrinsic factor level, gastrointestinal motility disorders, alteration in bowel bacterial flora, the inhibition at or alteration of the cubilin receptors(15). More appropriate explanation could be antagonism of the calcium mediated transport in the terminal ileum of the B12

complex. This is substantiatedby calcium supplementation improving the vitamin B 12 level in these patients(14).The cause of vitamin B12 deficiency with metformin is due to defective absorption of vitamin B12 at the terminal ileum. First it was believed that metformin caused modified proliferation of bacteria in the small bowel either due to intestinal dysmotility or an elevated intestinal glucose level.

The current, appropriate and more suitable explanation for metformin causing vitamin B12 malabsorption and deficiency is that metformin has the effect on calcium mediated membrane action in the terminal ileum(Fig 5).Absorption of the vitamin B12-intrinsic factor complex is calcium dependent and metformin interferes with this absorption. In support of this hypothesis, it is evident that dietary calcium supplementation brings back metformin-induced vitamin B12 malabsorption(Fig 5). In the view of varying pathogenic causes of this deficiency there is no absolute mode of treatment in these patients. The most common method of treatment used in these patients could be using intramuscular injection of Hydroxycobalamin as a loading dose, followed up with oral supplementation. But as the main interference occurs in the absorption process, it is wise to administer the sublingual form of vitamin B12. So parenteral followed by

sublingual supplementation is better than oral supplementation(16). But there are no studies that has clearly assessed this however. Intramuscular injections of vitamin B12 were proved superior than oral supplementation in studies with patients who have undergone gastric bypass surgery. But the relevance of sublingual group was not established in that also(17).



MECHANISM OF VITAMIN B12 MALABSORPTION

Fig 5

Of all the patients who were given metformin, 10–30% are having the evidence of low vitamin B12 absorption. Vitamin B12-intrinsic factor complex uptake by ileal receptors is mediated by a process dependent on calcium availability. Metformin deranges this calcium dependent uptake action (fig 5). Calcium supplementation in these patients is said to correct the malabsorption. However there is no parallel increase in biochemical B12 level. The effect of calcium was not researched independently without metformin, and therefore it is not known whether calcium increases B12 absorption or it corrects metformin induced deficiency. Despite improving the vitamin B12 absorption, calcium is said to increase the cardiac mortality and morbidity in already predisposed diabetic patients. In diabetic patients, there can be normal serum level of vitamin B12 with abnormal metabolites of vitamin B12 pathway within the cell. This indicates usage of metformin not only cures malabsorption but also clears the intracellular resistance to vitamin B12. This is shown by reduced level of vitamin B12 metabolites within the cell. With usage of metformin the serum B12 level appears to decrease because of increased intracellular utilization(18). This concept is proved with

analysis of RBC extracts and metabolite estimation in it(methylmallonic acid).

In diabetics not using metformin, the metabolite level is low within the cell, but the extracellular level of metabolites is elevated. So there is elevated vitamin B12 level in diabetics with deficiency inside the cell. This is manifested as increased serum homocysteine and this acts as a marker of vitamin B12 resistance in diabetics not taking metformin.

There is no recent evidence to suggest that the treatment with metformin in type 2 diabetics will unmask the vitamin B12 metabolic status of the patient. But it is suggested that by altering the metabolic marker levels within the cell, it improves the cobalamin metabolic pathways(18).

A study done by Greibi Etal was aimed at detecting the different available absorption pathways for vitamin B12 in presence of metformin. There they used rats as study population. Rats were given S.C injection of metformin and saline for subjects and control groups respectively for a period of three weeks. Following this procedure the level and the quantity of vitamin B12 was assessed and

the regions of distribution was detected using radioisotope B12. The results were analyzed and it showed similar patterns with other studies such that the treatment with metformin decreased vitamin B12 level. The results were significant as the 3week exposure to metformin reduced vitamin B12 in 22% of the rats. But the exact mechanism causing this reduction was not evaluated completely in this study. Regionalization of the vitamin B12 level in the subjects was done and it showed increased concentration of vitamin in the liver. But the total absorbed vitamin B12 level in both control and subject rats were similar. This suggest that there are no absorption defects induced by metformin rather the reduction in level is due to redistribution. Kidneys of the subject rats treated with metformin showed 36% reduction in vitamin B12 level than control rats treated with saline. This also reflects the redistribution of vitamin B12 to liver. But the shortcoming of the study is we cannot translate the exact findings into the humans because the pathogenesis of diabetes varies highly between two different species. As we have seen before, if the diabetic patients have B12 resistance at cellular level this study did not provide a solution to this resistance. Yet the crucial outcome of the study is the mode of administration that is used. It is a gastric bypass system.

Hence subcutaneous or intramuscular or sublingual administration will mask the malabsorption produced by metformin interaction at the small bowel or ileal level

Further studies where metformin treatment and its outcomes have resulted in useful data in decreasing the mortality and morbidity of different carcinomas(20). This is attributed to the glycemic control by metformin which increases glucose utilization by peripheral large tissues like muscles. And this limits the available glucose for proliferation of cancerous cells. Some investigations showed that there are some alternate mechanisms for metformin like inhibition of mTOR and AMPK or LKB1 axis in the cancer cells. These pathways are concerned with energy production and protein synthesis inside the cells. A meta-analysis showed reduction in the risk of developing hepatic, breast, colon and pancreatic tumors by 78%, 6%, 23%, 46% respectively(20).

Vitamin B 12

Cobalamin which is called as vitamin B12, is a member of B group of vitamins. It is said to play vital role in blood, brain, nerve function. The biochemical active form of vitamin is said to be in different forms depends on method of synthesis.

The richest sources of vitamin B12 are meat and eggs. Milk, fortified cereals, fish are some of the rich sources of vitamin B12. So vegetarians are at the risk of developing vitamin B12 deficiency. The daily required adult dose is around 1-3 microgram, which is only 0.1% of total body store. The total body stores are of 2-3 milligram. So the total body store is sufficient almost for half a decade, in the absence of external supplies.

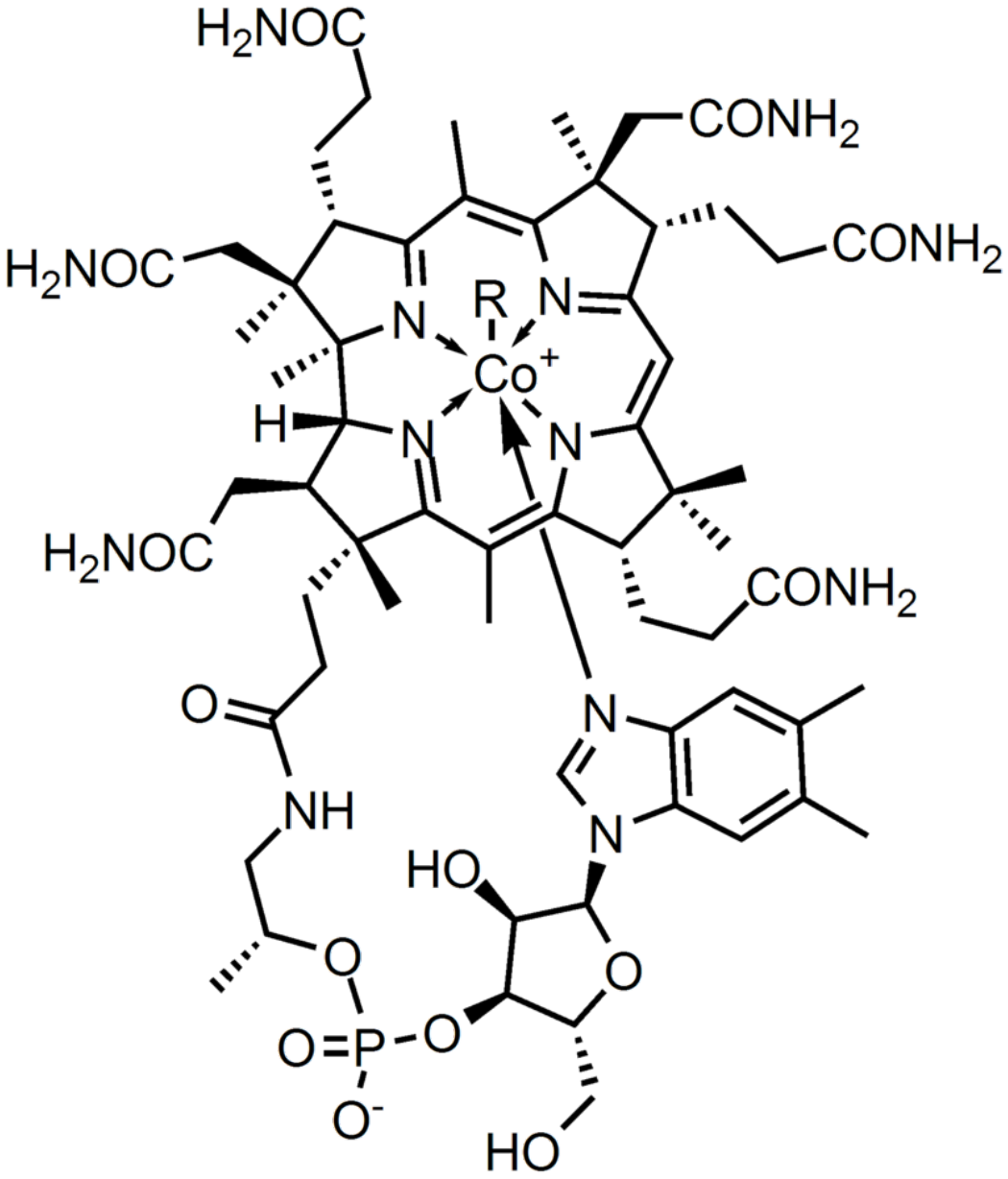
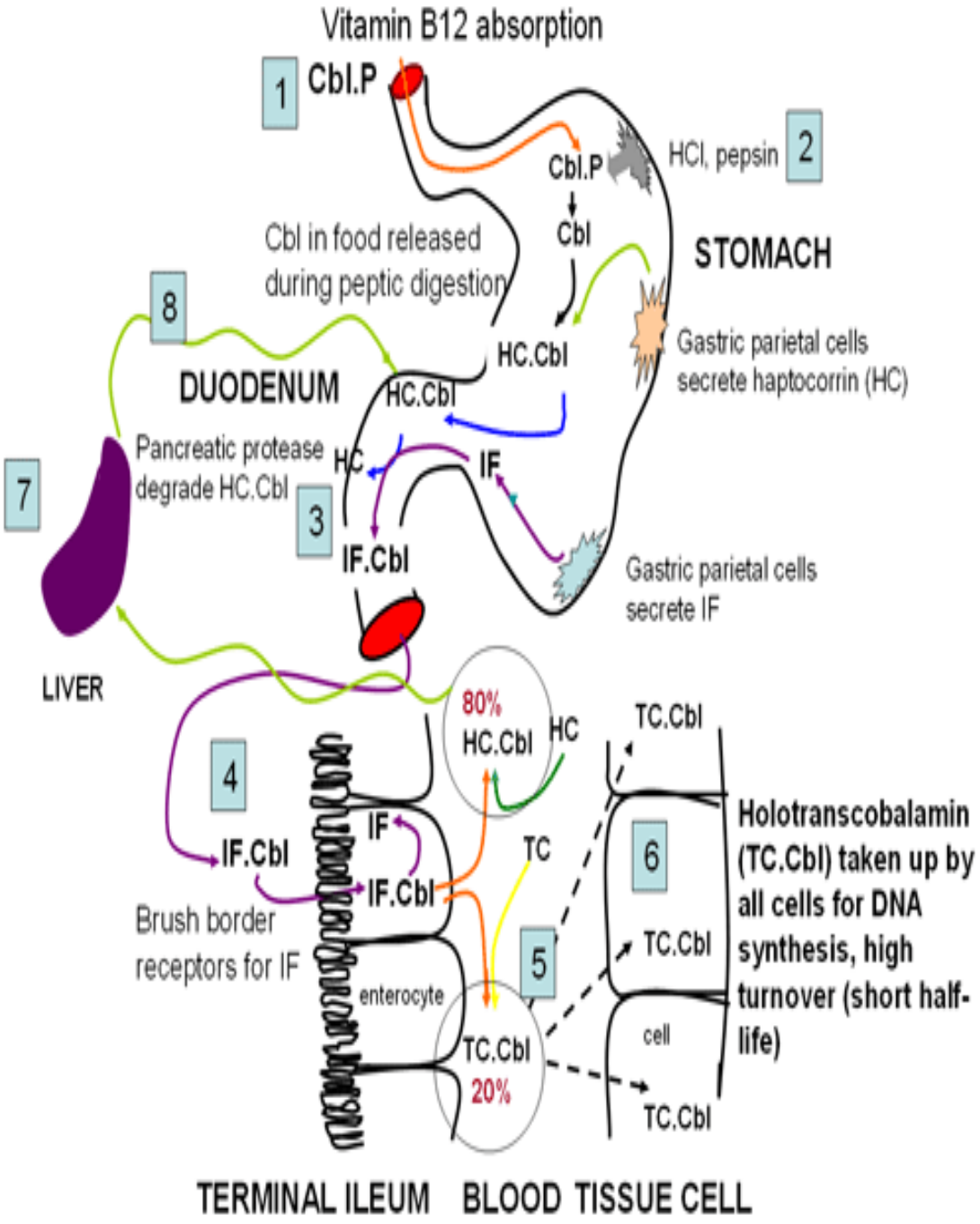

H2NOC
CONH2
H2NOC
CONH2
R
N
N
Co+
H
N
N
H2NOC
CONH2
O
N
NH
N
HO
O
O
O=P-O
O-
HO
R = 5'-deoxyadenosyl, Me, OH, CN

FIG 6

RECOMMENDED ALLOWANCES

Age	Male (mcg)	Female (mcg)	Pregnancy (mcg)	Lactation (mcg)
0-6 months	0.4	0.4		
7-12 months	0.5	0.5		
1-3 years	0.9	0.9		
4-8 years	1.2	1.2		
9-13 years	1.8	1.8		
14+years	2.4	2.4	2.6	2.8

FIG 7



VITAMIN B12 ABSORPTION

FIG 8

ABSORPTION AND TRANSPORT

There are two mechanisms by which vitamin B12 is absorbed. Passive process occurs through small intestine mucosa. But passive process is highly inefficient and extremely fast. Thus the normal physiological absorption process occurs by active diffusion at the level of terminal ileum in the presence of gastric intrinsic factor. Cobalamin in the diet is separated from its protein complexes by the action of gastric and small intestinal enzymes. Then it binds to the R- binder with which it is transported to the terminal ileum. Intrinsic factor is secreted from stomach parietal cells. It combines with cobalamin and forms IF-Cobalamin complexes and transported to terminal ileum. This complex attaches itself to receptor cubilin. It is an endocytic receptor protein which helps in the translocation of complex into the enterocytes, where the intrinsic factor is destroyed. With a lag period of six hours cobalamin appears in the portal circulation combined with transcobalamin II. High amount of cobalamin undergoes enterohepatic circulation from the denuded intestinal epithelial cells. Hence the risk of this vitamin deficiency is higher in patients with malabsorption than in vegetarians. The transport of cobalamin occurs in one to one molecule manner. Transcobalamin I is formed from the granules of

neutrophils. It has no primary role in transport of cobalamin into the tissues. The major transport protein is transcobalamin II. It is produced from the liver endothelial cells, ileal enterocytes and macrophages. This takes up cobalamin to areas of high demand like marrow, placenta.

TABLE 2. Clinical manifestations of vitamin B_{12} deficiency*

Type	Clinical manifestations
Haematological	Macrocytosis (frequent)
	Isolated thrombocytopenia and neutropenia, pancytopenia (rare)
Neuropsychiatric	Combined degeneration of the cord (classic)
	Peripheral neuropathy (frequency)
	Ataxia
	Optic atrophy (rare)
	Dementia
	Psychosis, depression
Digestive	Hunter's glossitis, angular stomatitis, jaundice, lactate and bilirubin elevation (classic)
Hyperhomocysteinaemia	Cardiovascular and thromboembolic risk

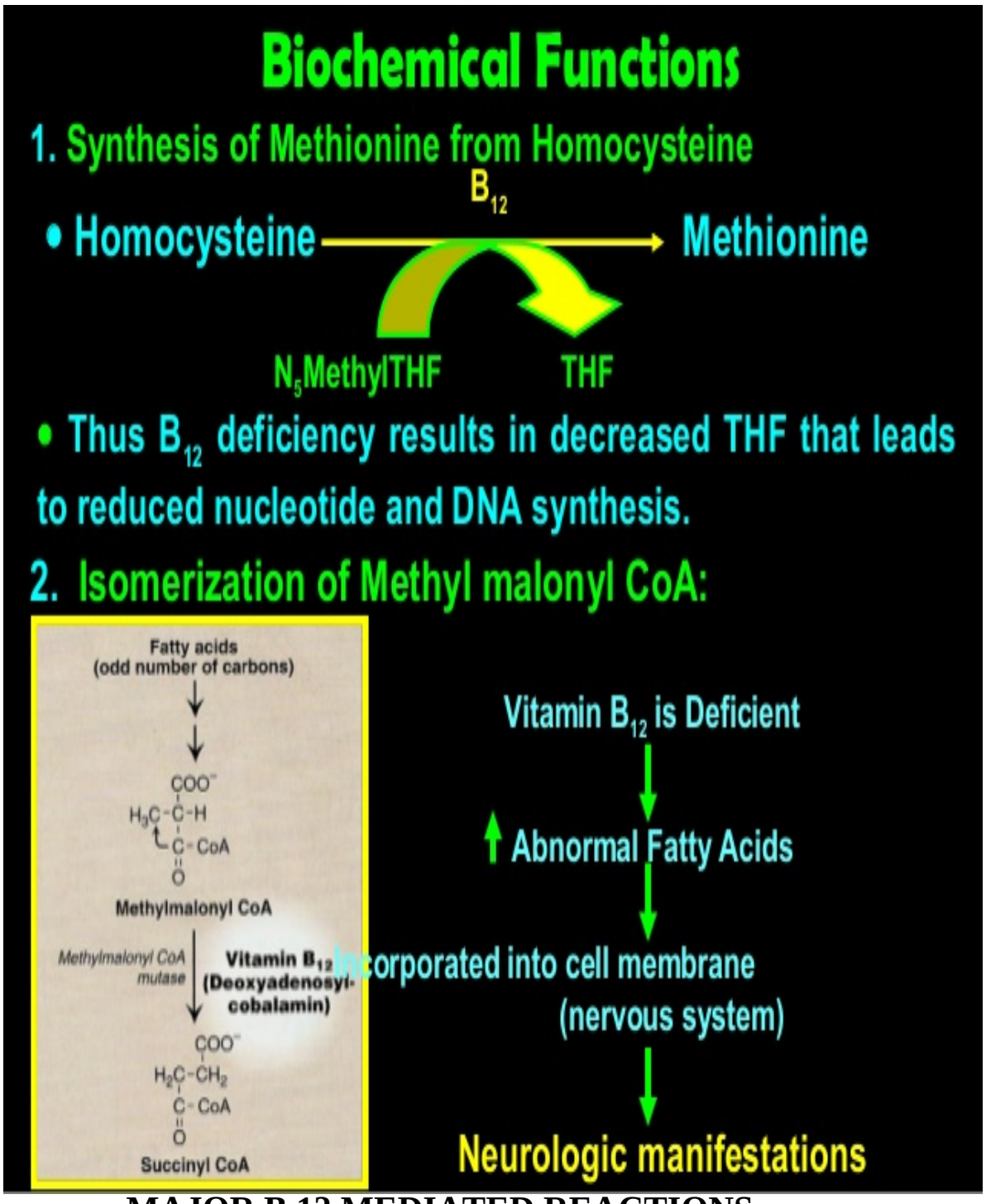
* Adapted from Reference 9

FIG 9

Causes of Cobalamine Deficiency

1. Reduced intake
2. Malabsorption – Addisonian pernicious anemia, Gastrectomy, pancreatic dysfunction, Tropical sprue, Zollinger Ellison syndrome
3. Food cobalamine malabsorption- Atrophic gastritis with achlorhydria
4. Abnormal transport protein- Tco I/II deficiency
5. Inborn error of cobalamine metabolism
6. Acquired drug effects

FIG 10



MAJOR B 12 MEDIATED REACTIONS

FIG 11

The different forms vitamin B12 is playing an important role in vital physiological chemical reactions in the body. Adenosylcobalamin is the coenzyme for, the methymalonyl-CoA conversion to succinyl-CoA, where methylmalonyl-CoA mutase acts as enzyme. Methylcobalamin is the co-enzyme in the conversion of homocysteine to methionine, where transfer of a methyl group occurs. The methyl group is donated by methylcobalamin. This methyl transfer is catalyzed by methionine synthetase. In the subsequent reactions methyl cobalamin is reproduced by one carbon transfer mechanism with the contribution from folate. SAM(S-Adenosyl methionine) is a derivative of methionine, where the methyl transfer to methionine has taken place. S-Adenosyl methionine is useful for life molecule synthesis like DNA and RNA, neuro transmitter formation and methylation that occurs in myelin synthesis(22). In the deficiency of vitamin B12, the generation of S-Adenosyl methionine is hampered which ultimately predisposes the patient for defective myelination and nucleotide synthesis. This is done by methyl transfer that occurs from SAM. The methionine synthase action needs a balance of carbon molecules which directs towards synthesis of nucleotides or the methylation of substrate compounds by S-adenosylmethionine

(SAM). The role of Cbl in this function may alter some epigenetic functions of an individual through specific methylation of DNA, RNA, histone and gene expression which can result in different physiological differences, depending on the site of methylation(21)

The clinical picture in a vitamin B12 deficiency patient is very different to differentiate as the symptoms of early disease is highly nonspecific. Most of the patients being of elderly age group and having long term diabetic history and they are predisposed to multiple micronutrient deficiency. Some of the most common presentations are easyfatigability or “general tiredness”. This shows the role of vitamin B12 in the regulation of sleep cycle. The two other routinely noted complications in these patients are burning sensation, numbness of feet and in very deficient cases,anemia.Both conditions overlap with the chronic conditions these patients may be having, yet they are one of the most important treatable conditions. This easy fatigability can be due to hypoglycemia due to drugs, or due to hyperglycemia, or the dietary habits of the patient like taking low carbohydrate diet. The peripheral nerve involvement can occur in the diabetic patients itself. So these are difficult to differentiate in the first clinical instance.The currently used biochemical markers for diagnosis of Vitamin B12

deficiency are under scanner and controversy. The routinely used serum B12 assay is said to be not sufficient for the diagnosis of vitamin B12(18). The use of vitamin B12 in the diagnosis of this depends on the range of values where level under 149 pmol/L is deficient status, with low or borderline levels being in the level of 148-220 pmol/L. Normal ranges of Vitamin B12range between 220-800 pmol/L. Values highly exceeding this values are to be seen with suspicion, as it may be associated with myeloproliferative status of these patients(23). The estimation of the metabolite biochemical markers such asmethyl malonic acid (MMA) and total homocysteine (tHcy) are said to be more appropriate for a correct diagnosis of deficiency with levels >0.28 μmol/L and >15 μmol/Lrespectively. These two are the markers of vitamin B12 deficiency. But when comparing the cost of both methods, estimation of MMA and tHcy is very costlier(23).

LABORATORY EVALUATION

The rise in MCV occurs early than the development of anemia in cases of vitaminB12 or folate deficiency. The detection of Macrocytosis in the pre auto analyzer era was not very frequent. But in this period, it is very easy to detect the macrocytosis with the help

of these automated analyzer. The clinical picture of vitamin B12 deficiency was not hematological previously. Now it becomes easier to pick the B12 deficiency cases with hematological picture. “ An unexplained rise in mean cell volume (MCV) of 5fl or more even within the normal age should also attract suspicion”. Dimorphic anemia is the coexistence of both iron deficiency anemia and vitamin B12 deficiency. In the presence of these blood picture the vitamin B12 blood picture will not be seen in a classical way.

“The earliest classical picture that is seen in the peripheral blood in the case of vitamin B12 and folate deficiency is Neutrophilic hypersegmentation. More than 5% of the neutrophils with five lobes or more than 1% of the neutrophils with 6 lobes is highly suggestive of vitamin B12 deficiency”. The blood picture can be similar in case of a myelodysplasia. In extreme deficiency status, the DNA synthesis is affected to such levels that, pancytopenia can occur in such conditions. Even an indolent leukemic process can occur which may mimic a vitamin B12 deficiency.

SERUM COBALAMIN (Pg/ml)	200-900	150-500	100-300	50-250	50-250
SerumMMA (Umol/L)	<0.4	<0.4	<0.4	0.4-20	1-20
Hypersegmented Neutrophils	0	0	0	+	++
MCV (fl)	80-95	80-95	80-95	90-110	100-130
Hemoglobin (Hb)	12-15	12-15	12-15	12-15	<12

Table 1

PERIPHERAL SMEAR PICTURE IDEALLY WOULD BE,

Peripheral Blood Smear – Pancytopenia, **macro-ovalocytes**, anisocytosis, poikilocytosis, Howell-Jolly bodies, polychromasia, **hypersegmented neutrophils**, may see teardrops and schistocytes.	
Increased ↑	MCV > 100; RDW
Normal	MCHC
Decreased ↓	Reticulocyte count

FIG 12

DIFFERENCE BETWEEN MEGALOBLASTIC ANEMIA AND HEMOLYTIC ANEMIA:

	Megaloblastic anaemia	Hemolytic anaemia
Reticulocyte count	Decreased	Increased
Macroovalocytes	Present	Absent
Hypersegmented neutrophils	Present	Absent
Features of intravascular haemolysis	Mild	Severe and prominent
Serum LDH	Very high	High
Pancytopenia	Common	Uncommon, except with hypersplenism and PNH
Peripheral smear	Hypersegmented neutrophils	Polychromasia, nucleated red cells
Bone marrow	Erythroid hyperplasia with orthochromatic megaloblasts	Erythroid hyperplasia
Red cell survival	Normal or nearly normal	Decreased
Complications of hemolysis	Generally absent	Present in cases with long history

FIG 13

LAB DIAGNOSTIC APPROACH TO MEGALOBLASTOSIS

STEPS IN THE EVALUATION OF MEGALOBLASTOSIS

1. Recognition of peripheral smear abnormality
2. Identification of causative vitamin deficiency
3. Recognition of the cause of the deficiency
4. Review following the therapy for the deficiency

The last part of this diagnostic approach is often not followed. Also in the early stages of deficiency, even when the values are low normal, it is prudent to follow the treatment of the deficiency. So not allowing the patient to the frank deficiency of the vitamin by treating the patient at the earliest even when the lab value starts to decrease is vital in the management of the vitamin deficiency.

1. RECOGNITION OF PERIPHERAL SMEAR ABNORMALITY

Blood count and morphology usually establishthe diagnosis of megaloblastic anemia. Occasionally, however, the picture may be modified or mild, and recognition may depend on active search for subtle morphologic changes. In general, the severity of megaloblastic morphologic abnormality is proportional to the severity of the anemia,

and early megaloblastosis may manifest only very mild changes.Consequently, 6-lobed neutrophils may be absent, and only an increaseof 4-lobed or 5-lobed neutrophils may be evident (24). Partially or incorrectly treated megaloblastosis may also look relatively normal morphologically, although Herbert has stated that incorrectly treated megaloblastosis never looks completely normal. A normal, or even low, mean corpuscular volume often resulting from microcytes coexisting with macrocytes-and masking of abnormal red cell morphology in bone marrow and peripheral blood is not unusual with coexisting iron deficiency though white cell changes are not thus masked. However, the MCV is otherwise a valuable screening tool and can occasionally uncover megaloblastosis in a patient whose hemoglobin level is still normal. Of course, macrocytosis occurs in non-megaloblastic states also, but examination of cell morphology is often sufficient to differentiate those from megaloblastosis. Megaloblastic changes found in erythroleukemia, various acute and chronic leukemia,some refractory anemias, and with antineoplastic drugs, may occasionally be impossible to differentiate from vitamin B12 or folate deficiency by morphology alone. The white cell changes may tend to be less classical (and in the leukemiaand refractory

anemias may be absent) and polyploidy may be more prominent in the conditions not associated with vitamin deficiency, but firm data on these aspects are lacking. It has also been suggested that vitamin-deficient megaloblasts tend to have a clock face chromatin pattern which is not seen in megaloblasts in some other conditions. Neutrophil hyper segmentation has been reported in iron deficiencyand renal failure. Whether subtle folic acid or vitamin B12 abnormalitiesin fact coexisted in most such cases is unsettled. Congenital neutrophil hyper segmentationhas also been described and may possibly occur in 1 percent of the population. Conversely, it has been claimed that neutrophil hyper segmentationmay not necessarily accompany folate deficiency in pregnancy and rare patients with the Pelger-Huet anomaly presented with 3-lobed or 4-lobed neutrophils rather than 6-lobed ones when megaloblastic.

Serum Vitamin B_{12}	*Serum Folate*	*Red Cell Folate*	*Status*
N	N	N	Normal
N	↓	N	Normal,* or early folate deficiency
N	↓	↓	Folate deficiency
↓	↓	↓	Folate deficiency, or combined folate and B_{12} deficiency
↓	N-↑	↓-N	Vitamin B_{12} deficiency

N = normal levels; ↓ = low levels; ↑ = elevated levels

*But usually with recent poor dietary intake.

FIG 14

2.IDENTIFICATION OF CASUATIVE VITAMIN DEFICIENCY

The development of vitamin assays has greatly simplified matters because vitamin B12 and folic acid deficiency can be differentiated neither morphologically, nor often, clinically. However, serum levels do not always reflect body stores and are affected by many factors. Further confusion may result from the interactions between serum vitamin B12 and folic acid levels which reflect in good part their metabolic interactions. Sometimes overlooked,is the coexistence of both vitamin deficiencies. Therefore, determination of both vitamins simultaneously is often desirable. Nevertheless, results can obviously be inconclusive even with all three tests combined.

SERUM FOLATE ASSAY

Most of the accumulatedexperience has been with the microbiologic assay, the most common using Lactobacillus casei.Because of the cumbersomeness ofmicrobiologic assay and the inhibitory artifact induced by antibiotic therapy, many radioisotopicassays have been developed and marketed commerciallyas kits. Unfortunately, great differences exist among the isotopic methods, and manyclinical laboratories have prematurely adopted and even modified kits without adequate testing. The effect and significance of many variables

remainscontroversial, and sufficient experience in comparing results to the standard microbiologic assay and to clinical data is lacking. Nevertheless, whilethe routine clinical use of folate radioassay is regrettable, the ultimate advantage of a reliable isotopic assay is clear. The microbiological assay too is subject tomany technical variables and results differ among laboratories.In fact, in some cases the "falsely" low radioisotopic assay, folate level due to serum folate binder abnormality may possibly reflect the true folate status more accurately thandoes the normal microbiologic assay level. Thecurrent status of radioisotopic folate assay has been recently reviewed. It bears emphasizing that low serum folatelevels need not, and often do not, mean actual folate deficiency and should be interpreted cautiously. Low levels have been found in apparently healthy control subjects. Too, an early rapid fall in serum folate, appearing not to reflect true depletion of body stores, has been described in subjects on a folate-poor diet.The mechanism for the fall appears to be the cessation of exchange between tissue stores and absorbed folate, andmay at least partially explain the unusually greatincidence of low serum folate levels in patientsin hospital. Such patients apparently require only reinstitution of a normal diet, but will progress to true

folate deficiency if the poor diet is continued. Identifying the level of depletion may sometimes be difficult, and much obviously depends on how one chooses to define deficiency.

SERUM FOLATE

Misleadingly Low Levels

1. Apparently normal persons.
2. Poor dietary intake, without actual folate deficiency.
3. Improper handling of blood sample (such as storage without freezing).
4. Drugs affecting assay: antibiotics, 5-fluorouracil, and possibly others.
5. Drugs not affecting assay: acetylsalicylic acid, alcohol, oral contraceptives.
6. Abnormality of serum folate-binding proteins: may give low results in pregnancy, oral contraceptive use, uremia and the like, with some radioassay systems butnot others.
7. Radioisotopic contamination of serum (for example, tracer injection for gallium or technetium scans).

Misleadingly Normal or Raised Levels

1. Ingestion of folate-rich food or vitamin supplementation.
2. Hemolysis of blood sample (red cell folate contentgreatly exceeds serum content).
3. Coexisting- vitamin B12 deficiency ("methyltetrahydrofolate trap" hypothesis, vitamin B12-dependententry of folate into red cells
4. Inborn errors of folate metabolism.

Red Blood Cell Folate

Red cell folate assay usually reflects tissuestores more accurately than does serum assay, it is less subjected to influence by extraneous factors, and correlates with severity of anemia and megaloblastosis whereas serum folate does not. Itsgreater specificity may be particularly useful when the existence of megaloblastosis is in question. Anadditional benefit is its helpfulness even after therapy has been started-thereby flooding the serum with folate-in the few days before folate repleted cells are released from bone marrow in significant numbers.Antibiotic use has little effect on red cell folate assay.

Misleadingly Low Levels

More than half of patients with vitamin B12 deficiency.

Improper handling of specimen.

? oral contraceptives.

Misleadingly Normal or High Levels

Early folate deficiency.

Blood transfusion.

Significant reticulocytosis (since reticulocytes are relatively richer in folate than older cells) &Iron deficiency.

SERUM B12

Misleadingly Low Levels

1. Folate deficiency
2. Pregnancy, near term-particularly seen with microbiologic assay.
3. Drugs: oral contraceptives, large amounts of vitamin C (by affecting assay).
4. Idiopathic: aplastic anemia, multiple myeloma, cancer.
5. Poor dietary intake in vegans (without true deficiency)
6. Transcobalamin I deficiency
7. ? Iron deficiency.
8. ? Old age.

Misleadingly Normal or Raised Levels

1. Vitamin administration before blood drawing.
2. Artifact of radioassay.
3. Serum vitamin B12-binding protein abnormality coexisting with vitamin B12 deficiency: chronic myelogenous leukemia,polycythemia vera.
4. ? Liver disease.
5. Transcobalamin II deficiency.
6. The generally higher results with some assay kits may be misinterpreted(must know reference values for individual kits).

3. RECOGNITION OF THE CAUSE OF THE DEFICIENCY

Once the deficient vitamin has been identified,the underlying disease must be sought. The differential diagnosis has been well covered in many reviews.

VITAMIN B12

1.Gastric studies - Direct assay of gastric juice for intrinsic factor content is conceptually the ideal test. In the absence & due to nonavailability of the intrinsic factor assay it is usually confirmatory to do an analysis on acidity study.

2.Radioactive vitamin B12 absorption test – Schilling test

3. Serum antibodies

Folate Deficiency

Determination of the cause of folate deficiency is more difficult than with vitamin B12 deficiency, and in fact is usually not even attempted. A major problem is the lack of a reliable, widely available test for folate malabsorption. The various methods, reviewed elsewhere, include measuring serum folate levels or urinary excretion of folate following a standard oral dose and tests resembling the radioactive vitamin B12 absorption tests in which isotopic folates are given. All require great expertise in carrying out and in interpreting results. A diagnosis of folate malabsorption, if made at all, is therefore usually established only indirectly by showing the presence of intestinal disease. However, in all truly deficientpatients without very obvious dietary cause of deficiency, further general investigation must be done for malabsorption. Drug effect or possibly increased utilization of folate (the last named, incidentally, has never been conclusively shown as a sole cause of folate deficiency) should also be considered.

DIABETES MELLITUS

Definition: It is an endocrine disorder which is characterized by polyuria, polyphagia, polydipsia clinically and features of hyperglycemia biochemically. It occurs due to absolute or relative insulin deficiency. Primarily it is divided into type 1 and type 2 diabetes mellitus.

Type 2 diabetes mellitus occurring in a middle aged to elderly people, is due to relative insulin deficiency and also insulin resistance.

SYMPTOMATOLOGY

Diagnosis of diabetes mellitus depends on medical history and few simple biochemical tests.

The hyperglycemic triad namely polyuria, polyphagia, polydipsia is an indirect evidence. Other associated features predominate in specific forms of diabetes. For example features of malabsorption and acute abdomen occurring in cases of pancreatitis. Most of the time the disease is asymptomatic, and it is identified in screening the patients for some other illness.

Generalized symptoms like weight loss, emaciation, paresthesias, acidotic breathing, recurrent vomiting, malaise, ocular disturbances, balanoposthitis, recurrent respiratory, urinary tract infections, dry

tongue, glossitis, tuberculosis, skin infections, poor wound healing, non healing ulcers, typical infections like sinonasal fungal infections all may indicate the underlying cause as diabetes mellitus. There can be acute presentations like ketoacidosis, diabetic coma , seizures.

The prevalence of diabetes has increased in the last two decades, presumably due to changing pattern of life style , changing dietary habits , increased consumption of refined sugar, high trans fatty acid intake, reduced physical activity etc. According to CDC estimation, the countries with maximum number of diabetics are China, India, United states, Brazil.

Diabetes mellitus is the reason behind most of the mortality that occurs. But the background evidence of diabetes being the cause of those deaths were underreported. Diabetes will be the cause of 11% of total medical expenditures in few decades worldwide.

CLASSIFICATION OF DIABETES MELLITUS (24)

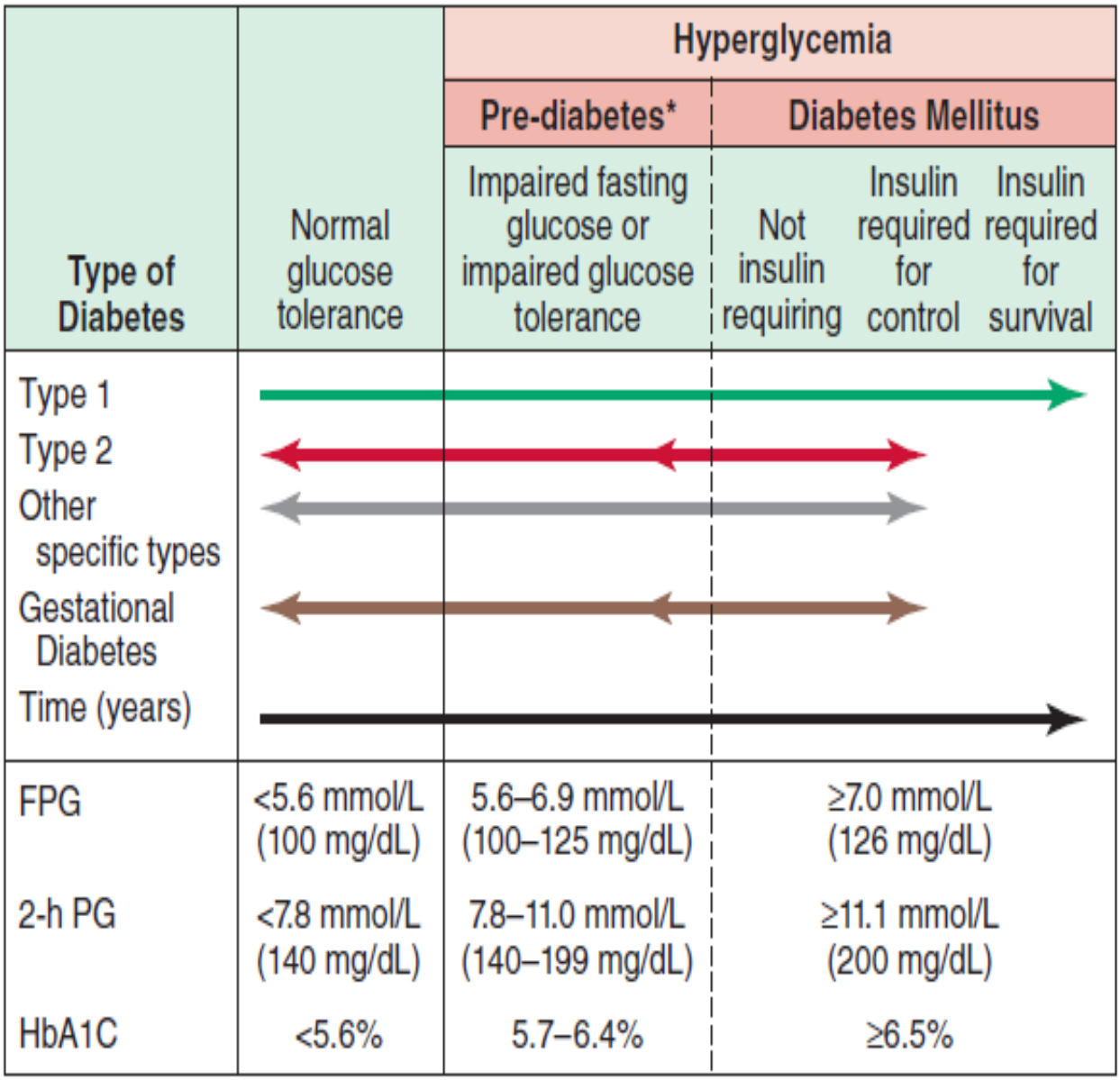


FIG 15

CRITERIA FOR DIAGNOSIS (24)

- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/L (200 mg/dL)[a] *or*
- Fasting plasma glucose ≥7.0 mmol/L (126 mg/dL)[b] *or*
- Hemoglobin A_{1c} ≥ 6.5%[c] *or*
- 2-h plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test[d]

[a]Random is defined as without regard to time since the last meal. [b]Fasting is defined as no caloric intake for at least 8 h. [c]Hemoglobin A_{1c} test should be performed in a laboratory using a method approved by the National Glycohemoglobin Standardization Program and correlated to the reference assay of the Diabetes Control and Complications Trial. Point-of-care hemoglobin A_{1c} should not be used for diagnostic purposes. [d]The test should be performed using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water, not recommended for routine clinical use.

Note: In the absence of unequivocal hyperglycemia and acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day.

FIG 16

SCREENING FOR DIABETES (24)

Rationales

1. Most of the diabetics are asymptomatic
2. Patients present with established complications at times
3. Early diagnosis and treatment will favor the outcome of the disease
4. Best for early diagnosis & prevention of progression of prediabetes to overt diabetes mellitus.

RISK FACTORS

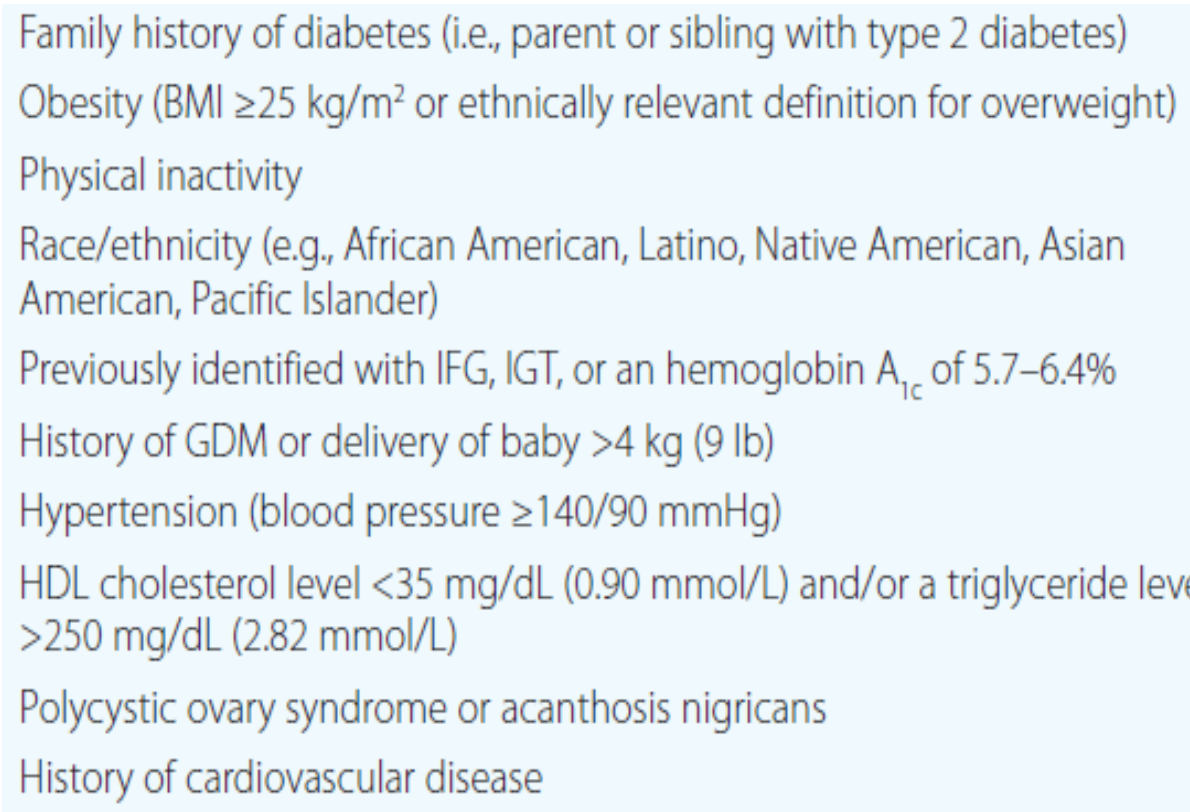


Fig 17

GLUCOSE HOMEOSTASIS

OVERALL MECHANISM INVOLVED

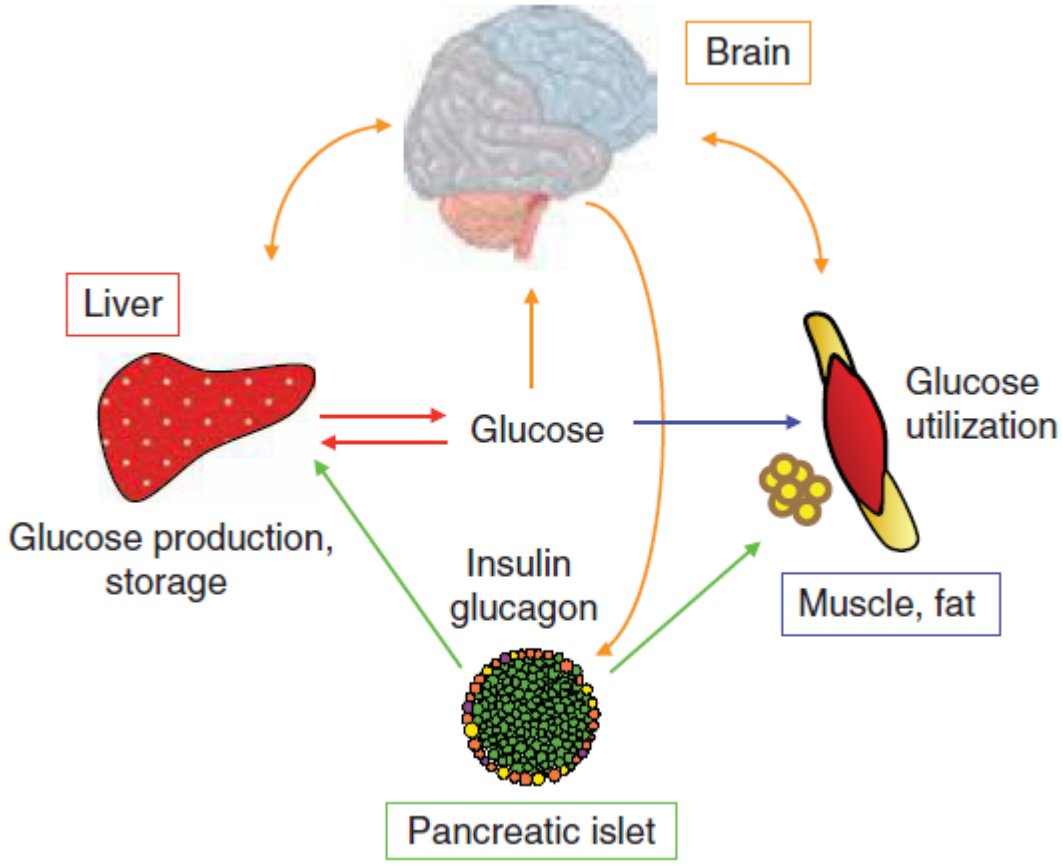


FIG 18

The overall glucose homeostasis is based on the balance between the liver glucose output and the peripheral utilization in the muscle, adipose tissue etc. The primary hormone involved in this homeostasis is Insulin. Other hormones, lipid derived factors, neural mechanisms are also involved in the glucose homeostasis.(24)

INSULIN PRODUCTION & SECRETION (24)

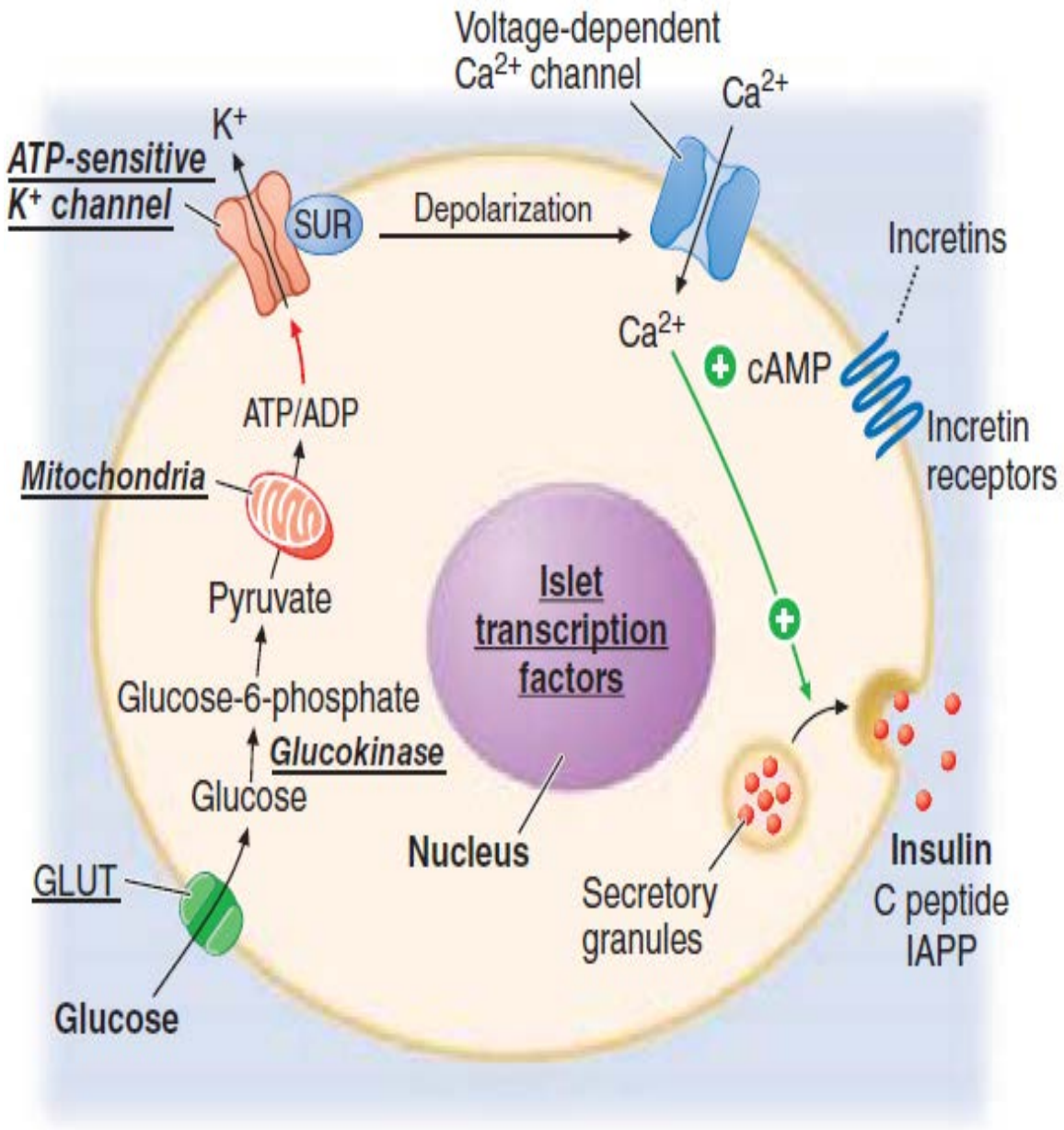


FIG 19

PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

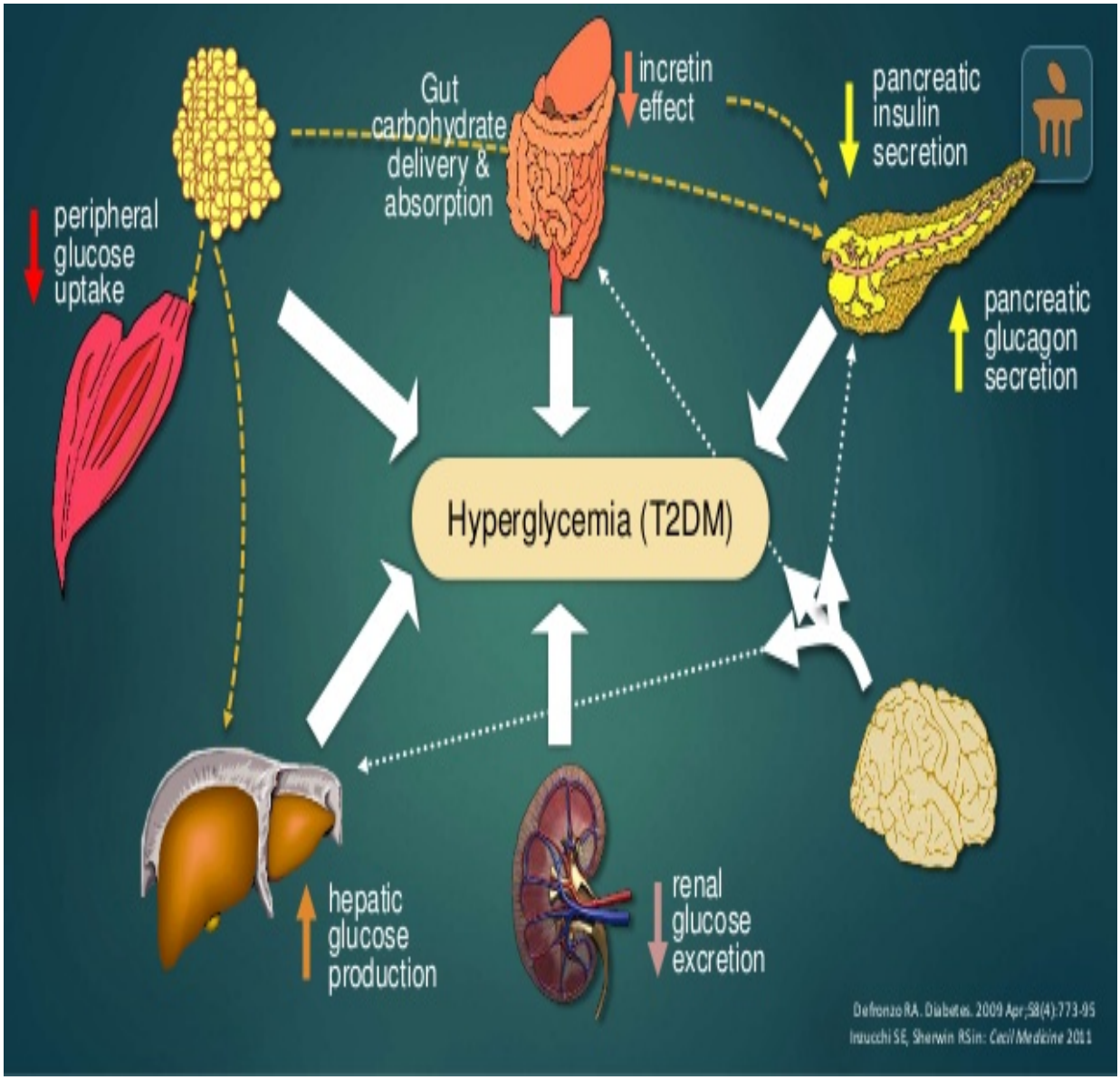


FIG 20

MATERIALS AND METHODS

The patients included in this study were recruited from the Government Mohan Kumaramangalam Medical College Hospital, Salem.

STUDY PERIOD:

August 2015- August 2016

STUDY DESIGN:

Cross sectional study

STUDY PLACE

Diabetic outpatient department, the Government Mohan Kumaramangalam Medical College Hospital, Salem.

STUDY POPULATION

Type 2 Diabetic patients, who were on Metformin therapy for more than 5 years

INCLUSION CRITERIA

50 cases of Type 2 diabetes mellitus patients who were on metformin therapy of more than 5 years

EXCLUSION CRITERIA

Patients with type 1 diabetes mellitus, pregnant women, prior vitamin B 12 injections, gastrectomy, colectomy, IBD, vegetarianism.

Patients with known chronic kidney disease, heart failure, liver cirrhosis, known malignancy.

Patients with over the counter medications, calcium supplements, histamine-2 blocker, proton pump inhibitor.

STUDY METHOD

- The work up will include history and physical examination of all patients,
- CBC ,
- Peripheral smear study,
- Renal function tests,
- Liver function tests,
- FBS, PPBS,
- Serum Vitamin B12 assay.

OBSERVATION & RESULTS:

MEAN VALUES OF ALL OBSERVATIONS:

MEAN VALUES OF PARAMETERS	
AGE	58.64 YRS
DURATION OF METFORMIN	9.3 YRS
DURATION OF DM	10.4 YRS
FBS	166.26
PPBS	267.8
BLOOD UREA	23.42
SERUM CREATININE	0.974
SGOT	23.04
SGPT	23.56
ALP	95.04
TOTAL BILIRUBUN	0.712
DIRECT BILIRUBIN	0.344
TOTAL PROTEIN	6.924
ALBUMIN	3.8
WBC	7380 CELLS
RBC	3.27 MILLION
PLATELET	2.3 LAKHS
ESR 1 HR	13.16
ESR 2 HR	24.16
HB%	10.43
PCV	32.16
POLYMORPHS	65.40%
LYMPHOCYTES	32.40%
EOSINOPHILS	2.20%
MCV	99.16
MCH	32.22
MCHC	31.88
VITAMIN B12	296.62

Table 2

CHART & BAR DIAGRAM SHOWING THE SEX DISTRIBUTION IN THIS STUDY

	SEX
MALE	24
FEMALE	26

Table 3

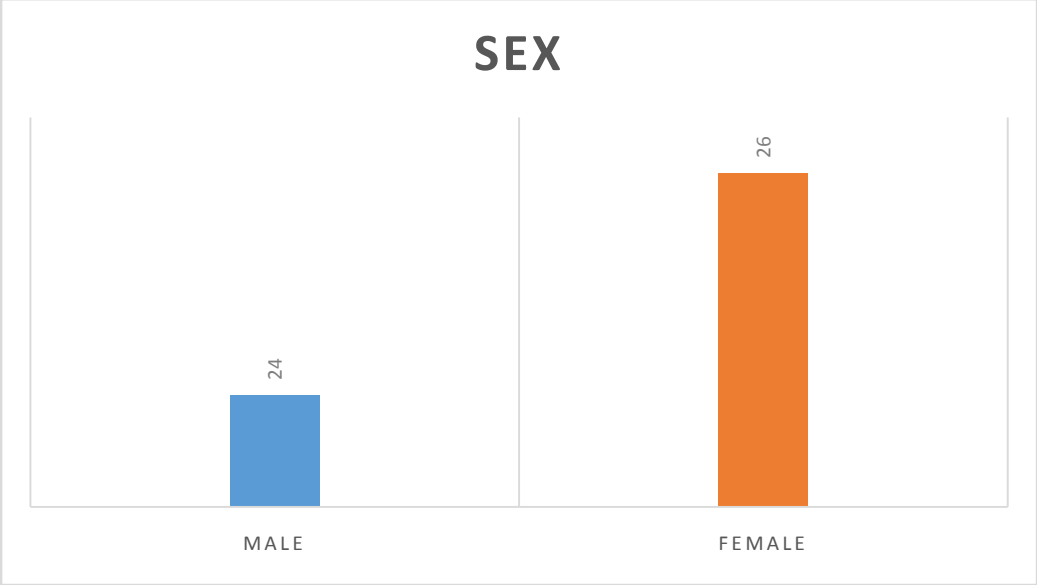


CHART 1

The distribution of both sex is equal in the study. The study included 50 total no. of diabetic patients, out of which 24 were males and 26 were females.

DIFFERENTIATION BASED ON AGE - TABLE & CHART

	AGE
< 60 YRS	35
> 60 YRS	15

Table 4

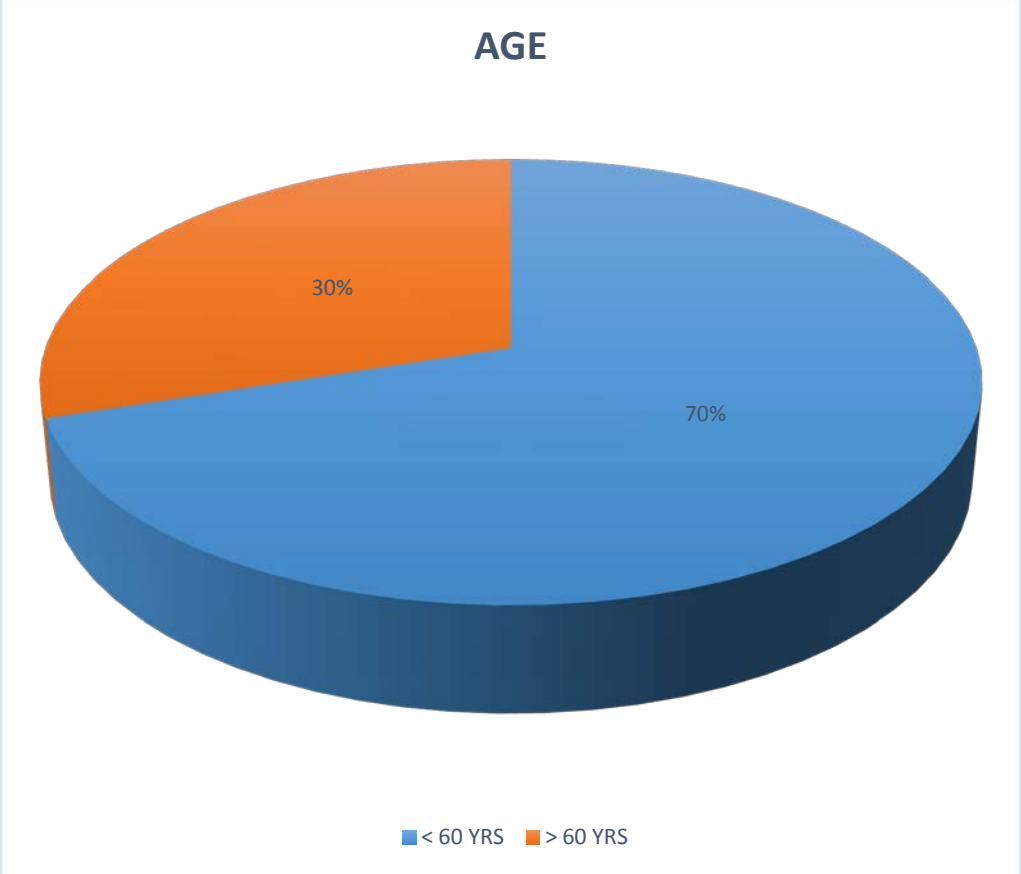


CHART 2

Most of the people were more than 60 years of age in this study.

DIFFERENTIATION BASED ON DURATION OF METFORMIN– TABLE & CHART

	DURATION OF METFORMIN
< 10 YRS	37
>10 YRS	13

Table 5

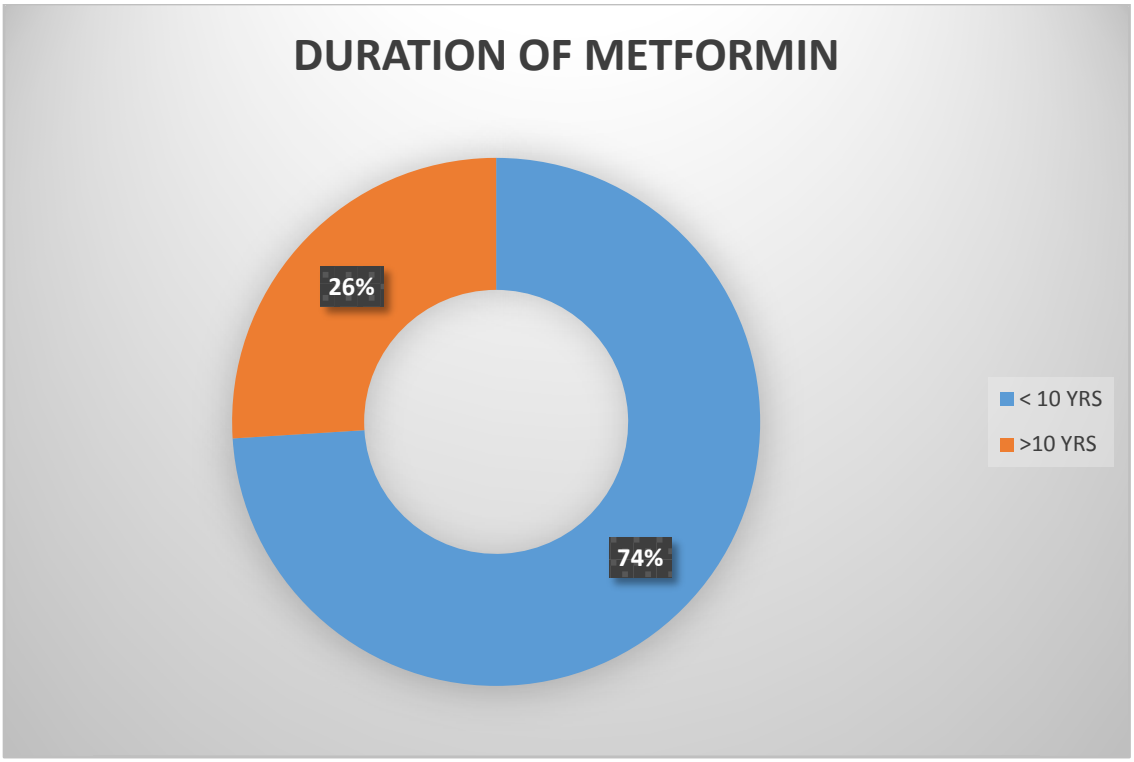


CHART 3

Majority of study population were taking metformin less than 10 years of duration(74%). 26% of population were taking metformin less than 10 years of duration.

DISTRIBUTION OF MACROCYTOSIS – TABLE & CHART

	MCV
NORMAL	32
HIGH	18

Table 6

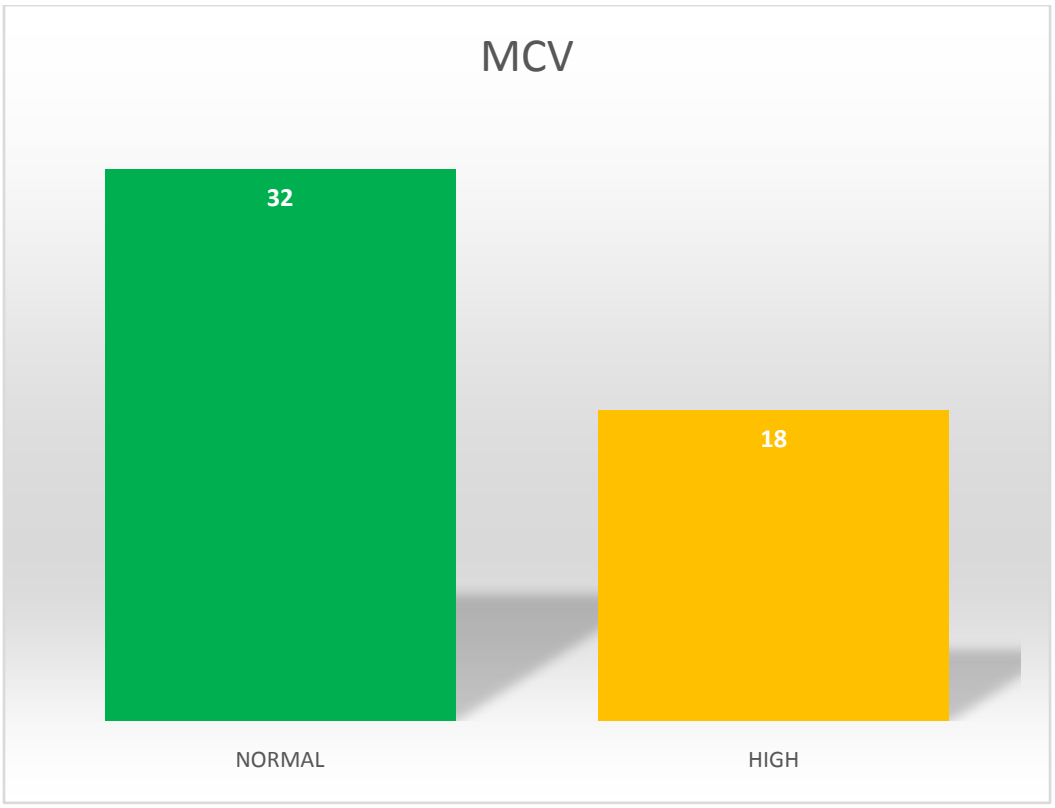


CHART 4

Macrocytosis was noted in peripheral smear in 18 patients.

DISTRIBUTION BASED ON VITAMIN B12 LEVEL

	VITAMIN B12
NORMAL	8
BORDERLINE	30
DEFICIENT	12

Table 7

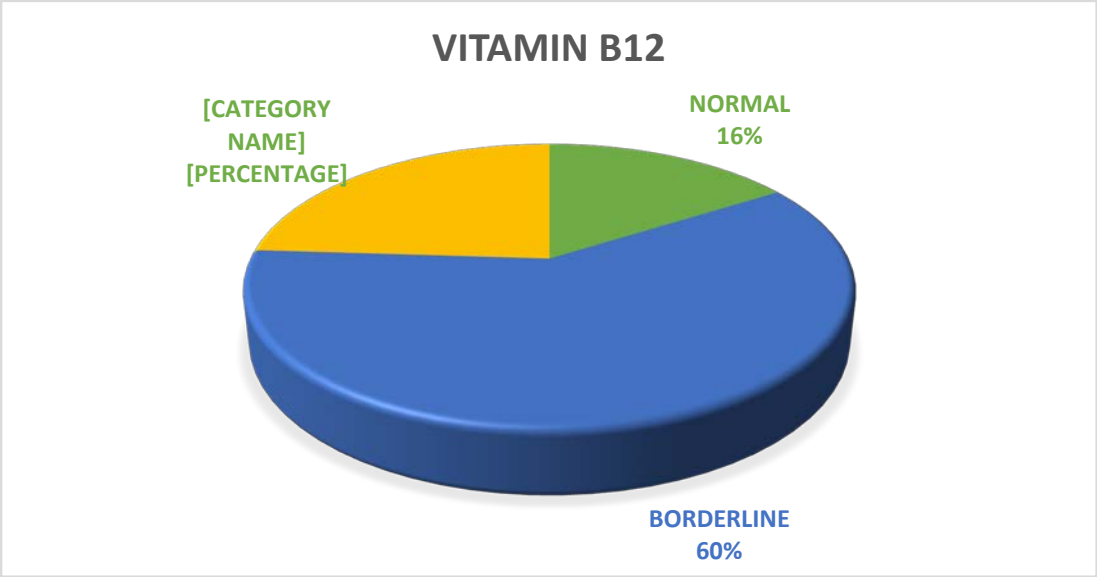


CHART 5

Out of the 50 patients, almost 30 had borderline deficiency in vitamin B12. Irrespective of the glycemic status, duration of diabetes and metformin therapy, 60% of the total patients had borderline deficiency.

DISTRIBUTION BASED ON PERIPHERAL SMEAR PICTURE – TABLE & CHART

	RBC - PERIPHERAL SMEAR
MACROCYTIC CELLS	12
MICROCYTIC HYPOCHROMIC	6
NORMAL	32

Table 8

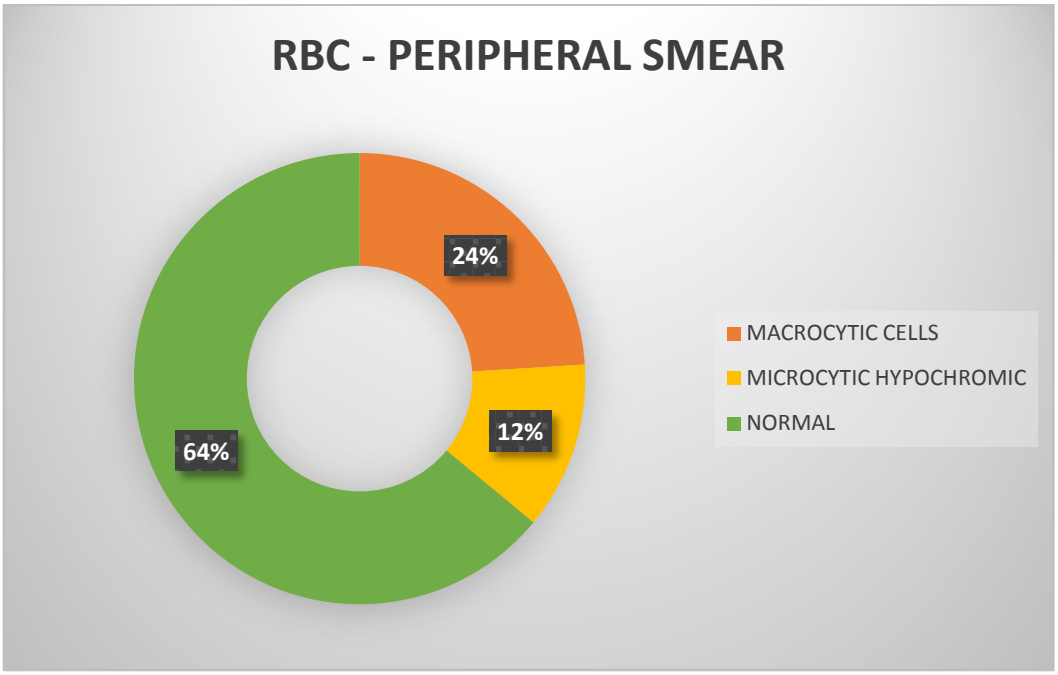


CHART 6

Majority of the population was having a Normal blood picture. Macrocytosis is noted in 24% of patients.

RELATIONSHIP BETWEEN VITAMIN B12 & PERIPHERAL SMEAR - TABLE & CHART

SMEAR	VIT B12 LEVEL		
	DEFICIENT	BORDERLINE	NORMAL
MACROCYTOSIS	7	2	3
MICROCYTIC	0	6	0
NORMAL	5	22	5

Table 11

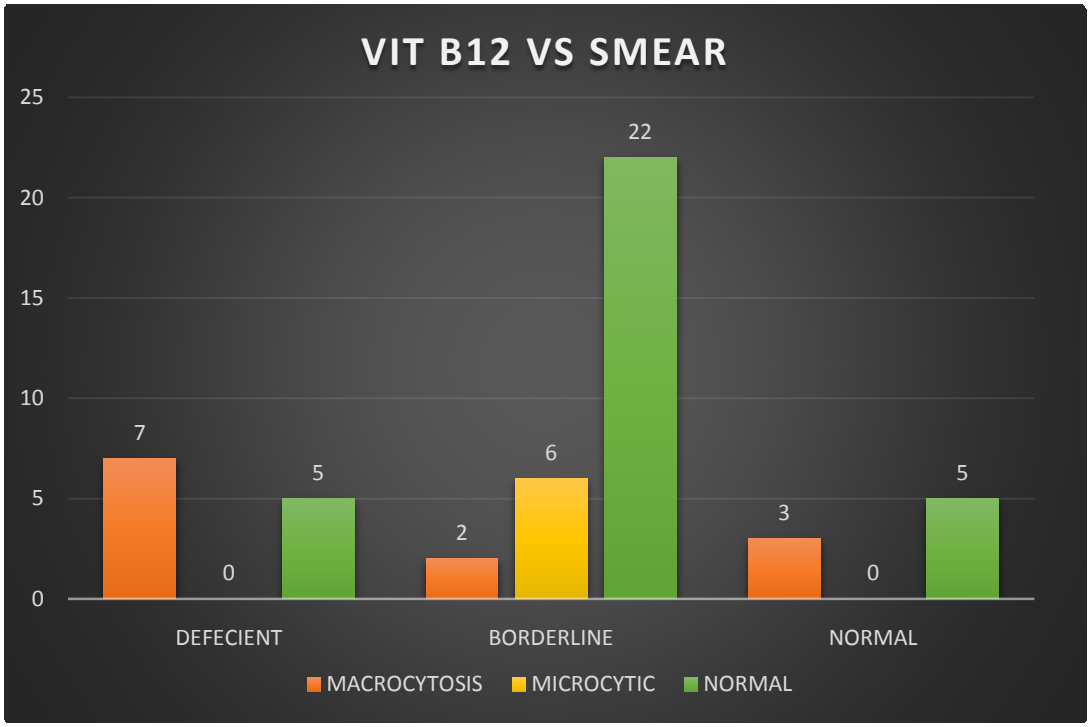


CHART 7

MACROCYTOSIS & VITAMIN B12

VIT B 12 LEVELS	AMONG MACROCYTOSIS(N=12)
DEFECIENT	7
BORDERLINE	2
NORMAL	3

Table 10

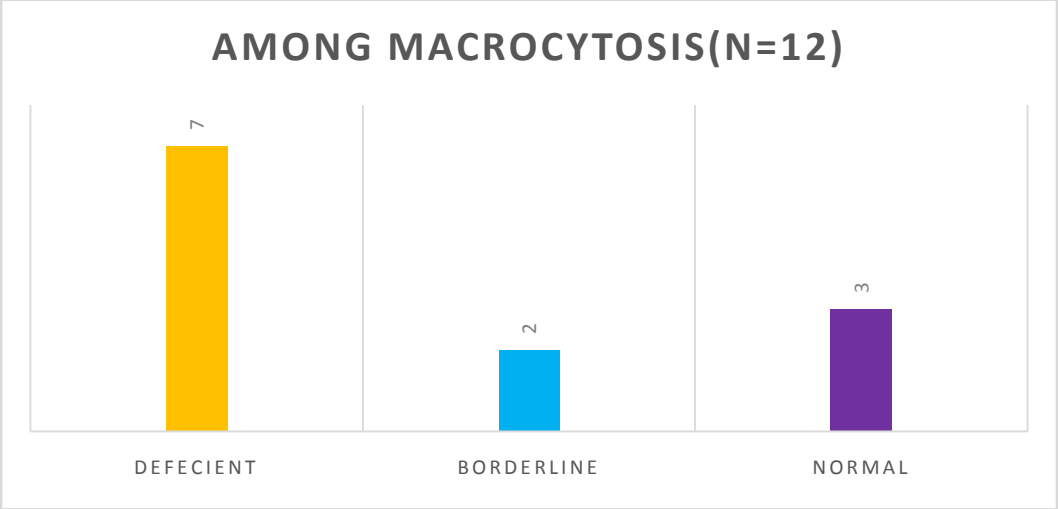


CHART 8

Predominant vitamin B12 is seen in the borderline deficient groups. Macrocytosis is predominantly seen in the deficient group. Other groups also have macrocytosis, but in lower number. P value is not significant for difference between males and females.

SIGNIFICANCE OF VALUES

SIGNIFICANCE OF VITAMIN B12 & SEX

VITAMIN B12 LEVELS	SEX	
	MALE	FEMALE
DEFICIENT	7	5
BORDERLINE	12	16
NORMAL	5	3
P VALUE	0.416	
SIGNIFICANCE	NON SIGNIFICANT	

Table 11

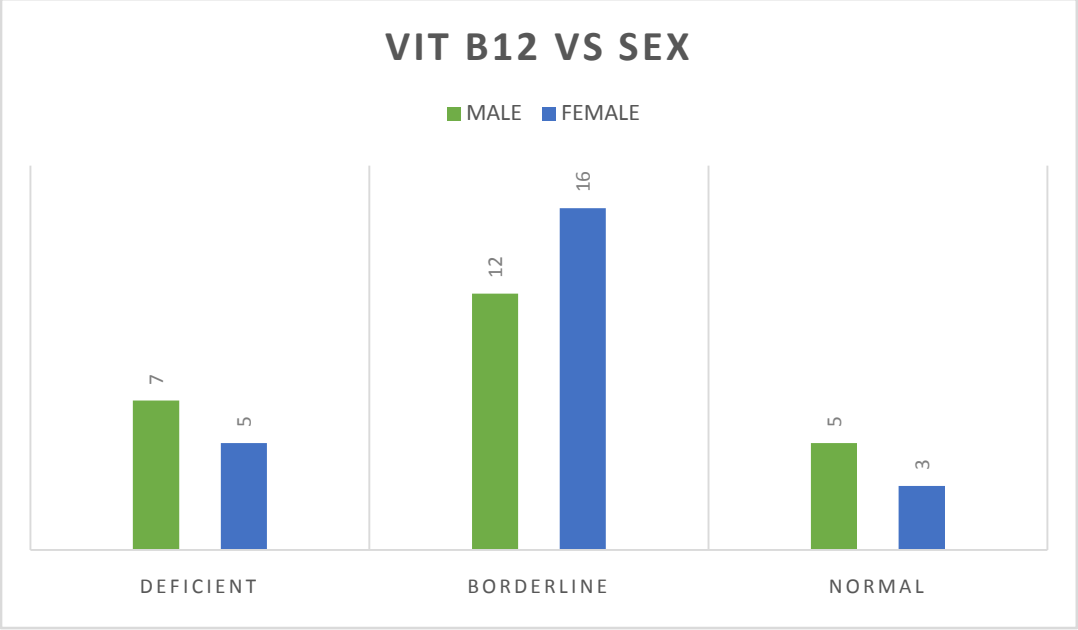


CHART 9

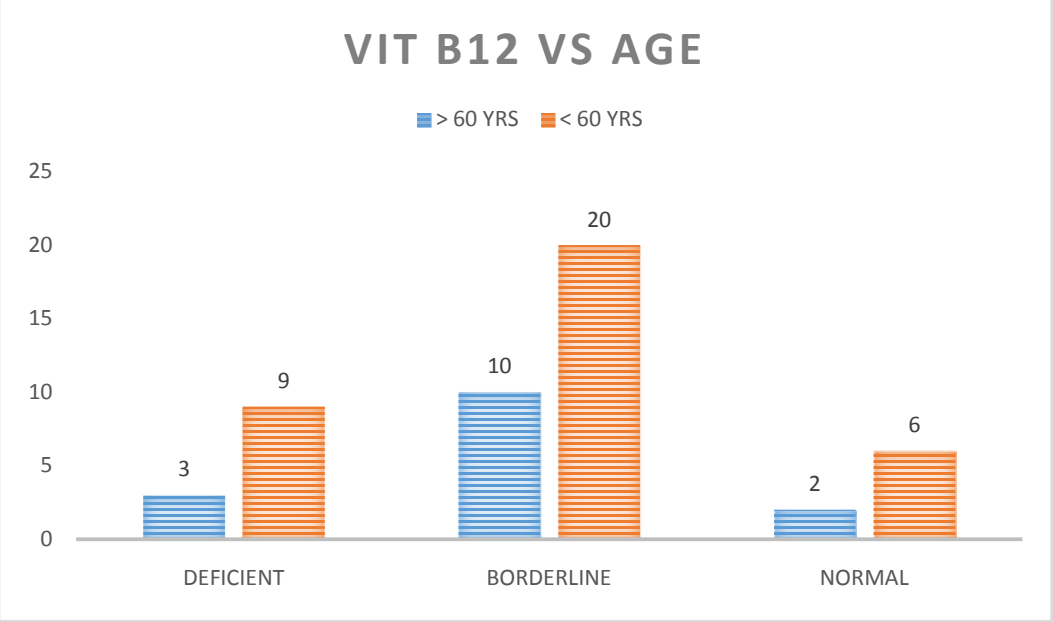
There is no significant correlation with sex & vitamin B12 in this study.

Predominantly patients are from borderline (female sex).

SIGNIFICANCE OF VITAMIN B12 LEVEL& AGE – TABLE & CHART

VITAMIN B12 LEVELS	AGE	
	> 60 YRS	< 60 YRS
DEFICIENT	3	9
BORDERLINE	10	20
NORMAL	2	6
P VALUE	0.668	
SIGNIFICANT	**NON SIGNIFICANT**	

Table 12



There was no significant correlation between age & vitamin B12 deficiency.

CHART 10

SIGNIFICANCE OF VITAMIN B12 LEVEL& DURATION OF METFORMIN

VITAMIN B12 LEVELS	DURATION OF METFORMIN	
	> 10 YRS	< 10 YRS
DEFICIENT	4	8
BORDERLINE	7	23
NORMAL	2	6
P VALUE	0.511	
SIGNIFICANT	**NON SIGNIFICANT**	

Table 13

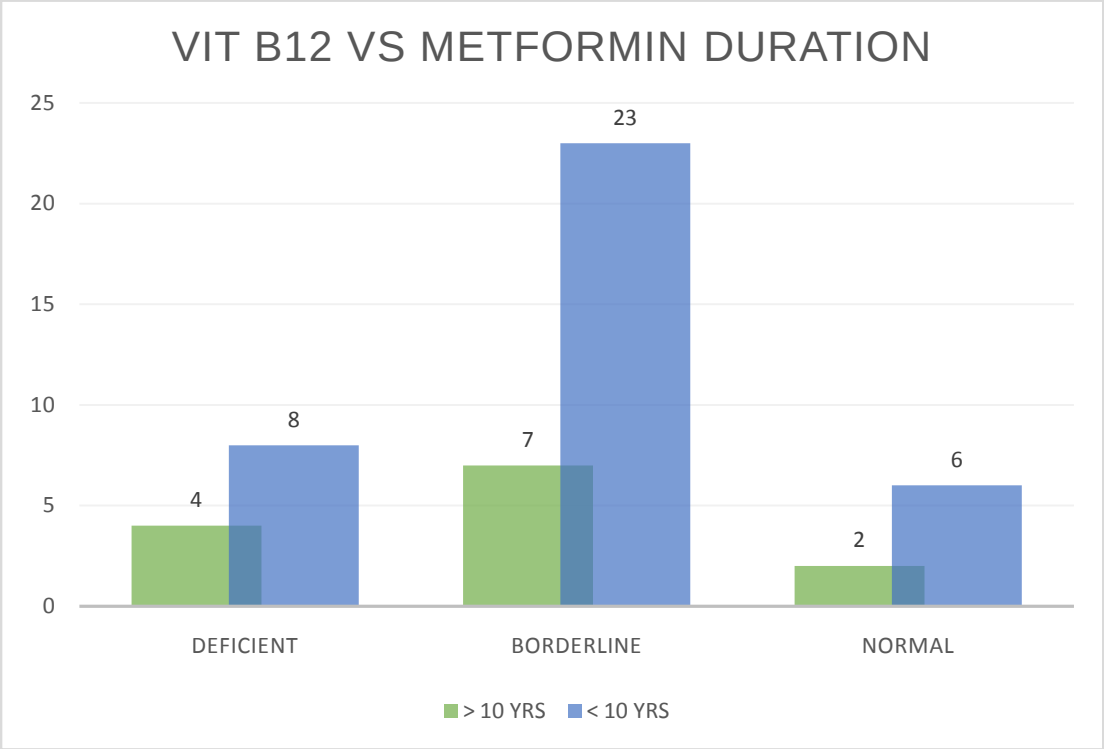


CHART 11

There was no significant correlation between duration of metformin & development of vitamin B12 deficiency.

SIGNIFICANCE OF VITAMIN B12 LEVEL& DURATION OF DIABETES.

VITAMIN B12 LEVELS	DURATION OF DM	
	> 10 YRS	< 10 YRS
DEFICIENT	6	6
BORDERLINE	14	16
NORMAL	2	6
P VALUE	0.634	
SIGNIFICANT	NON SIGNIFICANT	

Table 14

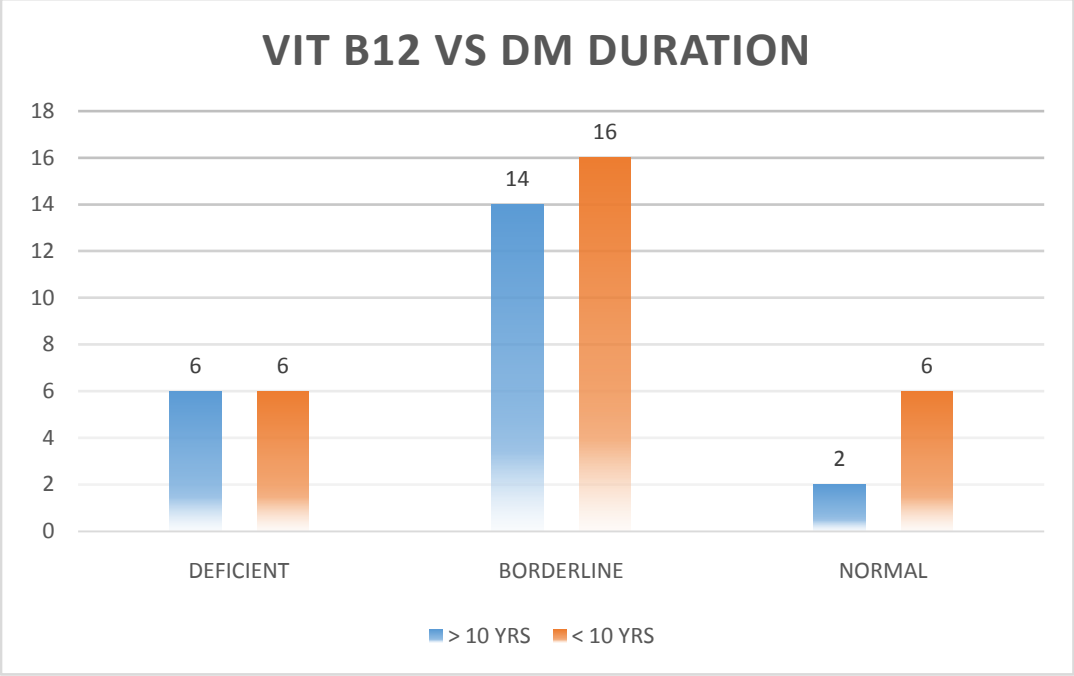


CHART 12

The relation between duration diabetes & B12 deficiency was not statistically significant.

MEAN AGE IN SUBGROUPS

VITAMIN B 12 LEVELS	MEAN AGE
DEFICIENT	57.25
BORDERLINE	59.03
NORMAL	59.25

Table 15

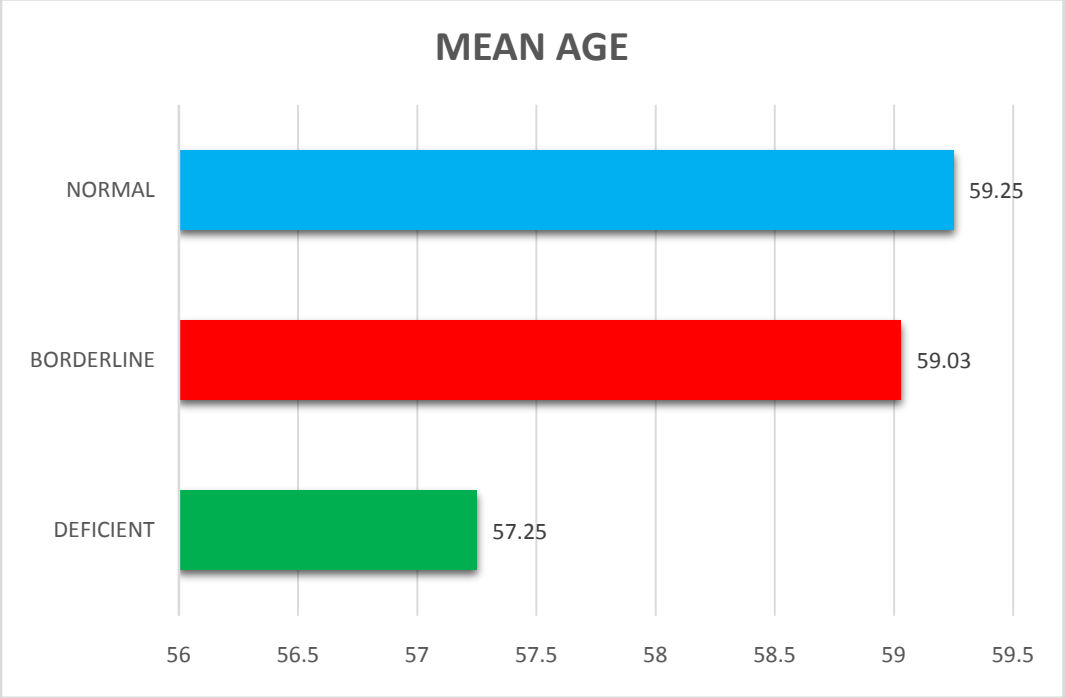


CHART 13

The mean age in all the three subgroups were assessed which did not show much difference. Mean age in deficient population is 57.25, in borderline group is 59.03, in normal group is 59.25

MEAN DURATION OF METFORMIN IN SUBGROUPS

VITAMIN B 12 LEVELS	MEAN DURATION OF METFORMIN
DEFICIENT	10.33
BORDERLINE	9.07
NORMAL	8.63

Table 16

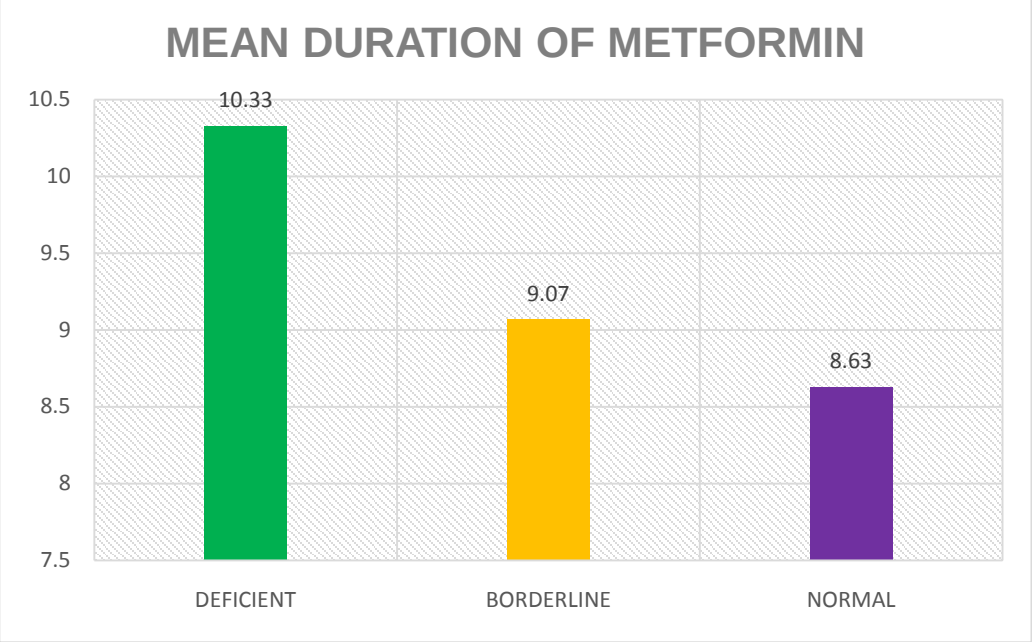


CHART 14

The mean duration of metformin was the highest in deficient group.

2nd highest in borderline group. Lowest in the normal group

MEAN DURATION OF DM WITH VITAMIN B 12 LEVELS

VITAMIN B 12 LEVELS	MEAN DURATION OF DM
DEFICIENT	11.17
BORDERLINE	10.4
NORMAL	9.25

Table 17

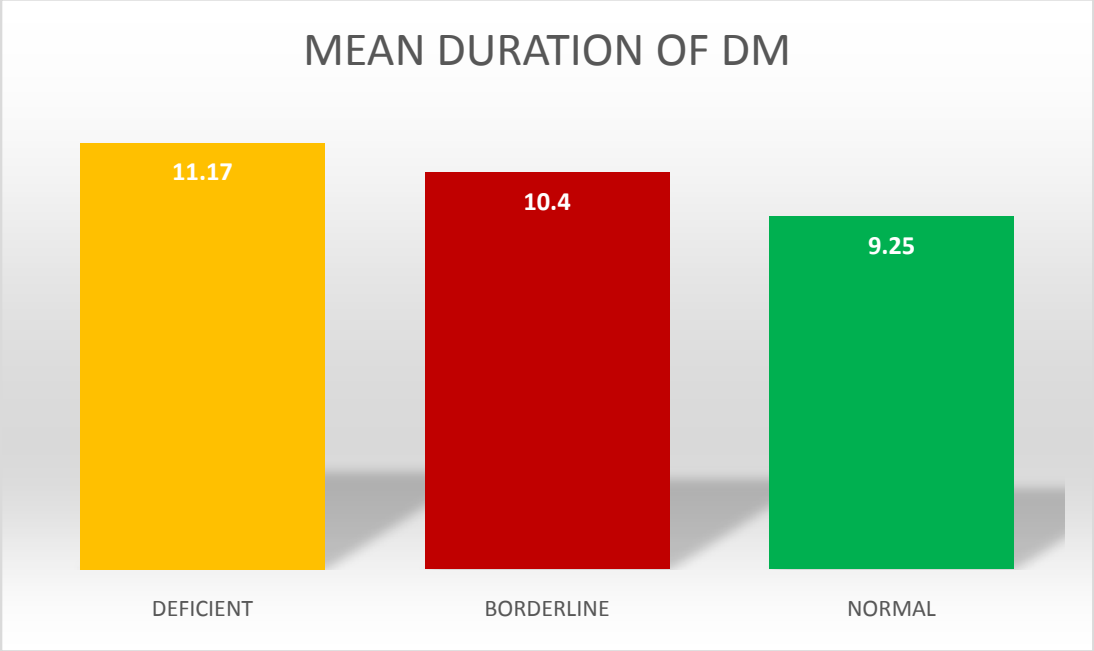


CHART 15

The mean duration of diabetes is higher in the deficient group as seen in mean duration of metformin. Lowest in normal group.

So both mean duration of diabetes & mean duration of metformin is higher in deficient group.

MEAN FBS, PPBSLEVELS WITHVITAMIN B 12 LEVELS

VITAMIN B 12 LEVELS	MEAN FBS	MEAN PPBS
DEFICIENT	172.68	273.67
BORDERLINE	170.97	271.67
NORMAL	139.98	244.5

Table 18

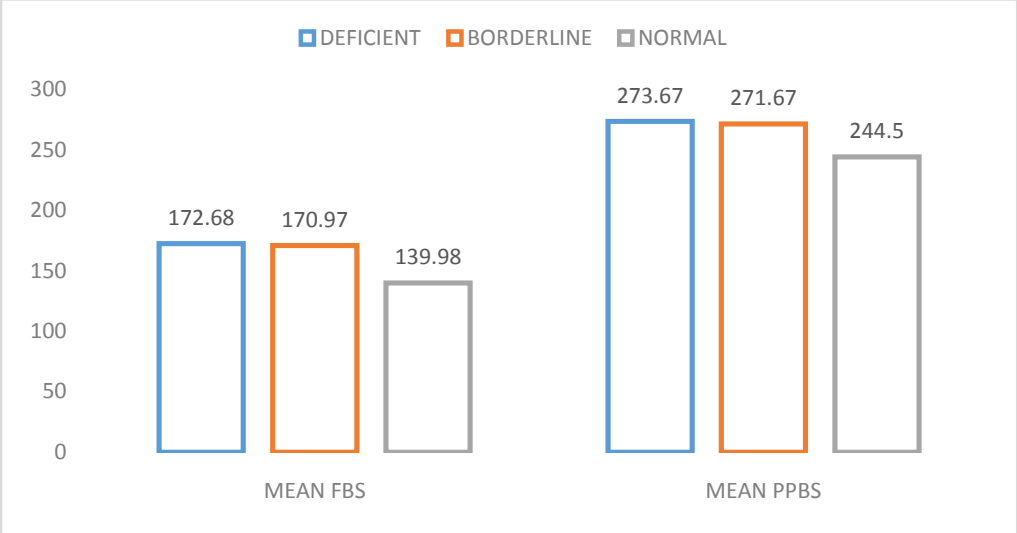


CHART 16

The mean fasting & post prandial blood sugar levels are also high in both deficient and borderline groups which may indicate poor glycemic index and may predispose to development of Vitamin B12 deficiency

MEAN BLOOD UREA ,SERUM CREATININE WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN UREA	MEAN CREATININE
DEFICIENT	21.75	0.958
BORDERLINE	23.87	0.997
NORMAL	24.25	0.913

Table 19

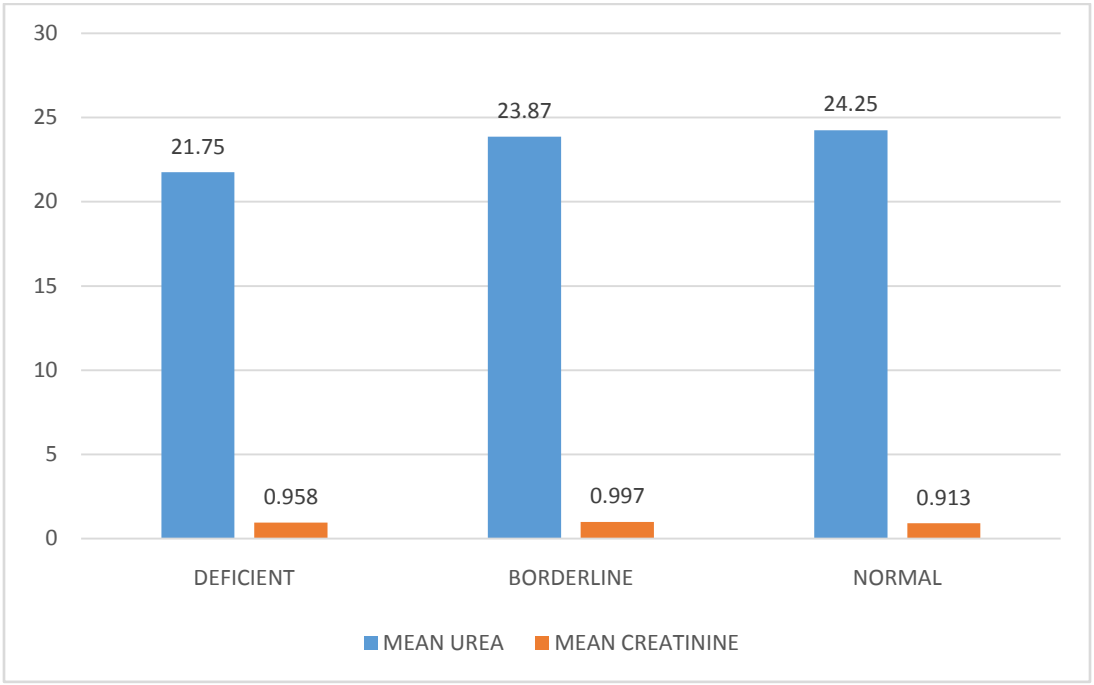


CHART 17

The mean creatinine & urea level did not differ much in these subgroups.

MEAN SGOT, SGPT,ALP WITH VITAMIN B 12 LEVELS

VITAMIN B 12 LEVELS	MEAN SGOT	MEAN SGPT
DEFICIENT	24	26.92
BORDERLINE	23.3	22.73
NORMAL	20.63	21.63

Table 20

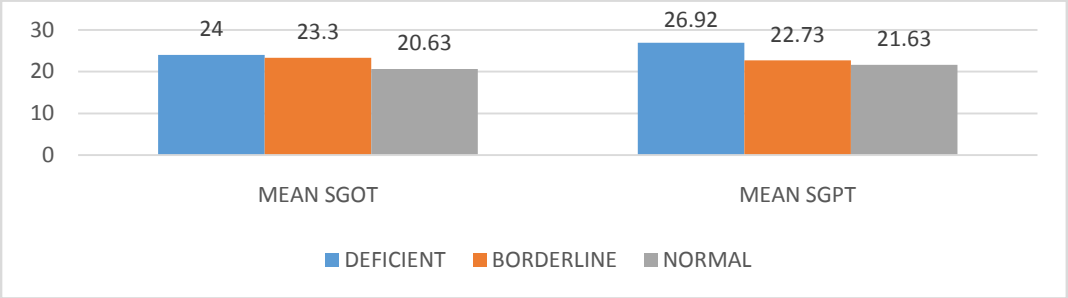


CHART 18

VITAMIN B 12 LEVELS	MEAN ALP
DEFICIENT	88.42
BORDERLINE	98.7
NORMAL	91.25

Table 21

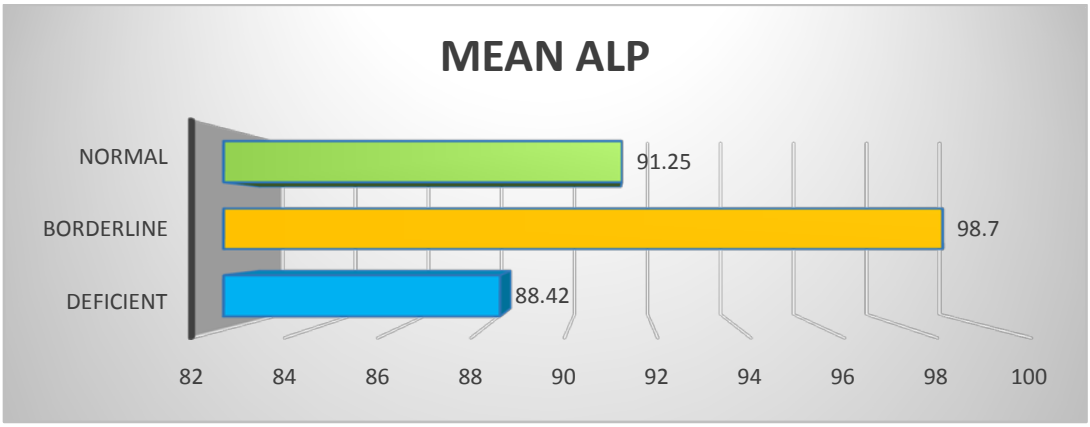


CHART 19

MEAN BILIRUBIN, PROTEIN LEVEL WITH VITAMIN B12 LEVELS

VITAMIN B 12 LEVELS	MEAN TOTAL BILIRUBIN	MEAN DIRECT BILIRUBIN
DEFICIENT	0.73	0.38
BORDERLINE	0.69	0.32
NORMAL	0.75	0.35

Table 22

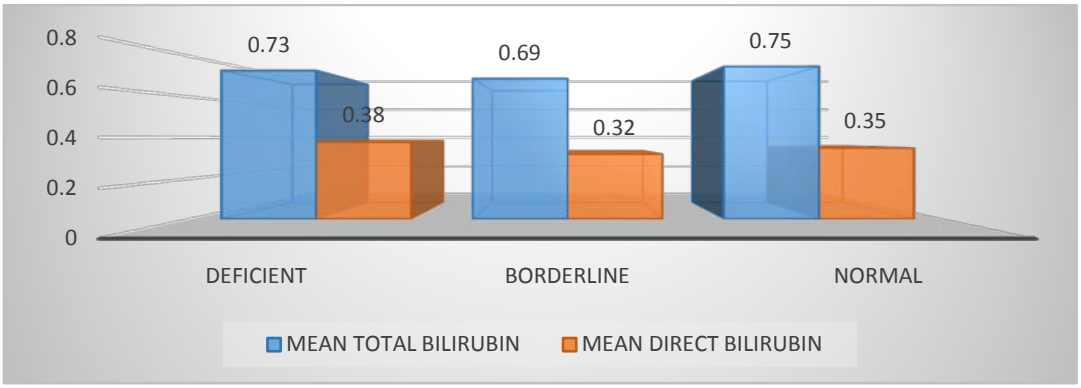


CHART 20

VITAMIN B 12 LEVELS	MEAN TOTAL PROTEIN	MEAN ALBUMIN
DEFICIENT	6.23	3.42
BORDERLINE	7.15	3.91
NORMAL	7.1	3.98

Table 23

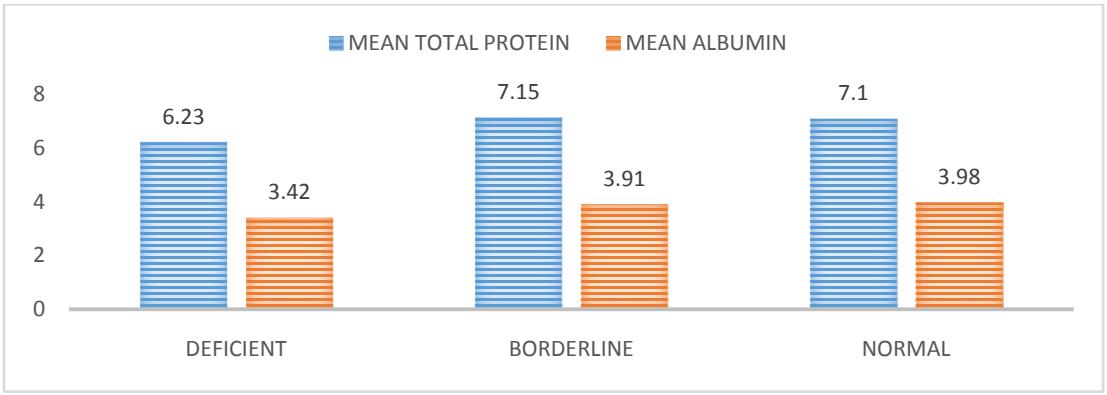


CHART 21

MEAN TOTAL COUNT WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN WBC
DEFICIENT	6466
BORDERLINE	7715
NORMAL	7490

Table 24

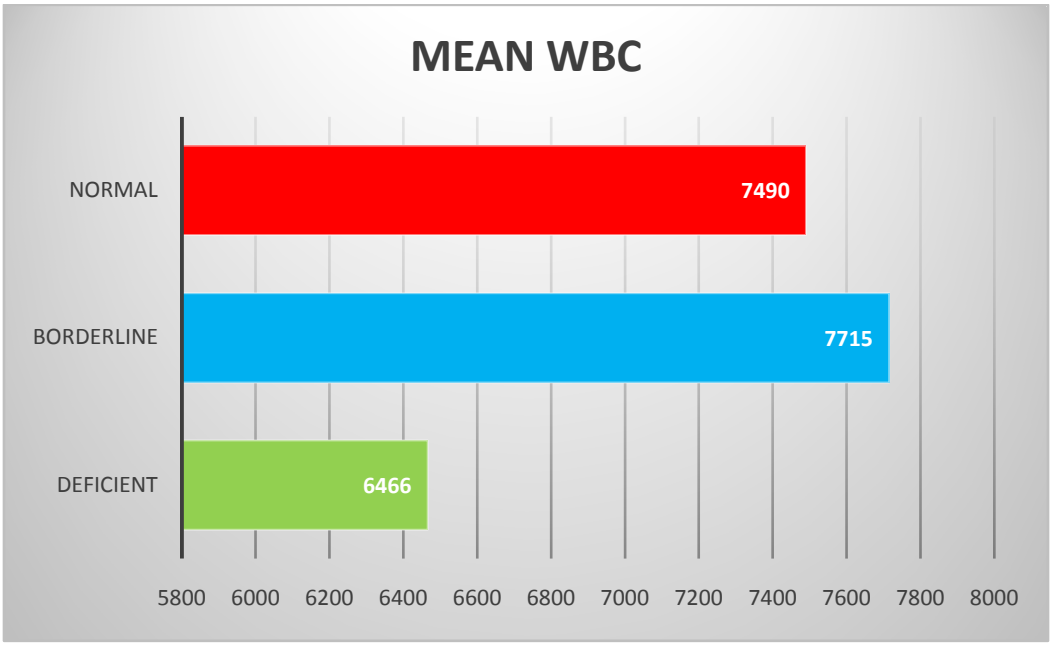


CHART 22

The mean total count value was low in the deficient group. But it did not show similar pattern in borderline group

MEAN RBC COUNT(IN MILLIONS) WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN RBC(IN MILLIONS)
DEFICIENT	2.86
BORDERLINE	3.47
NORMAL	3.14

Table 25

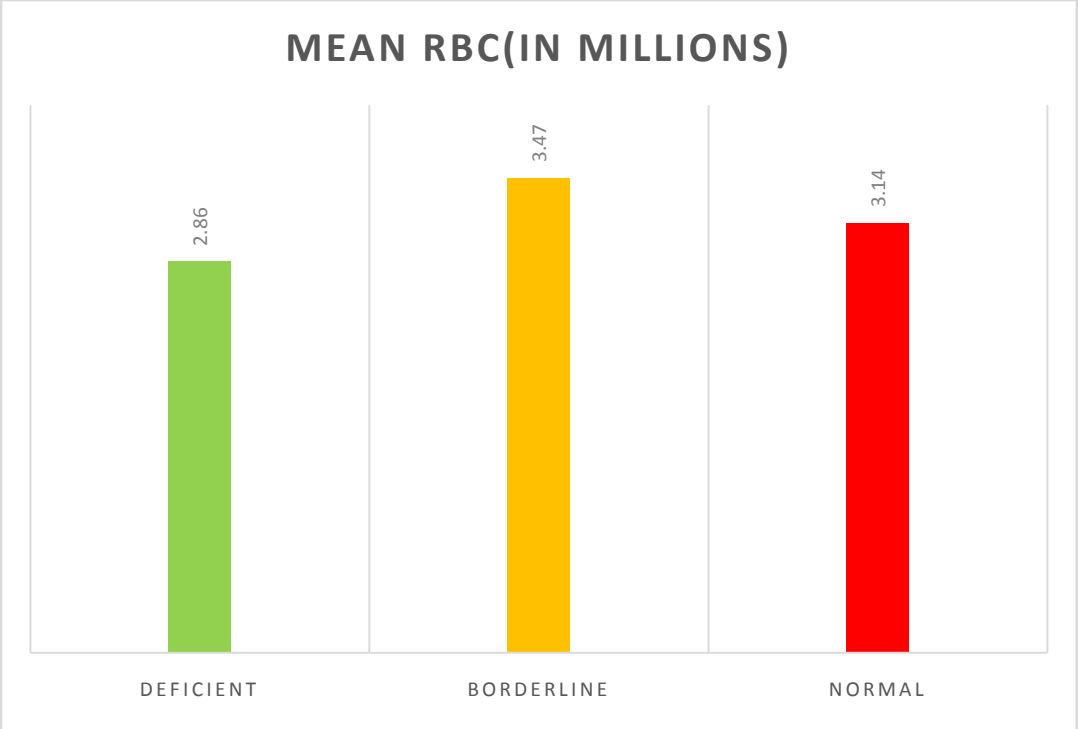


CHART 23

The mean RBC count is low in case of deficient group but it did not show similar reduction pattern in the borderline group as expected.

MEAN PLATELETCOUNT WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN PLATELETS(IN LAKHS)
DEFICIENT	2.14
BORDERLINE	2.48
NORMAL	2.35

Table 26

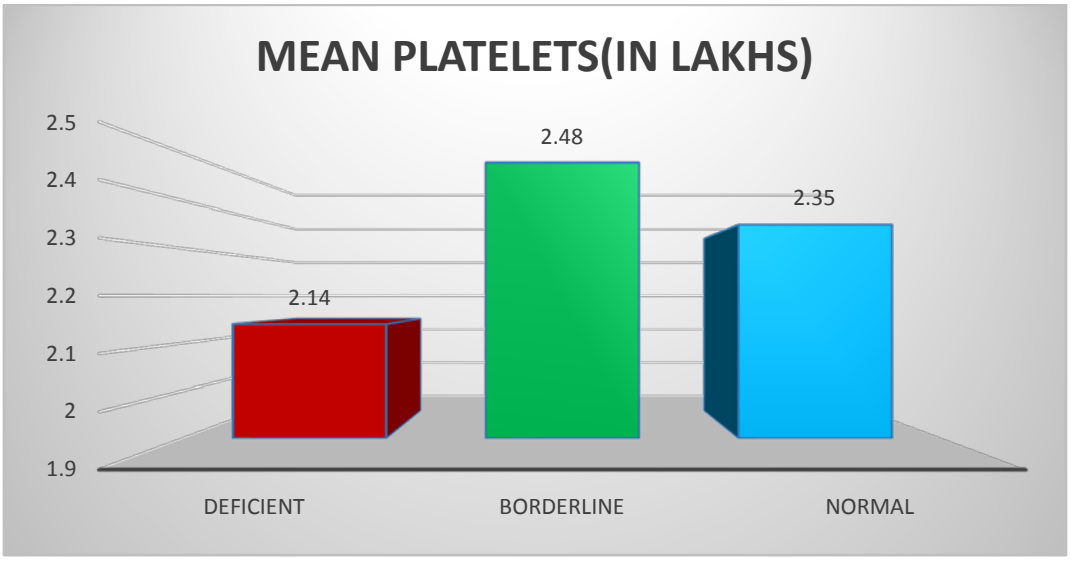


CHART 24

The pattern noted in Total count & RBC count is reflected in mean platelet count too. The mean platelet count is lower in the deficient group. Hence when seeing the deficient group alone, the mean values of all count was reduced, indicating the suppression of all cell line formation in vitamin B12 deficiency.

MEAN ESR & DIFFERENTIAL WITH VITAMIN B12

VITAMIN B 12 LEVELS	MEAN ESR 1 HR	MEAN ESR 2 HR
DEFICIENT	14	25.83
BORDERLINE	12.76	24.56
NORMAL	13.37	20.12

Table 27

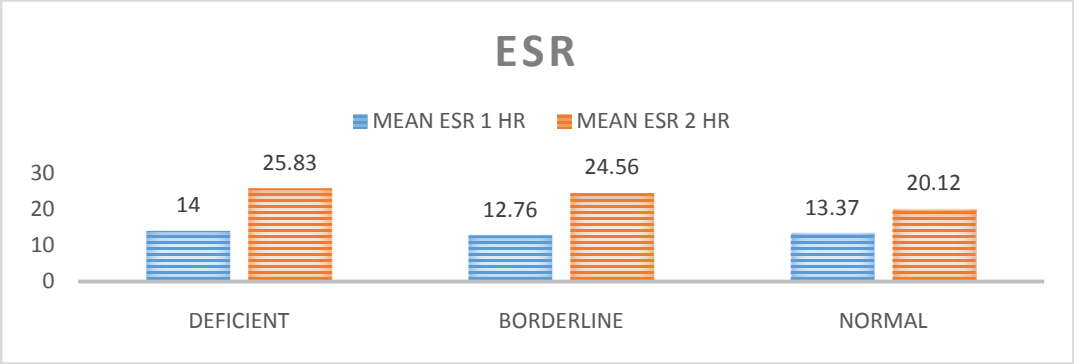


CHART 25

VITAMIN B 12 LEVELS	MEAN POLYMORPHS	MEAN LYMPHOCYTES	MEAN EOSINOPHILS
DEFICIENT	63.75	34.33	1.91
BORDERLINE	65.83	31.63	2.33
NORMAL	64.75	32.87	2.37

Table 28

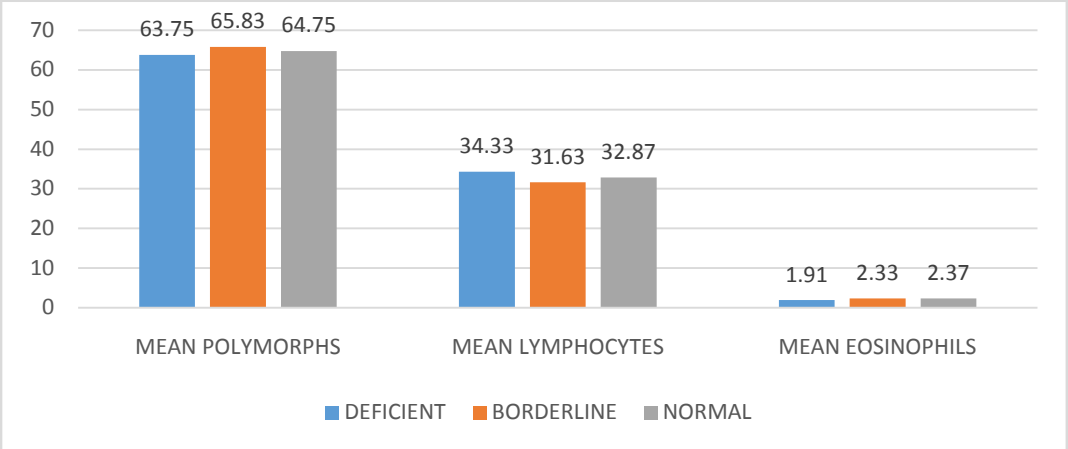


CHART 26

MEAN HEMOGLOBIN WITH VITAMIN B12

VITAMIN B 12 LEVELS	MEAN HB%
DEFICIENT	10.49
BORDERLINE	10.4
NORMAL	10.45

Table 29

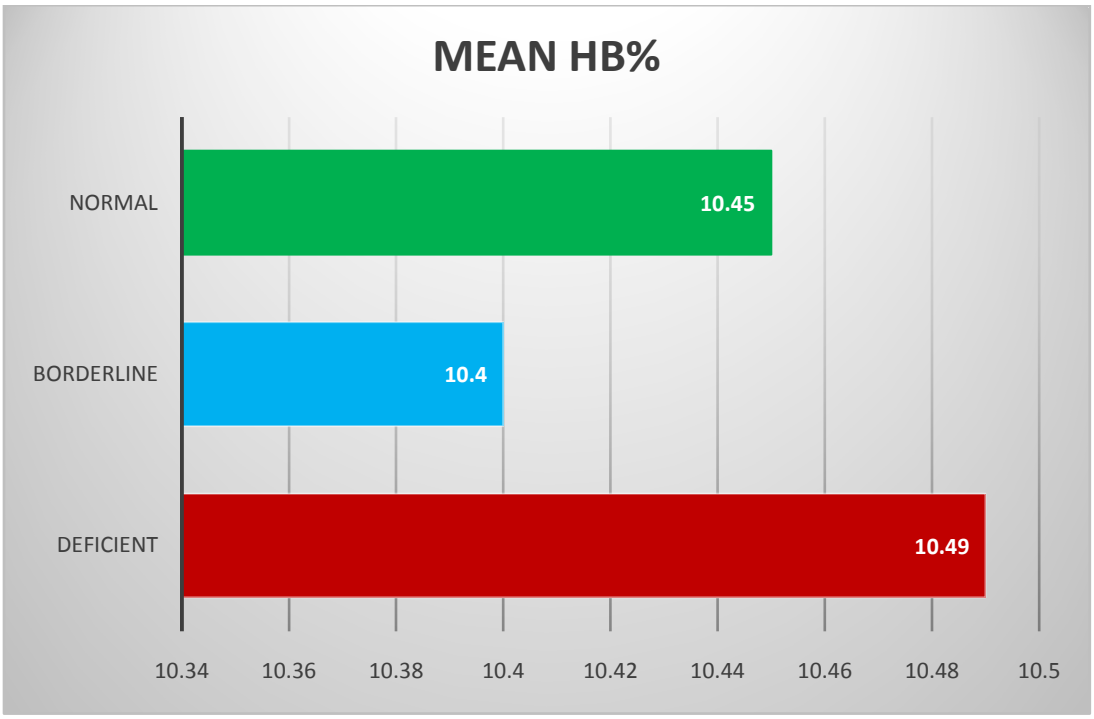


CHART 27

Hemoglobin deficiency is noted more in the vitamin B12 borderline group.

Yet paradoxically more number of vitamin B12 patients had higher hemoglobin level.

.

MEAN HEMATOCRIT WITH VITAMIN B12

VITAMIN B 12 LEVELS	MEAN PCV
DEFICIENT	32.17
BORDERLINE	32.17
NORMAL	32.13

Table 30

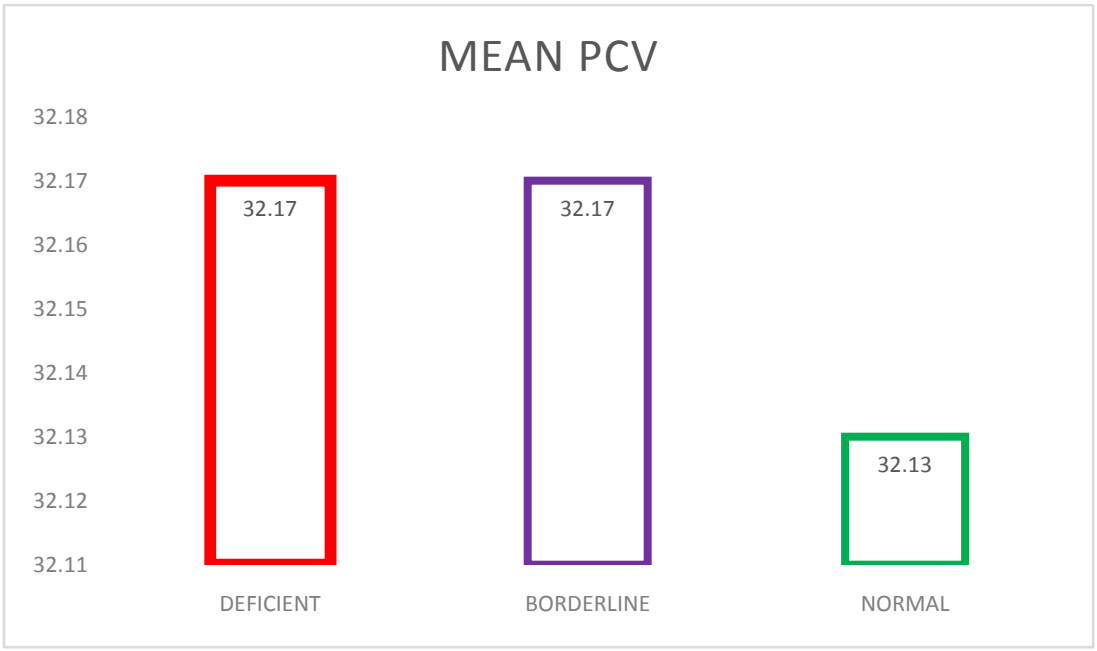


CHART 28

Mean hematocrit values are more similar in all subgroups, even though there was some difference in hemoglobin deficiency

MEAN MCV WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN MCV
DEFICIENT	114.05
BORDERLINE	91
NORMAL	106.08

Table 31

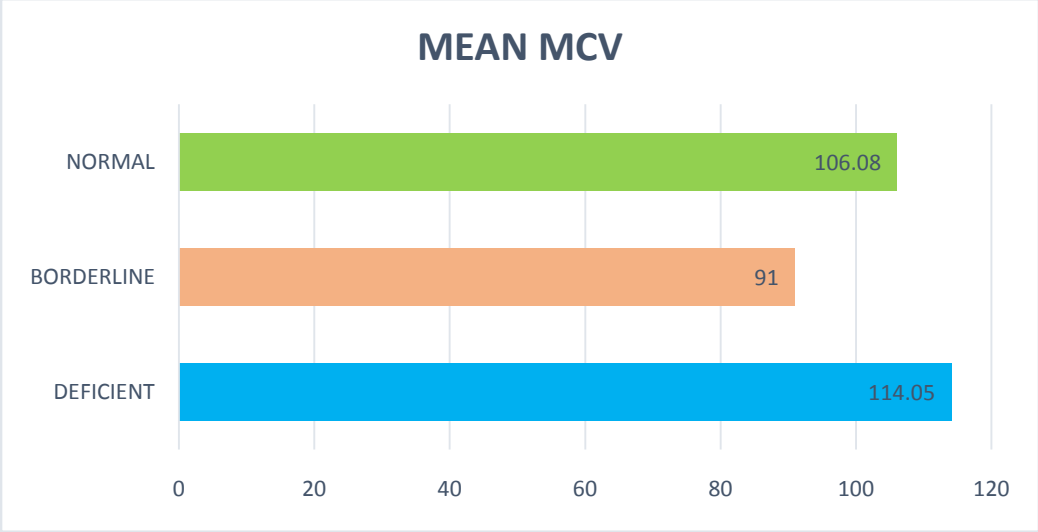


CHART 29

Mean MCV shows higher level in deficient group as expected in the study. But the borderline group have low MCV level.

MEAN MCH WITH VITAMIN B12 LEVEL

VITAMIN B 12 LEVELS	MEAN MCH
DEFICIENT	36.61
BORDERLINE	29.83
NORMAL	32.22

Table 32

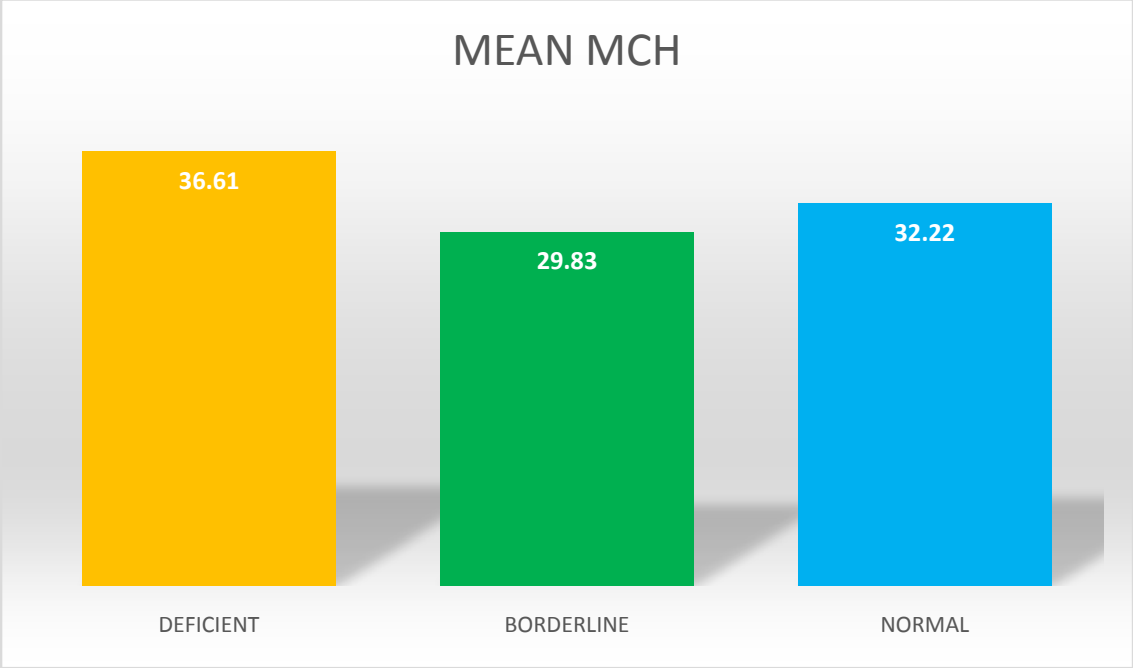


CHART 30

Mean MCH value is highest in deficient group , then followed by Normal subgroup.

MEAN MCHC WITH VITAMIN B12 LEVELS

VITAMIN B 12 LEVELS	MEAN MCHC
DEFICIENT	29.42
BORDERLINE	32.69
NORMAL	32.55

Table 33

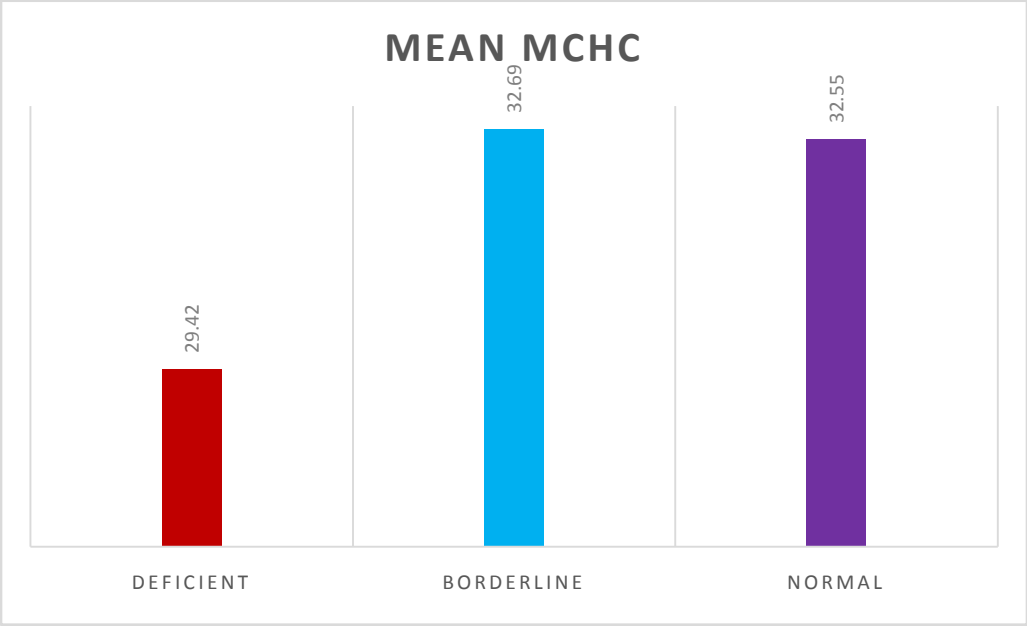


CHART 31

The Mean MCHC was almost equal in normal & borderline group. But lower in the Vitamin B12 deficient group.

DISCUSSION

Metformin being the most commonly prescribed drug for the treatment of diabetes mellitus and there are proposed mechanism through which metformin interacts with vitamin B12 absorption, it causes deficiency of vitamin B12. It is a cross sectional study where the serum B12 level of type 2 Diabetic patients who are on metformin therapy(>5yrs) was measured and correlated with various other basic parameters like fasting , post prandial status, duration of metformin therapy, duration of diabetes mellitus.

Our study comprised of 50 type 2 diabetic population. Out of which 24 are males & 26 are females.

All patients recruited under the inclusion criteria. Hence all patients were taking metformin of more than 5 years

22 out of 50 people were having duration of more than 10 years for type 2 diabetes mellitus

18 out of 50 patients had high Mean corpuscular volume. But only 12 of the population had macrocytosis demonstrable by the peripheral blood picture.6 people were having microcytic picture. Out of the twelve patient, 7 had vitamin B12 deficienxy, 2 had borderline

deficiency, 3 hadnormal level. Even in the vitamin B12 deficient group, there were 5 patients who did not show macrocytosis in the peripheral picture. Hence we come to a conclusion, that even though the treatment with vitamin B12 caused Macrocytosis, there are other mechanisms that could be operating in the development of macrocytosis in those patients.

The P value calculated for finding the significance between the sex and vitamin B12 deficiency has not been established. The total number of males and females were almost equally distributed. But there was no specific relation between sex and development of vitamin B12 deficiency.

Similarly based on the age of the patient it is divided into two subgroups and P value was calculated for them. It was not significant. Yet this could be due to differences among these populations in various other parameters like duration of diabetes,dosage of metformin which was not assessed in this study group.

The P value for duration of diabetes is calculated by dividing the group into two subgroups. There were more number of people below 10 years of duration of treatment with metformin. Here also the duration did not show significance which could be because of small

sample size. The dosage of the metformin was not calculated. So the significance of duration in this small group may be affected by total dose of metformin. So it is not possible to cometo a conclusion at this level saying that the duration of metformin will not affect the vitamin B12 status. The mean duration of therapy with metformin is high in the deficient group followed by the borderline group. Hence the duration of therapy with metformindetermines the development of vitamin deficiency.

The nonsignificant P value with duration of metformin and duration of diabetes may occur due to the small sample size. This being a cross sectional study, the subgroup population is very low and it is difficult to study the P value in this subgroup. As we do subgroup study in these populations the number of patient decreases in each subgroup and the significance decreases further. But the mean value shows good correlation with duration of metformin therapy and duration of diabetes.

The National health & Nutritional survey done from US 1999-2006 had documented vitaminB12 deficiency in both diabetics with and without metformin therapy. But the biochemical B12 deficiency is higher in group with metformin. It is correlated with our study where

we found some relation with mean duration of metformin and B12 deficiency.

Matthew C Pflipsen et al , showed higher prevalence of vitamin B12 deficiency among 22% of diabetics taking chronic metformin. They measured methyl malonic acid and homocysteine in the borderline B12 deficient cases, to confirm those cases as deficiency cases. In our study we had high borderline patients. If we do methyl malonic acid and homocysteine in these subgroup we can also diagnose higher number of absolute deficient cases(13).

Vineetha Shobha et al, showed higher prevalence of vitamin B12 deficiency in aymptomatic urban population of diabetics with metformin therapy(2). Hence there can be associated pre existing vitamin B12 deficiency due to causes other than diabetes and Metformin, like vegetarianism.

The mean urea, creatinine when compared to vitamin B12 has not shown any significant relation.

The Mean values of all the Liver function parameters has not shown any significant relation with vitamin B12 deficiency.

Total count & RBC count, platelet count mean value is lower in the deficient group, which may correlate with the depressed marrow function in the vitamin B 12 deficiency.

The Mean ESR, Differential count has not shown any significance with the vitamin B12 status. There is a decreased hemoglobin level in the borderline, deficient groups. It correlates well with borderline group (10.4) than the deficient group (10.49). This shows that there can be additional factors influencing the development of anemia which are not addressed in this study.

Mean MCH were similar in all subgroups. Mean MCHC was low when compared to other groups which may correlate with decreased hemoglobinization with increased cell size in macrocytosis patients.

CONCLUSION:

- Chronic metformin therapy causes asymptomatic vitamin B12 deficiency.
- Vitamin B12 assay in the patients who were on chronic metformin therapy helps in assessing Vitamin B12 deficiency.
- Vitamin B12 deficiency is associated with macrocytosis
- The duration of metformin therapy has affected the vitaminB12 status significantly

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PROFORMA

Name:
Address:

Age/sex:

Occupation:

PAST HISTORY:

Duration of diabetes:

No of years of Metformin therapy :

Drug h/o:

h/o abdominal surgery:

PERSONAL HISTORY:

Alcohol Y/N

Smoking Y/N

DIET Veg/Nonveg

GENERAL PHYSICAL EXAMINATION:

anaemia cyanosis

Jaundice pedal oedema

Clubbing knuckle hyperpigmentation

Lymphadenopathy

Vitals: PR :`

BP :

Weight : ( ) kg

SYSTEMIC EXAMINATION:

1.Cardiovascular System: S1 S2 murmurs

2. Respiratory system:

3. Abdomen:

4. Central Nervous System:

INVESTIGATIONS:

CBC , Peripheral smear study,

Renal function tests, serum Electrolytes,

Urine complete,

Liver function tests,

FBS, PPBS,ECG all leads.

Serum vitamin B12 assay.

KEY TO MASTER CHART

MET DUR - Metformin duration

DIAB DUR – Diabetes duration

FBS – Fasting blood sugar

PPBS – Postprandial blood sugar

CREAT – Creatinine

OT – Serum glutamic oxaloacetic transaminase

PT – serum glutamic pyruvaic transaminase

ALP – Alanine transaminase

TOTAL BI – total bilirubin

TP – total protein

WBC – white blood cell count

RBC – red blood cell counyt

P – polymorphs

L- Lymphocyte

E -Eosinophils

MCV – Mean corpuscular volume

MCH – Mean corpuscular hemoglobin

MCHC – mean corpuscular hemoglobin concentration

VITB12 – Vitamin B 12

ESR – erythrocyte sedimentation rate