CHAPTER 2
REVIEW OF LITERATURE

Diabetes mellitus (DM) is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Fully expressed diabetes is characterized by fasting hyperglycemia, but the disease can be recognized during less overt stages, most usually by the presence of glucose intolerance. The effect of diabetes mellitus includes long term damage, dysfunction and failure of various organs like eyes, kidneys, heart and blood vessels. The characteristic symptoms of diabetes are thirst, polyuria, blurring of vision, weight loss and polyphagia and in its most severe forms, with ketoacidosis or non ketotic hyperosmolarity, which in the absence of effective treatment, leads to stupor, coma and death (Bennett & Knowler, 2006)

CLASSIFICATION OF DM

As per ADA, 2017 diabetes can be classified into the following general categories:

1. Type 1 diabetes (due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency)
2. Type 2 diabetes (due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance)
3. Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation)
4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)

The two broad categories of DM are designated Type 1 and Type 2 (Table 2.1). Both types of diabetes are preceded by a phase of abnormal glucose homeostasis as the disease

progress. Type 1 DM is the result of complete or near -total insulin deficiency. (ADA, 2017)

Type 2 Diabetes Mellitus (T2DM) is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. T2DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose(IFG) or impaired glucose tolerance(IGT). (ADA, 2017)

OTHER TYPES OF DM

Other etiologies for DM include specific gene defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities, and a host of conditions that impair glucose tolerance (Table 2.1). Maturity-onset diabetes of the young(MODY) is a sub type of DM characterized by autosomal dominant inheritance, early onset of hyperglycemia, and impairment in insulin secretion. Mutations in the insulin receptor cause a group of rare disorders characterized by severe insulin resistance. (Powers, 2012)

DIAGNOSIS

Glucose tolerance is classified into three broad categories: normal glucose homeostasis, diabetes mellitus and impaired glucose homeostasis. Glucose tolerance can be assessed using the fasting plasma glucose (FPG), the response to oral glucose challenge, or HbA1c.An FPG<100mg/dl (5.6 mmol/l), a plasma glucose<140 mg/dl (11.1 mmol/l) following an oral glucose challenge (OGTT-oral glucose tolerance test), and HbA1c< 5.6% are considered to define normal glucose tolerance. The International Expert committee with members appointed by the European Association for the study of Diabetes, the International Diabetes Federation and American Diabetes Association, has issued diagnostic criteria for DM based on the criteria given in Table 2.2 (ADA, 2020):

Abnormal glucose homeostasis is defined as:

(1) FPG= 100-125mg/dl (5.6-6.9mmol/l), which is defined as IFG (note that the WHO uses an FPG of 110-125mg/dl (6.1-6.9 mmol/l)

TABLE 2.1: ETIOLOGIC CLASSIFICATION OF DISORDERS OF GLYCEMIA

Type 1 (β cell destruction, usually leading to absolute insulin deficiency) A. Autoimmune B. Idiopathic
Type 2 (May range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin deficiency)
Genetic defects of β cell function • Chromosome20, HNF4α (MODY1) • Chromosome 7, glucokinase (MODY 2) • Chromosome 12, HNF1α (MODY 3) • Chromosome 13, IPF1(MODY 4) • Chromosome 17, HNF3β(MODY 5)
Genetic defects in insulin action
Disease of exocrine pancreas
Endocrinopathies
Drug or chemical induced
Infections
Uncommon forms of immune- mediated diabetes
Other genetic syndromes sometimes associated with diabetes
Gestational diabetes

(Powers, 2012)

TABLE 2.2: DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS

• Symptoms of diabetes plus random blood glucose concentration≥200mg/dl(11.1 mmol/l)(Random is defined as without regard to time since the last meal) or
• Fasting Plasma glucose≥126mg/dl(7.0mmol/l)(Fasting is defined as no caloric intake for at least 8 hrs or
• HbA1c>6.5% or
• Two-hr plasma glucose≥200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test. The OGTT should be performed using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in 250-300ml water.

(Adapted from ADA, 2020)

(2) Plasma glucose levels between 140 and 199 mg/dl (7.8 and11 mmol/l) following an oral glucose challenge, which is termed impaired glucose tolerance(IGT)or
(3) HbA1c = 5.7-6.4%

An HbA1c of 5.7-6.4%, IFG, and IGT do not identify the same individuals, but individuals in all three groups are at a greater risk of progressing to T2DM and have an increased risk of cardiovascular disease. Some use the term "pre diabetes", "increased risk of diabetes(ADA) or "intermediate hyperglycemia" (WHO) for this category.

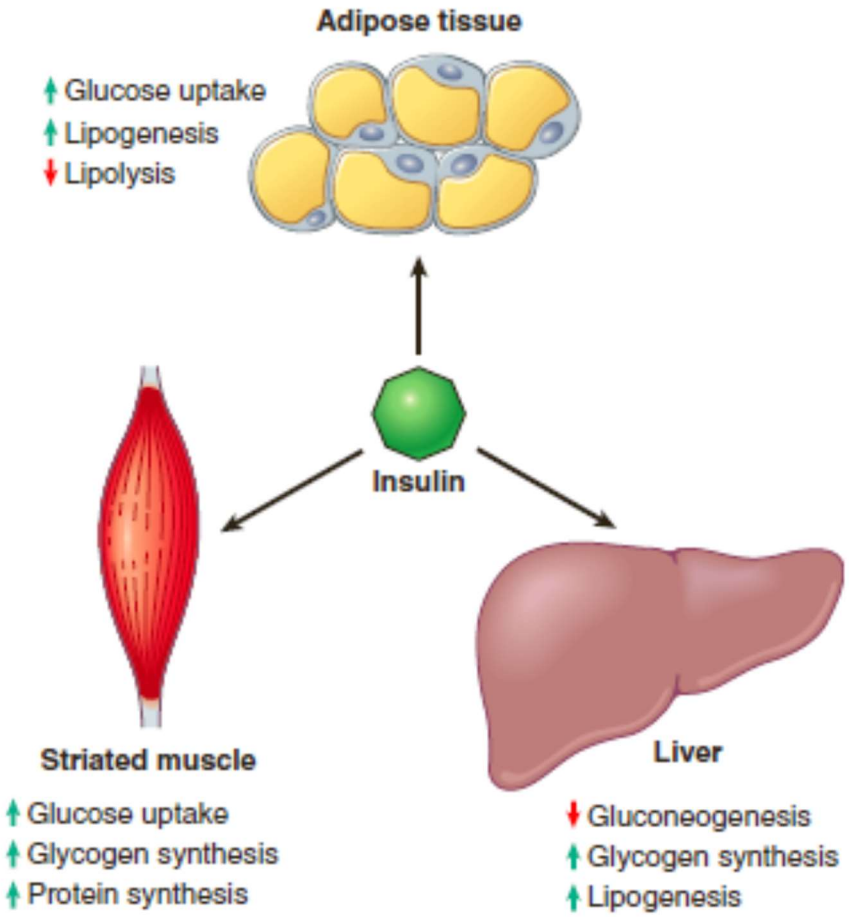
The current criteria for the diagnosis of DM emphasize that the HbA1c or FPG as the most reliable and convenient tests for identifying DM in asymptomatic individuals. Oral glucose tolerance test, although still a valid means for diagnosing DM, is not often used in routine clinical care (Powers, 2018).

NORMAL INSULIN PHYSIOLOGY AND GLUCOSE HOMEOSTASIS

Before discussing the pathogenesis of diabetes, we briefly describe normal insulin physiology and glucose metabolism. Normal glucose homeostasis is tightly regulated by three interrelated processes:
(1) Glucose production in the liver,
(2) Glucose uptake and utilization by peripheral tissues, chiefly skeletal muscle, and
(3) Action of insulin and counter regulatory hormones like glucagon.

The principal metabolic function of insulin is to increase the rate of glucose transport into certain cells in the body (Fig. 2.1) like striated muscle cells (including myocardial cells) and, to a lesser extent, adipocytes (representing about two thirds of total body weight). Glucose uptake in other peripheral tissue like brain is insulin independent. In muscle cells, glucose is then either stored as glycogen or oxidized to generate adenosine triphosphate (ATP). In adipose tissue, glucose is stored primarily as lipid. Besides promoting lipid synthesis (lipogenesis), insulin also inhibits lipid degradation (lipolysis) in adipocytes. Insulin promotes amino acid uptake and protein synthesis while inhibiting protein degradation. Thus insulin is an anabolic hormone which increases synthesis and reduces degradation of glycogen, lipid, and protein. In addition to these metabolic effects,

FIG. 2.1: METABOLIC ACTIONS OF INSULIN IN STRIATED MUSCLES, ADIPOSE TISSUE, AND LIVER



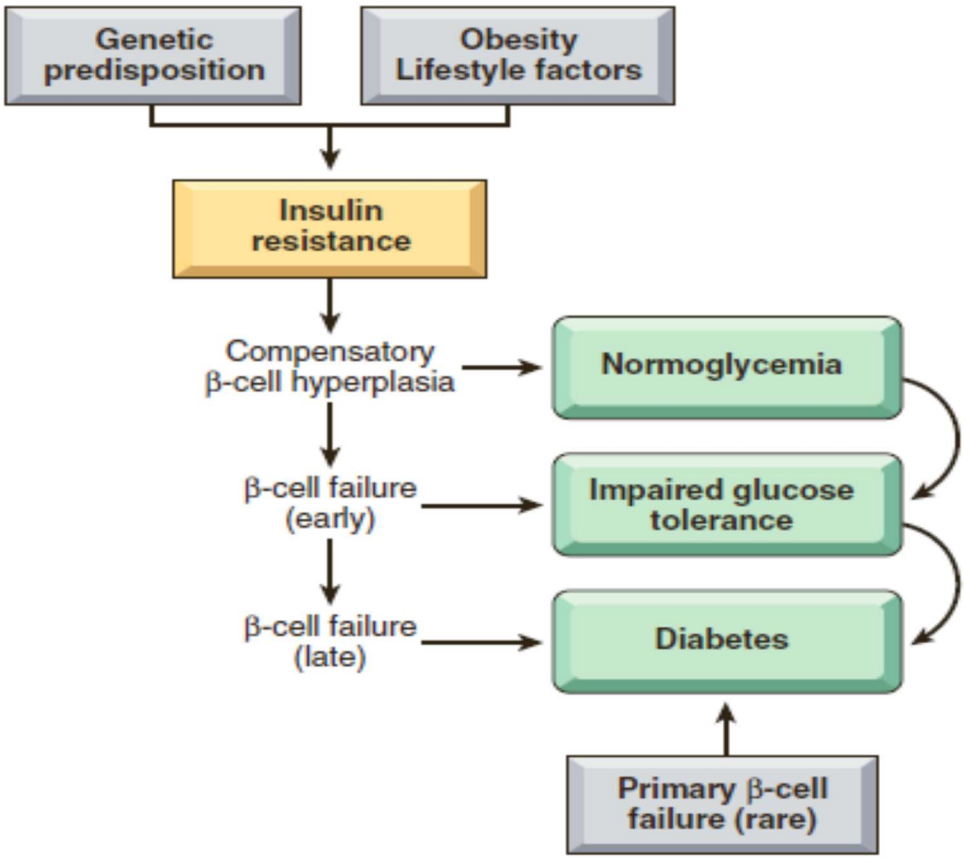
(Adapted from Kumar, Abbas, & Aster, 2013)

insulin has several *mitogenic* functions like initiation of DNA synthesis in certain cells and stimulation of their growth and differentiation. Insulin reduces the production of glucose from the liver. Insulin and glucagon have opposing regulatory effects on glucose homeostasis. In fasting state, low insulin and high glucagon levels facilitate hepatic gluconeogenesis and glycogenolysis (glycogen breakdown) while decreasing glycogen synthesis, thereby preventing hypoglycemia. Thus, *fasting* plasma glucose (FPG) levels are determined primarily by hepatic glucose output. After ingestion of food, insulin levels rise and glucagon levels fall in response to the large glucose load. The most important stimulus is glucose itself which initiates insulin synthesis in the pancreatic beta cells. In peripheral tissues (skeletal muscle and adipose tissue), secreted insulin binds to the insulin receptor, triggering a number of intracellular responses that promote glucose uptake and postprandial glucose utilization, thereby maintaining glucose homeostasis. Abnormalities at various points along this complex signaling cascade, from synthesis and release of insulin by beta cells to insulin receptor interactions in peripheral tissues, can result in the diabetic phenotype (Kumar, Abbas, & Aster, 2013).

PATHOGENESIS

Type 2 diabetes is a prototypical complex multifactorial disease. Environmental factors, such as a sedentary life style and dietary habits and obesity play a role. Genetic factors are also involved in the pathogenesis, as evidenced by the disease concordance rate of 35% to 60% in monozygotic twins compared with nearly half that in dizygotic twins. Such concordance is even greater than in type 1 diabetes, suggesting perhaps an even larger genetic component in type 2 diabetes. Additional evidence for a genetic basis has emerged from recent large-scale genome wide association studies, which have identified more than a dozen susceptibility loci called "diabetogenic" genes. Unlike type 1 diabetes, however, the disease is not linked to genes involved in immune tolerance and regulation (e.g., *HLA, CTLA4*), and evidence of an autoimmune basis is lacking. The two metabolic defects that characterize type 2 diabetes are (1) a decreased ability of peripheral tissues to respond to insulin (insulin resistance) and (2) beta cell dysfunction that is manifested as inadequate insulin secretion in the face of insulin resistance and hyperglycemia (Fig.2.2). Insulin resistance predates the development of hyperglycemia and usually is accompanied

FIG. 2.2: PATHOGENESIS OF TYPE 2 DIABETES



(Adapted from Kumar, Abbas & Aster, 2013)

EPIDEMIOLOGY OF DIABETES

GLOBAL SCENARIO: In 2015 International Diabetes Federation (IDF) Atlas declared that diabetes is one of the largest global health emergencies of the 21st century.

According to the 9th edition of International Diabetes Federation Diabetes atlas (IDF 2019), it is estimated that currently **9.3%** (463.0 million) adults aged **20–79 years** worldwide have diabetes (**both type 1 and type 2**). Based on the 2019 estimates, by 2030 a projected 10.2% (578.4 million), and by 2045 a projected 10.9% (700.2 million) adults aged 20–79 years, will be living with diabetes (Fig 2.3).

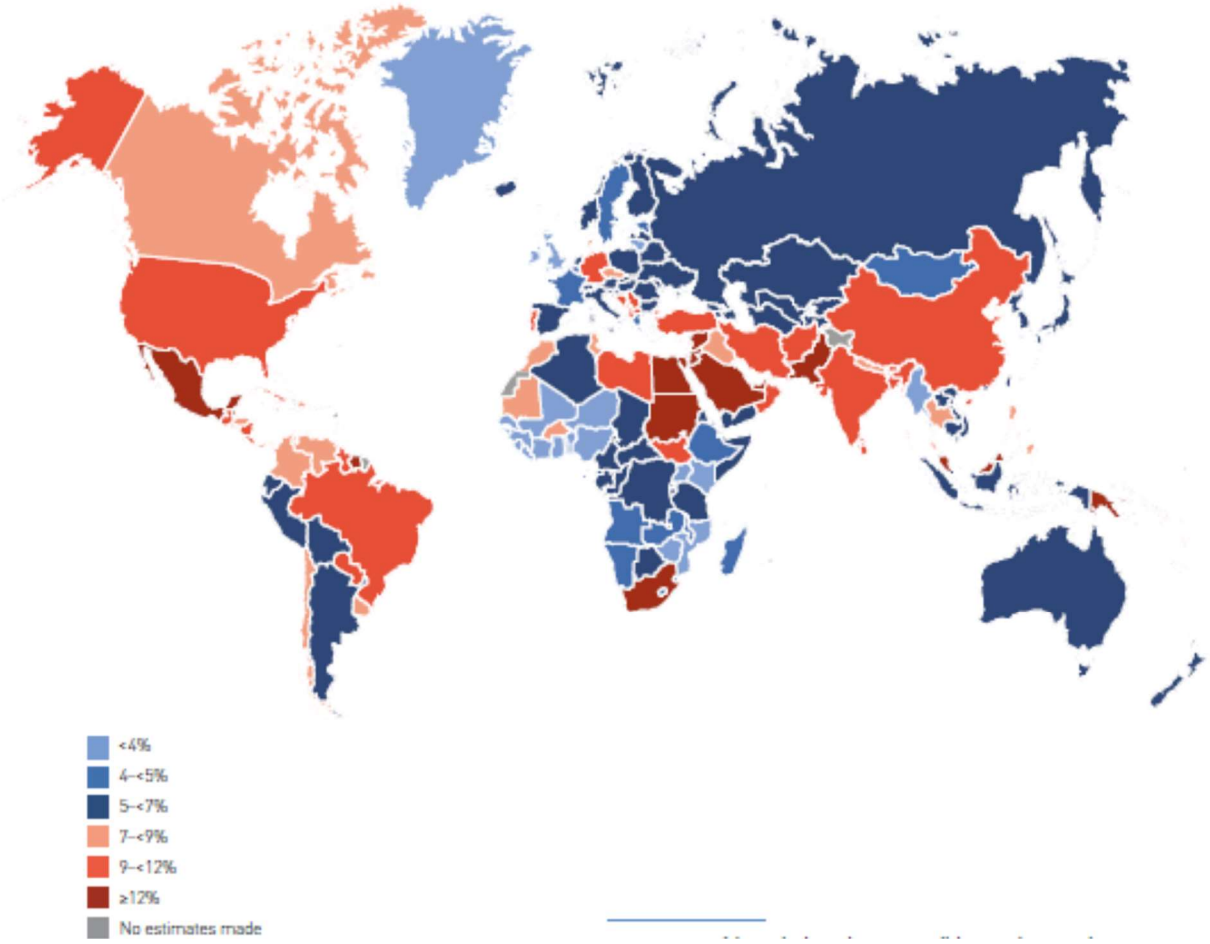
In 2000, the global estimate of diabetes prevalence in the 20-79 yrs age group was 151 million, which was close to WHO estimate at the time (150 million). Estimates have since shown alarming increases (Fig 2.3), tripling in 2019 estimate of 463 million. Projections for the future have clearly indicated that the global impact of the diabetes likely to continue increasing considerably.

Further the IDF 2019 states that the global prevalence of impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8% (454 million) by 2030 and 8.6 % (548 million) by 2045.

Although the prevalence of both type 1 and type 2 diabetes is increasing worldwide, the prevalence of T2DM is rising much more rapidly presumably because of increasing obesity, reduced activity levels as countries become more industrialized. The major proportion of this increase will occur in developing countries of the World (about 80%), where the disorder predominantly affects younger adults in the economically productive age group. (Powers, 2012).

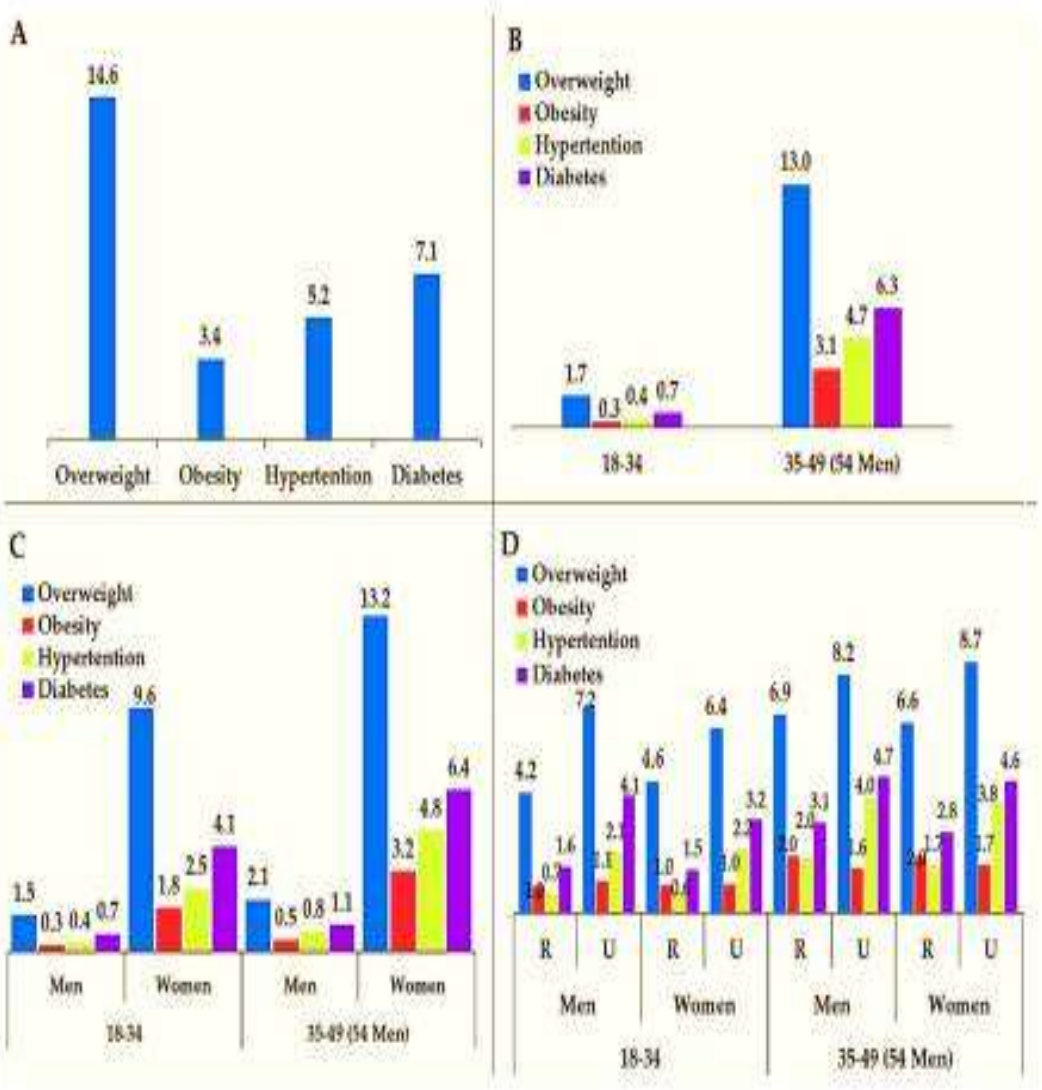
NATIONAL SCENARIO: The latest NFHS-4 data from 29 states and 7 UTs between 2015-2016 stated that the national prevalence of diabetes was 7.1% (Fig 2.4) and at the national level, the 35–49-years (54 years for men) age group, women, and urban areas had a greater prevalence of diabetes than individuals aged 18–34 years, men, and rural areas (Vennu et. al, 2019) (Fig 2.4).

FIG. 2.3: ESTIMATED TOTAL NUMBER OF ADULTS (20–79 YRS) WITH DIABETES IN 2019



(Adapted from IDF, 2019)

FIG. 2.4: THE % PREVALENCE OF OVERWEIGHT, OBESITY, HYPERTENSION AND DIABETES AMONG ADULTS BY (A) DISEASE/CONDITION, (B) AGE GROUP,(C) AGE GROUP AND SEX, AND (D) AGE GROUP, SEX, AND RESIDENCE.



(Adapted from NFHS-4 data, Vennu et. al, 2019).

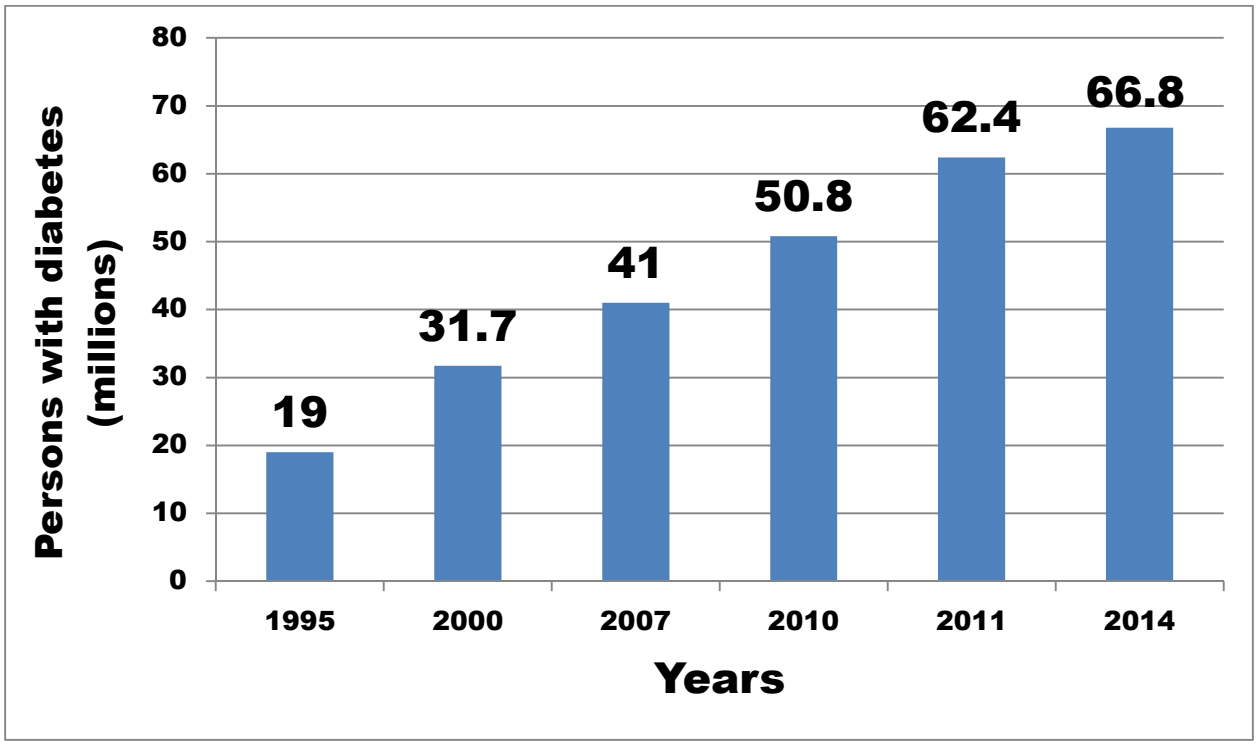
These results were consistent with previous studies that reported a higher prevalence of diabetes among adults in urban (11.2%) compared with rural (5.2%) India. (Anjana et. al, 2011; Anjana et al, 2017; Ramachandran et al, 2010)

Before this the National Urban Diabetes Survey (NUDS) reported the prevalence of type 2 diabetes to be 12.1% and impaired glucose tolerance to be 14% after the national survey of diabetes by ICMR in 1975 (Ramchandran et al, 2001). The NUDS was carried by the Diabetes Epidemiology Study group in India (DESI) in six major cities of India covering all regions of the country although some regional studies have shown rise in type 2 diabetes prevalence.

Preliminary results from a large community study conducted by the Indian Council of Medical research (ICMR) revealed that a lower proportion of the population is affected in states of Northern India (Chandigarh 0.12 million, Jharkhand 0.96 million) as compared to Maharashtra (9.2 million) and Tamil Nadu (4.8 million). The National Urban Survey conducted across the metropolitan cities of India reported similar trend: 11.7 per cent in Kolkata (Eastern India), 6.1 per cent in Kashmir Valley (Northern India), 11.6 per cent in New Delhi (Northern India), and 9.3 per cent in West India (Mumbai) compared with (13.5 per cent in Chennai (South India), 16.6 per cent in Hyderabad (south India), and 12.4 per cent Bangalore (South India). In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. Rough estimates show that the prevalence of diabetes in rural populations is one-quarter that of urban population for India and other Indian sub-continent countries such as Bangladesh, Nepal, Bhutan, and Sri Lanka (Kaveeshwar, & Cornwall, 2014).

Last twenty years have shown a rising trend of diabetes in India. The number of people with diabetes in India has increased from 19 million in 1995 to 66.8 million people in 2014 (Fig 2.5) (Mohan et al, 2015). The first Phase of National Family Health Survey for the reference year 2019 – 20 (NFHS-5) has covered 17 States and 5 Union Territories and the survey for the remaining 14 States/Union Territories of India in the second phase is in progress. Data for Delhi is not yet reported.

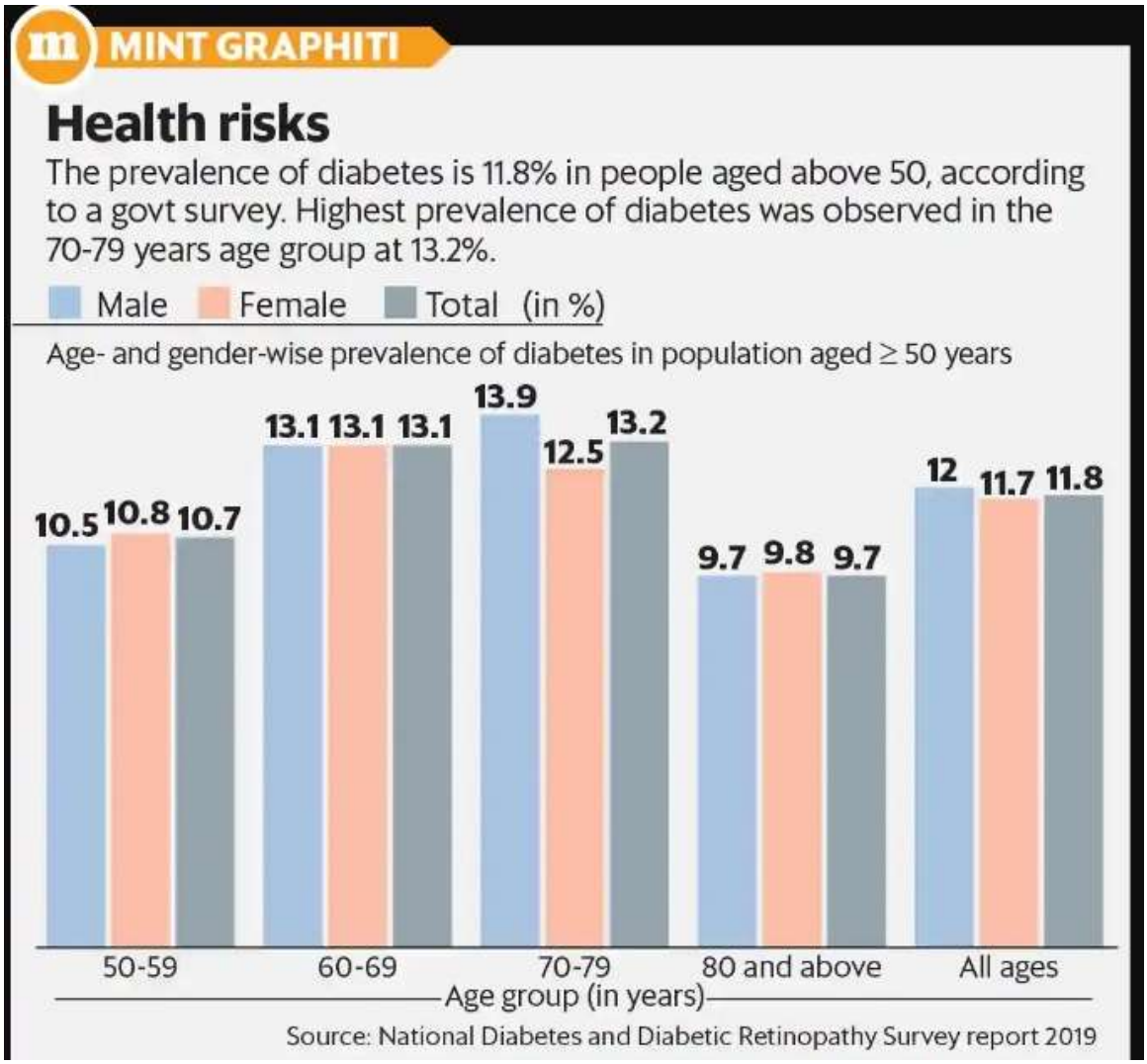
FIG. 2.5: THE RISING PREVALENCE OF DIABETES IN INDIA FOR LAST TWENTY YEARS



(Adapted from Mohan et al, 2015)

Recently a report released by the health and family welfare ministry stated the prevalence of diabetes in India has remained at 11.8% (Fig 2.6) in the last four years, according to the National Diabetes and Diabetic Retinopathy Survey. Males showed a similar prevalence of diabetes (12%) as females (11.7%). Highest prevalence of diabetes was observed in 70-79 years' age group at 13.2%. This survey was conducted in 21 districts during 2015-2019 by Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of medical sciences. (Sharma, 2019)

FIG. 2.6: PREVALENCE OF DIABETES IN INDIA BY NATIONAL DIABETES AND DIABETIC RETINOPATHY SURVEY REPORT (2019)



DRUGS IN TYPE 2 DIABETES MELLITUS

Drugs in T2DM are generally known as oral glucose lowering agents which are subdivided into agents that increase insulin secretion, reduce glucose production, increase insulin sensitivity, and enhance GLP-1 action as shown in Table 2.3.

METFORMIN HISTORY

Metformin, a biguanide, was introduced in the United Kingdom in 1958, in Canada in 1972, and in the United States in 1995. As stated in the commentary on historical overview of metformin by Bailey, 2017, the history of metformin is linked to *Galega officinalis* (Fig 2.7) (also known as French lilac or goat's rue or Spanish sainfoin or professor weed), a traditional herbal medicine in Europe, found to be rich in guanidine, which, in 1918, was shown to lower blood glucose. Guanidine derivatives, including metformin, were synthesized and some (not metformin) were used to treat diabetes in the 1920s and 1930s but were discontinued due to toxicity and the increased availability of insulin. Metformin was rediscovered in the search for antimalarial agents in the 1940s and, during clinical tests, proved useful to treat influenza when it sometimes lowered blood glucose. This glucose lowering property of metformin was pursued by the French physician Jean Sterne, who first reported the use of metformin to treat diabetes in humans in 1957. (Bailey, 2017)

Metformin is representative of the class biguanides which works on liver by reducing the hepatic glucose production and improves peripheral glucose utilization slightly. The mechanism for functionality of metformin on glucose metabolism is complex and not yet fully understood. Though the available evidence suggests that metformin activates AMP dependent protein kinase and enters cell through organic cation transporters. Metformin reduces fasting plasma glucose and insulin levels, improves the lipid profile, and promotes modest weight loss. (Rena et al, 2017).

FIG. 2.7: *GALEGA OFFICINALIS* / FRENCH LILAC OR GOAT'S RUE: HERBAL SOURCE OF METFORMIN

(Adapted from Bailey, 2017)

MEDICALLY PRESCRIBED DOSE OF METFORMIN: The initial starting dose of 500 mg once or twice a day can be increased to 1000mg bid. An extended -release form of metformin is available and it may have fever, GI side effects (diarrhoea, anorexia, nausea, metallic taste). Because of its relatively slow onset of action and GI symptoms with higher dose, the dose should be escalated every 2-3 weeks based on Self-Monitoring Blood Glucose (SMBG) measurements (Powers, 2012, 2018). Metformin is effective as monotherapy and can be used in combination with other oral agents or with insulin. The major toxicity of metformin, lactic acidosis is very rare and can be prevented by careful patient selection. (Marin-Penalver et. al, 2016).

METFORMIN CONTRAINDICATIONS: Metformin should not be used in patients with moderate renal insufficiency (GFR<45ml/min) or any form of acidosis, CHF, liver disease or severe hypoxemia. The National Institute of health and Clinical excellence in the U.K. suggest that metformin may be safe at GFR> 30 ml/min, with reduced dose when the GFR < 45ml/min. (Powers , 2018)

Metformin should be discontinued in patients who are seriously ill, in patients who can take nothing orally, and in those receiving radiographic contrast material. Insulin should be used until metformin can be restarted. (Power, 2012)

Till date metformin is the drug of first choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function. (ADA, 2020).

RECENT INDICATION OF METFORMIN: It is regarded as the first-line treatment for type 2 diabetes in most guidelines around the world (IDF, 2019). Metformin therapy for prevention of type 2 diabetes should be considered in those with prediabetes, especially for those with BMI ≥35 kg/m^2, those aged ,60 years, and women with prior gestational diabetes mellitus. Long-term use of metformin may be associated with biochemical vitamin B12 deficiency, and periodic measurement of vitamin B12 levels should be considered in metformin-treated patients, especially in those with anemia or peripheral neuropathy (ADA, 2020)

TABLE 2.3: DRUGS USED IN MANAGEMENT OF T2DM

Category of Drugs	Mechanism of action	Drug specific benefits	Drug specific side effects	Contraindications
Biguanides e.g. Metformin, Phenomorfin,	Decrease hepatic glucose production	Weight neutral, Do not cause hypoglycemia, Inexpensive	Diarrhea, Nausea, Lactic acidosis	Serum creatine >1.5mg/dl(men) >1.4mg/dl(women) CHF, acidosis, severely ill patients
α- glucosidase inhibitors e.g.Acarbose, Miglitol	Decrease GI glucose absorption	Reduce postprandial glycemia	GI flatulence, Affects liver functions	Renal or liver disease
Dipeptidyl Peptidase IV inhibitor(DPP-IV) e.g. Saxagliptin, Sitagliptin, Vildagliptin	Prolong endogenous GLP-1 action	Do not cause hypoglycemia	-	Reduce dose with renal disease
Insulin secretagogues: Sulfonylurea e.g.Glimepride Glipizide Glipizide (Extended Release) Glyburide	Increase insulin secretion	Inexpensive	Hypoglycemia, Weight gain	Renal/Liver disease
Insulin secretagogues: Non- Sulfonylurea e.g.Repaglinide Nateglinide	Increase insulin secretion	Lower postprandial glucose, short onset of action	Hypoglycemia	Renal/liver disease

Thiazolidinediones e.g.Rosiglitazone Pioglitazone	Decrease insulin resistance, Increase glucose utilization	Lower insulin requirements	Peripheral edema, CHF, Weight gain, fractures, roziglitazone may increase cardiovascular risk	CHF, Liver disease
Bile acid sequestrants e.g.Colesevelam	Bind bile acids, mechanism of glucose lowering not known	-	Constipation, Dyspepsia, Abdominal pain, nausea, increased triglyceride, interfere with absorption of other drugs, intestinal obstruction	High plasma triglycerides

(Adapted from Powers, 2012)

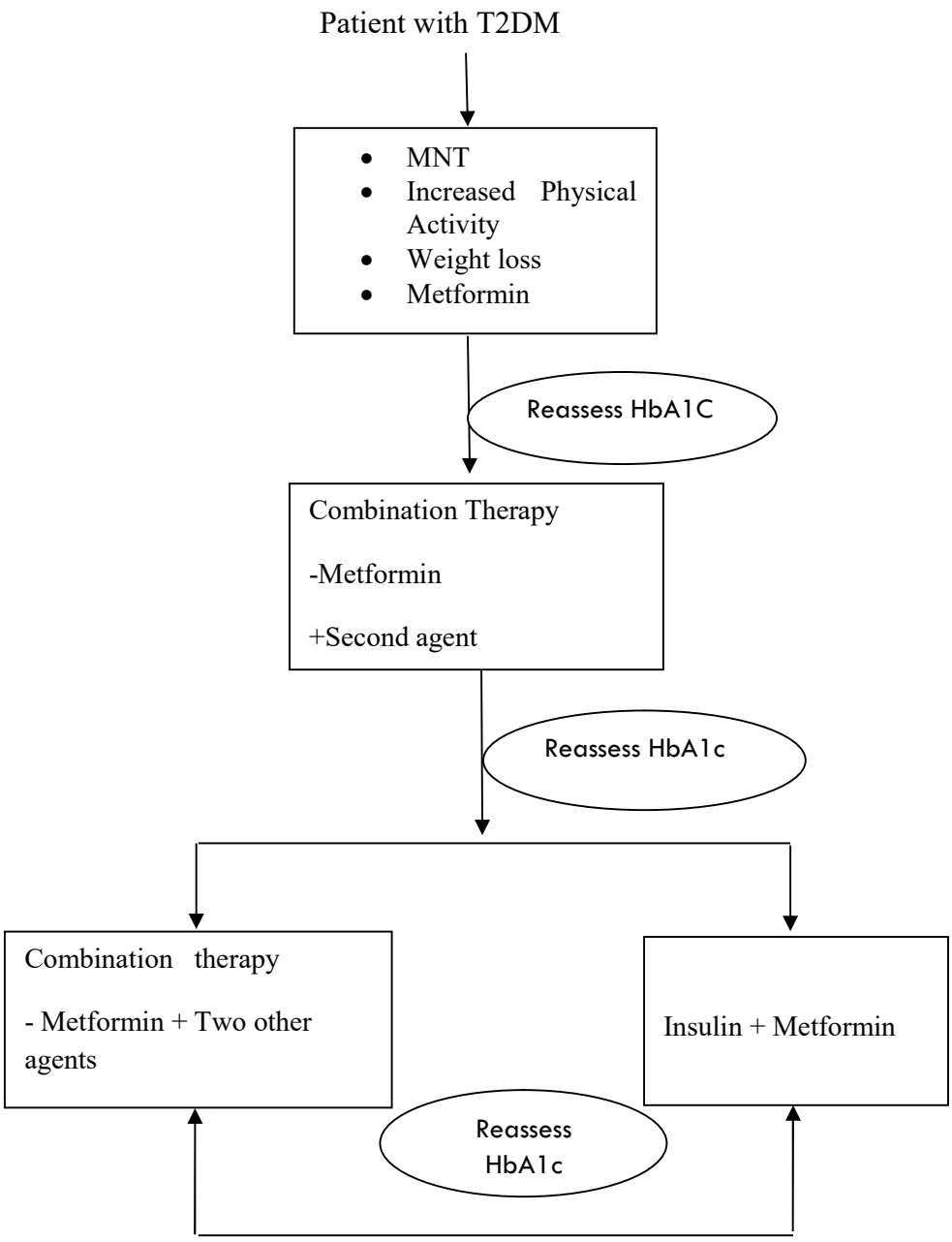
CHOICE OF INITIAL GLUCOSE LOWERING AGENT

Choice of initial glucose lowering agent depends on the level of hyperglycemia. Fig 2.8 depicts the glycemic management of type 2 diabetes which suggests that prime goal is to initiate Medical Nutrition Therapy (MNT) and increased physical activity. If we assume maximum benefit from MNT and increased physical activity, patients with mild to moderate hyperglycemia (FPG= 200-250mg/dl) respond well to single oral glucose lowering agent. Patients with more severe hyperglycemia (FPG>250mg/dl) are unlikely to achieve normoglycemia with oral monotherapy. Insulin can be used as initial therapy in individuals with severe hyperglycemia (FPG>250-300mg/dl). This approach is based on the fact that more rapid glycemic control will reduce 'glucose toxicity' to the islet cells. This improves insulin secretion and allow glucose lowering agents to be more effective. Further insulin may be discontinued. (Powers, 2012)

Insulin secretagogues, DPP-IV inhibitors and α glucosidase inhibitors begin to lower plasma glucose immediately, whereas the glucose lowering effects of the biguanides and thiazolidinediones are delayed by several weeks. Not all agents are effective in all individuals with T2DM. Most individuals will eventually require treatment with more than one class of oral glucose lowering agents or insulin. (Powers, 2012)

Considerable clinical experience exists with metformin and sulfonylureas as they have been available since decades. The newer classes of drugs including α glucosidase inhibitors, DPP-IV inhibitors, and thiazolidinediones are assumed to reduce DM related complications by improving glycemic control, although long term data are not yet available. However, all of these agents are currently costlier than metformin and sulfonylureas. Fig 2.9 depicts that metformin is used for initial therapy because of its efficacy and relatively lower cost. Metformin has the advantage that it promotes mild weight loss, lowers insulin levels and improves the lipid profile slightly. Based on HbA1c results, the dose of metformin should be increased until the glycemic target is achieved or maximum dose is reached. (Powers, 2012 and Powers, 2018)

FIG. 2.8: GLYCEMIC MANAGEMENT OF TYPE 2 DIABETES



(Adapted from Powers, 2018)

COMBINATION THERAPY WITH GLUCOSE LOWERING AGENTS

The effect of combination therapy on glycemic control is usually additive because mechanisms of action of the first and second agents used are different. The dosing of agents in combination is the same as when the agents are used alone. Several fixed dose combinations of oral agents are available but evidence of its superiority to titration of single agent to a maximum dose and then addition of second agent is lacking. If adequate control is not achieved with the combination of two agents, a third oral agent or basal insulin should be added. (Powers, 2012)

METFORMIN INDUCED LOW B12 LEVELS AND TYPE 2 DIABETES MELLITUS

According to the ADA 2020 guidelines, metformin and lifestyle modifications are the first line therapies in the treatment of T2DM. However, Metformin causes vitamin B-12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency, a clinically important and treatable condition. Despite of this fact any guidelines for annual monitoring or screening of the B12 is not reflected in clinical practice though some studies suggest for screening of B12 in T2DM adults on biguanide drugs have been reported in past decades as described in following literature.

The published literature suggests that intestinal malabsorption in T2DM patients on metformin was first studied by Berchtold et. al, 1969. He studied the disturbance of intestinal absorption following metformin therapy by observing the mode of action of biguanides. His study can be summarized as follows: In 21 patients with clinical or chemical diabetes mellitus and obesity, urinary excretion of vitamin BI~ and Dxylose, and the levels of blood lipids were determined before and 10 days after metformin treatment. In addition, fat tolerance and fat balance studies were carried out in 7 patients. In a long term study of 9 patients, the Schilling test was repeated 2--3 months after the beginning of the metformin treatment in a) 7 patients with permanent metformin treatment, and b) 2 patients after cessation of initial metformin treatment. The results were as follows: vitamin B~ and D-xylose absorptions were pathologically low after metformin treatment. Blood lipids and body weight were reduced. There was also some

indication of disturbed fat absorption, which, however, could not be proved. Based on these results Berchtold stated that it is possible that the decrease in blood sugar after treatment with metformin is partly induced by malabsorption.

Tomkin et al, 1971 took up studies on long term effect of metformin in T2DM adults where he studied the long term effect of metformin (more than 2 years). Vitamin-B12 malabsorption has been found in 21 (30%) of 71 diabetic patients taking long-term metformin therapy in addition to dietary management. The patients with evidence of B12 malabsorption had significantly lower haemoglobin levels (and significantly higher serum folic acid levels) than those with normal B12 absorption. Steatorrhoea was found in only one patient. Stopping metformin therapy resulted in reversion of B12 absorption to normal in most patients examined. Four patients with B12 malabsorption were found to have pathologically low serum B,2 levels. The causes and implications of these findings are discussed and it is concluded that all patients on long-term metformin therapy should have annual serum B12 estimations. It remains to be shown whether the malabsorption of B12 is due to competitive inhibition by metformin in the distal ileum or whether the enzyme system involved in the active absorption.

The malabsorption of vitamin B12 in diabetic patients treated with another biguanide drug phenformin: a comparison with metformin was studied by Tomkin, 1973 which is described as follows. An investigation to study B12 absorption in diabetic patients on long-term phenformin therapy was undertaken and the results were compared to a similar investigation previously reported in diabetics on long-term metformin. 46% of the patients were found to have B12 malabsorption as shown by abnormal results of Schilling tests. The mechanism of B12 malabsorption is unknown but it is suggested that all patients on long-term phenformin therapy should, like the patients on metformin, have annual serum B12 estimations until the results of a longer follow-up series are known.

In another study, low serum levels of vit B12 were reported in 17.5% of patients using 2 g of metformin daily for at least 2 years. (Stowers and Smith, 1971)

In 1995, one early randomized controlled trial by De Fronzo, R.A. and Goodman, A., M. metformin decreased the serum vitamin B12 levels by 22% and 29% compared to placebo and glyburide respectively.

Wulffele et. al, 2003 studied short term effect of metformin in type 2 diabetes where he studied a total of 196 subjects who were randomized to receive metformin and 194 to receive placebo. The actual mean dose in the metformin-treated group was 2163 mg. Each patient in this group maintained his/her maximally tolerated daily dose (one, two or three tablets of 850mg) during the trial. 26 experienced adverse effects of metformin. Of these 26, 11 experienced diarrhoea, five flatulence, four fatigue, one pruritus, one headaches, one pyrosis, one nausea, one myocardial infarction and one patient died suddenly. This was the first randomized, placebo-controlled study that reports on the effects of treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12. We found that 16 weeks of metformin treatment in patients with type 2 diabetes was associated with an increase in serum homocysteine of ∼4% (about 0.7μmol L−1) and with decreases in serum folate and vitamin B12 of ∼7 and ∼14%, respectively. In addition, further analysis indicated that the increase in serum homocysteine was mediated by the decreases in serum folate and vitamin B12.

Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as the 4 month. This study by Wulffele et al, 2003 is the first randomized, placebo-controlled study that reports on the effects of treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12. It was found that 16 weeks (four months) of metformin treatment in patients with type 2 diabetes was associated with an increase in serum homocysteine of 4% (about 0.7μmol L−1) and with decreases in serum folate and vitamin B12 of 7 and 14%, respectively (Wulffele et. al, 2003).

The B12 lowering side effect of metformin has been demonstrated again in several ensuing cross sectional studies (Pflipsen et al, 2009; Sparre Hermann. et al, 2004; Nervo et al, 2011), case reports (Liu et. al, 2006; Bell 2010; Kumthekar et al 2012) and randomized controlled trials (Kos et. al, 2012; de Jager et. al, 2010). The risk of

developing metformin associated vitamin B12 deficiency is greatly influenced by increasing age, metformin dose and duration of use (de Jager et al, 2010; Ting et. al, 2006). Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as fourth month. Metformin induced B12 deficiency (< 200pg/ml) has been found to be 5.8% to 33% (Reinstatler et al, 2012; Qureshi et al, 2011) and it is said that this wide range of B12 deficiency is due to diverse study definition of B12 deficiency.

In the cross sectional study on 203 outpatient type 2 diabetic patients at a large military primary care clinic in USA, definite vitamin B12 deficiency was defined as serum vitamin B12 concentrations of <100 pg/ml or elevated serum methylmalonic acid of >243 nmol/L or homocysteine concentrations of >11.9 nmol/L if serum vitamin B12 concentrations were between 100 to 350 pg/mL (Pflipsen et al, 2009).

Clinically overt features of vitamin B12 deficiency manifest by 5–10 years owing to the large body stores in the liver mainly that are not quickly depleted (Andre's et. al, 2002).

The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor (Wile & Toth, 2010).Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum. This inhibitory effect is reversed with calcium supplementation (Bauman, et al, 2000).

In a nested case control study performed among 155 adult Chinese DM patients on metformin and 310 controls, every 1 g/day increase in the metformin dose conferred an odds ratio of 2.9 (95% confidence interval, 2.15-3.87) for developing vitamin B12 deficiency. Among patients using metformin for ≥ 3 years, the adjusted odds ratio was 2.4 (95% confidence interval, 1.46-3.91) compared with those who had received metformin for ≤ 3 years (Ting et al, 2006).

In one population based study among 1048 elderly Finnish subjects aged 65–100 years, the total prevalence of definite vitamin B12 deficiency was 12.1% (Loikas et. al, 2007). In this study, vitamin B12 deficiency was defined as total serum vitamin B12 concentrations <150 pmol/l or total serum vitamin B12 of 150–250 pmol/l and holotranscobalamin ≤37 pmol/l and homocysteine ≥15 μmol/l.

In a study by Singh et al, 2013 serum B12 level (in pg/ml) was significantly higher in non-metformin group as compared with metformin group. (549.2±244.7 versus 410±230.7, mean difference=139.6 95% CI 56.86-221.54, P=0.0011). The proportion of patients with possible B12 deficiency (150-220 pg/ml) in metformin exposed group was significantly higher than proportion in non-metformin exposed group [18/84 (21.4%) versus 3/52 (5.7%), mean difference=15.7% 95% CI 4.9-26.5%, P=0.026).

In the National Health and Nutrition Examination Survey (1999–2006 USA) by Reinstatler et al 2012 defined definite and borderline biochemical vitamin B12 deficiency as serum vitamin B12 concentrations of ≤148 pmol/l and >148-221 pmol/l respectively.

In one cross sectional study that documented a high prevalence of vitamin B12 deficiency of 33% among adult patients with T2DM by Qureshi et al, 2011, vitamin B12 deficiency was defined as serum vitamin B12 concentrations <150pg/ml. However, patients enrolled in this study were those who were on high dose (>2 g/day) and long-term (4 years) metformin treatment, both clinical factors known to be associated with vitamin B12 deficiency.

In one study by Kumar et al, 2017 where 161 T2DM subjects were studied over a period of 6 months at Karnataka institute of endocrinology and research Bangalore reported a prevalence of definite vitamin B12 deficiency(B12<200pg/ml) in 27.3% and biochemical B12 deficiency (200-300pg/ml) in 26.3%. Further analysis showed that 23.2% on 1000 mg metformin and 36.73% on 2000 mg metformin were deficient in vitamin B12 respectively. There was no correlation between vitamin B12 deficiency and duration of

metformin therapy.

In one study by Raizada et al 2017 a total of 183 T2DM patients were recruited from the endocrinology outpatient department of a tertiary care hospital(AIIMS) where 121 were in metformin group while 62 were in no metformin group. The mean age was 49.8 ± 10.2 y. and duration of metformin use was 27.3 ± 35.8 months (range 3–180 months). Maximum daily dose of metformin was 834.1 ± 754.2 mg (range 500–2550 mg). The cumulative dose of metformin was 980.6 ± 1576.1 g (range 75–10,950 g). Vitamin B12 deficiency prevalence was 35.5% (B12<150 pmol/L) and borderline deficiency was 22.3% levels between 150 and 221pmol/L.

Owing to the diverse definitions of vitamin B12 deficiency used in most studies in different regions of the world, comparison of the prevalence of vitamin B12 deficiency among T2DM patients and healthy general populations is difficult.

There are various mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM. These proposed mechanisms from reviewed literature can be summarized as below:

- ✓ Alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency (Akinlade, 2015)
- ✓ Competitive inhibition or inactivation of vitamin B12 absorption (Akinlade, 2015),
- ✓ Alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor (Andre`s et al, 2002).
- ✓ Biguanides can induce malabsorption by two different mechanisms. One of these is temporary and unrelated to intrinsic factor secretion and the other is permanent and mediated by depression of intrinsic factor secretion. (Adams et. al, 1983)
- ✓ Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum. This inhibitory effect is reversed with calcium supplementation (Bauman et al, 2000).

VITAMIN B12 METABOLISM

Vitamin B12, also known as cobalamin, is a water soluble vitamin that has a crucial role in the normal formation of RBCs and the functioning of the brain and nervous system. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism (Yamada, 2013)

Sources of B12: No fungi, plants, or animals (including humans) are capable of producing vitamin B12.Vitamin B12 is not present in plant foods Human dietary sources include milk, eggs, fish and meat in quantities in excess of a few micrograms a day (Rosenblatt and Fenton, 2001).

ABSORPTION AND DISTRIBUTION OF VITAMIN B12 IN HUMAN BODY

In humans, the absorption, transport and cellular uptake of cobalamin is complex. Food-bound cobalamin is released in the stomach with the help of peptic activity, where it is subsequently bound by haptocorrin (Quadros, 2010). In the small intestine, cobalamin is released from haptocorrin by pancreatic protease digestion and bound by intrinsic factor (IF) to form an IF–Cbl complex. The IF–Cbl complex passes through the small intestine, where it is bound on the apical surface of ileal epithelial cells by a receptor composed of a heterodimer of amnionless and cubilin, called cubam, which aids in the endocytosis of IF–Cbl (Moestrup et al, 1998 and Fyfe et al, 2004). Once inside the cell, IF is degraded in the lysosomes and cobalamin is released into the cytosol (Kapadia et al, 1983). Thereafter it is transported across the ileal receptor cell and released into the bloodstream. In the bloodstream, cobalamin binds to either haptocorrin or transcobalamin (Morkbak et al, 2006). Although haptocorrin binds the bulk (75%-90%) of plasma cobalamin, it is not involved in cellular cobalamin uptake apart from uptake in hepatocytes (Morkbak et al, 2006). Therefore, individuals who have deficient or absent haptocorrin have serum cobalamin values in the deficient range, but show no sign of cobalamin deficiency. Transcobalamin binds only a minor fraction (10-25%) of circulating cobalamins, it is the protein responsible for facilitating the uptake of cobalamin by cells (Hall and Finkler, 1963). Transcobalamin acts as a final screening mechanism because, like IF,

transcobalamin is very specific for cobalamin forms that have the lower DMB intact (Allen 1975 and Fedosov et al, 2002). Treatment of transcobalamin deficiency requires very high serum cobalamin levels, ranging from 1000 to 10000 pg/ml, achieved by oral or intramuscular delivery of 0.5–1.0 mg of CNCbl or HOCbl once or twice weekly (Cooper & Rosenblatt, 1987). There is some evidence that at sufficiently high concentrations, at least some tissues are capable of taking up unbound cobalamin (Rosenblatt and Fenton, 2001). From the bloodstream, cobalamin is taken up into cells through receptor-mediated endocytosis as a complex of Cbl–TC bound to the transcobalamine receptor (TCblR) (Youngdahl-Turner et al, 1979).

The vitamin B12 –IF complex is highly resistant to proteolytic degradation. The complex attaches at its specific receptors on the mucosa of the terminal ileum, a site where its absorption occurs. This stage of vitamin B12 absorption is calcium mediated.

The intracellular vitamin B12 is released following IF degradation. This free vitamin B12 attaches to another protein carrier, transcobalamin –II (TC-II) and is later released into the circulation. This vitamin B12 – TC-II complex, also referred to as holo TC-II is then actively taken up by the liver, bone marrow and other vital body cells. The liver serves as the principal storage site of up to 90% of the body's total vitamin B12.

A enzymatic pathways disruption in any of the described steps above will result into clinical or biochemical vitamin B12 deficiency. This includes insufficient dietary intake especially among alcoholics and vegetarians and malabsorption due to several conditions like chronic atrophic gastritis mainly in the elderly, pernicious anemia, celiac disease, chronic pancreatitis and drugs like metformin and proton pump inhibitors (PPIs).

PHYSIOLOGICAL FUNCTIONS OF VITAMIN B12

Vitamin B12 has its physiological effects in human body through mediating two principal (Fig 2.9):

i. The methylation process of homocysteine to methionine and

ii. The conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA.

Vitamin B12 act as a co-factor in the methylation of homocysteine to methionine which is later activated into S-adenosyl-methionine that donates its methyl group to methyl acceptors such as membrane phospholipids, myelin and neurotransmitters. Metabolically significant vitamin B12 deficiency hence will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine causing hyperhomocysteinemia. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurons and the vascular endothelium. This reaction is also essential in the conversion of dietary folate (methyl-tetrahydrofolate) to its active metabolic form, tetrahydrofolate. In another essential enzymatic pathway, vitamin B12 as a co-factor mediates the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. In the presence of vitamin B12 deficiency, this conversion pathway is diminished and an increase in the serum methylmalonic acid (MMA) ensues. This is followed by defective fatty acid synthesis of the neuronal membranes (Malouf & Areosa Sastre, 2003). Vitamin B12 is also essential in the synthesis of monoamines or neurotransmitters like serotonin and dopamine (Bottiglieri, 2000). This synthesis is impaired with vitamin B12 deficiency.

All the above collectively explain the resultant neurocognitive manifestations that accompany vitamin B12 deficiency. Axonal demyelination, degeneration and later death are the hallmark of vitamin B12 deficiency induced neuronal damage that manifests as severe peripheral or autonomic neuropathy, sub-acute combined degeneration of the spinal cord, delirium and dementia (Selhub et al, 2009). Evidence demonstrates that hyperhomocysteinemia is also associated with an increased risk of cardiovascular events due to its cellular and vasculo-toxic effects (Melhem et al, 2009, Selhub, 2008 and Sadeghian et al, 2006). Vitamin B12 is an essential nutrient required in DNA synthesis, cellular repair and normal haemopoesis along with other micronutrients like folate and iron.

Vitamin B12 deficiency is classically associated with overt haematological findings like macrocytic red blood cells (mean cell volume [MCV]> 100 fl) with/without anaemia,

ovalocytes, hyper segmented white blood cells (i.e. >5% of neutrophils with ≥5 lobes) and pancytopenia (Aslinia et al, 2006). Due to defective cell repair processes, atrophic glossitis, stomatitis and malabsorption due to villi atrophy and mucositis are also common.

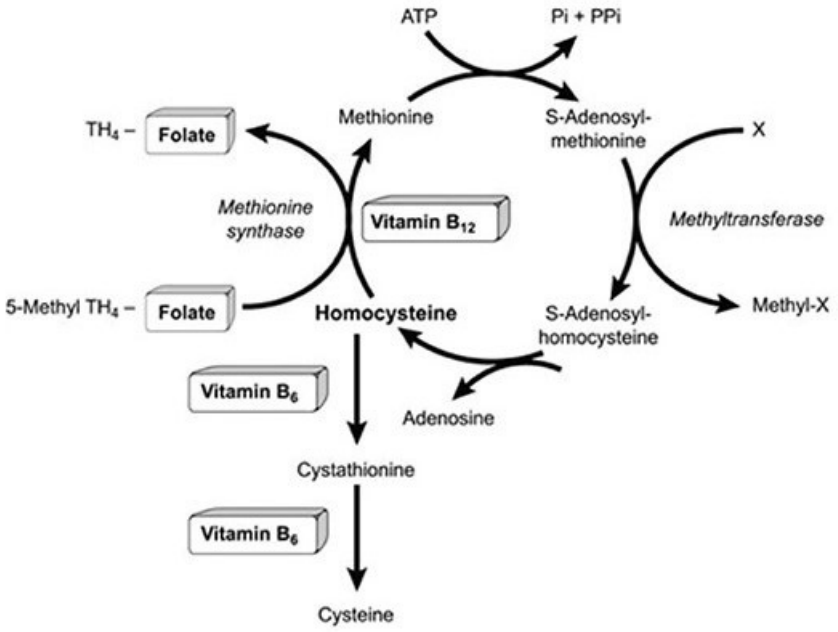
VITAMIN B12 DEFICIENCY IN DIABETES

Till date no published guidelines exist to advocate routine screening for vitamin B12 deficiency among patients with T2DM. Despite its superior glycemic lowering effect, metformin has been shown to decrease vitamin B12 levels.

The problem of B12 deficiency due to metformin in T2DM adults is known since 1971. In a study by Tomkin et al (1971) vitamin B12 malabsorption has been found in 21 (30%) of 71diabetic patients taking long-term metformin therapy in addition to dietary management. The patients with evidence of B12 malabsorption had significantly lower haemoglobin levels (and significantly higher serum folic acid levels) than those with normal B12 absorption. Stopping metformin therapy resulted in reversion of B12 absorption to normal in most patients examined. The study concluded that all patients on long-term metformin therapy should have annual serum B12 estimations.

In one early randomized controlled trial by DeFronzo et al metformin decreased the serum vitamin B12 levels by 22% and 29% compared to placebo and glyburide respectively (DeFronzo and Goodman 1995).

FIG 2.9: FUNCTIONS OF VITAMIN B12 IN HUMAN BODY



Methionine synthase is a vitamin B_{12}-dependent enzyme that catalyzes the formation of methionine from homocysteine using 5-methyltetrahydrofolate (5-methyl TH_4), a folate derivative, as a methyl donor. Another pathway catalyzed by betaine homocysteine methyltransferase also remethylates homocysteine to methionine using betaine as a methyl donor (not shown here). Methionine, in the form of S-adenosylmethionine, is required for most biological methylation reactions, including DNA methylation.

(Adapted from website of Micronutrient Information Centre, Linus Pauling Centre, Oregon State University: https://lpi.oregonstate.edu/mic/vitamins/vitamin-B12)

Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as the 4th month (Wulffele et al, 2003). Clinically overt features of vitamin B12 deficiency manifest by 5–10 years owing to the large body stores in the liver mainly that are not quickly depleted (Andre et al, 2002).

The vitamin B12 lowering side effect of metformin has been demonstrated again in several cross sectional studies (Pflipsen et al. 2009; Sparre Hermann et al 2004; Nervo et al 2011) and case reports (Liu et al, 2006, Bell, 2010 and Kumthekar, 2012) and randomized controlled trials (Kos et al, 2012 and de Jager et al, 2010)

In a study by Pflipsen et al (2009) 22% (n = 44) of diabetic patients had metabolically confirmed B(12) deficiency. Patients on metformin had lower serum B12 levels (425.99pg/mL vs 527.49pg/mL; P = .012) and were at increased risk for B12 deficiency (P = .04), as defined by a serum B12 level <350pg/mL. Prevalence of B12 deficiency was significantly lower for patients using a multivitamin (odds ratio, 0.31; 95% CI, 0.15-0.63).

The development of metformin associated vitamin B12 deficiency is influenced by several factors like increasing age, metformin dose and duration of use (de Jager et al 2010 and Ting et al 2006). In a nested case control study done among 155 adult Chinese DM patients on metformin and 310 controls, every 1 g/day increase in the metformin dose conferred an odds ratio of 2.9 (95% confidence interval, 2.15-3.87) for developing vitamin B12 deficiency. Among patients using metformin for ≥ 3 years, the adjusted odds ratio was 2.4 (95% confidence interval, 1.46-3.91) compared with those who had received metformin for ≤ 3 years (Ting et al, 2006).

In a study by Reinstatler et al (2012) biochemical B12 deficiency was present in 5.8% of those with diabetes using metformin compared with 2.4% of those not using metformin (P = 0.0026) and 3.3% of those without diabetes (P = 0.0002). Among those with diabetes, metformin use was associated with biochemical B12 deficiency (adjusted odds ratio 2.92; 95% CI 1.26–6.78). Consumption of any supplement containing B12 was not

associated with a reduction in the prevalence of biochemical B12 deficiency among those with diabetes, whereas consumption of any supplement containing B12 was associated with a two-thirds reduction among those without diabetes.

In another recent study conducted in Nigeria by Akinlade et al (2015) vitamin B12 deficiency and borderline deficiency were recorded in 8.6% and 26.0% of the patients respectively. Vitamin B12 deficiency was defined as serum concentration of <200 pg/dl, borderline deficiency as 200 – 300pg/dl and >300pg/dl as normal**.** Vitamin B12 level was significantly lower in patients who have been on metformin for greater than 10 years compared with patients with less than 10 years history of metformin use. Similarly, patients who were on metformin at a dose of >1000 mg/day had significantly lower vitamin B12 level when compared with patients on <1000 mg/day. To conclude it can be stated that low serum vitamin B12 level is associated with longer duration and higher dose of metformin use. Therefore, routine determination of vitamin B12 level in patients with T2DM on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from vitamin B12 supplements.

In another recent study conducted in 27 study centers in U.S.by Aroda et al, 2016 low B12 (≤203 pg/mL) occurred more often in metformin group than placebo group at 5 years (4.3 vs 2.3%; $P = .02$) but not at 13 years (7.4 vs 5.4%; $P = .12$). Combined low and borderline-low B12 (≤ 298 pg/mL) was more common in metformin group at 5 years (19.1 vs 9.5%; $P < .01$) and 13 years (20.3 vs 15.6%; $P = .02$). Years of metformin use were associated with increased risk of B12 deficiency (odds ratio, B12 deficiency/year metformin use, 1.13; 95% confidence interval, 1.06–1.20). Anemia prevalence was higher in metformin group, but did not differ by B12 status. Neuropathy prevalence was higher in MET with low B12 levels.

In a study by de Groot-Kamphuis et al (2013) conducted in Netherland in the total cohort (n=298), the overall prevalence of vitamin B12 concentrations <150pml/l was 9.7% (95% CI 6.6-13.7%). In type 2 diabetes patients not taking metformin (n=134), the prevalence was 4.4% (95% CI 1.6-9.4%) been compared with 14.1% in metformin users (n=164)

(95% CI 9.2-20.4%; p=0.006). Each 100 mg step in metformin dose increased (OR=1.081, p=0.014), whereas proton pump inhibitor (PPI) use lowered (OR=0.322, p=0.037) the odds of having a vitamin B12deficiency in logistic regression. Nevertheless, metformin use did not predict the chance on having anaemia or neuropathy. To conclude it can be stated that among patients with type 2 diabetes using metformin, the prevalence of vitamin B12 deficiency is higher than compared with patients not using metformin. However, metformin use did not predict the chance of having anaemia or neuropathy.

In a study by Ting et al (2006) a total of 155 patients with metformin-related vitamin B deficiency (mean ± SD serum vitamin B concentration, 148.6 ± 40.4 pg/mL [110 ± 30 pmol/L]) were compared with 310 matched controls (466.1 ± 330.4 pg/mL [344 ± 244 pmol/L]). After adjusting for confounders, the authors found clinically important and statistically significant association of vitamin B deficiency with dose and duration of metformin use. Each 1-g/d metformin dose increment conferred an odds ratio of 2.88 (95% confidence interval, 2.15-3.87) for developing vitamin B deficiency (P<.001). Among those using metformin for 3 years or more, the adjusted odds ratio was 2.39 (95% confidence interval, 1.46-3.91) (P = .001) compared with those receiving metformin for less than 3 years. After exclusion of 113 subjects with borderline vitamin B concentration, dose of metformin remained the strongest independent predictor of vitamin B deficiency.

In another study by Kos et al, 2012 at the department of General Internal Medicine, Loyola University Medical Center, Maywood, Illinois, USA the effect of metformin therapy was seen on B12 levels and it was found that patients taking metformin had statistically significant lower vitamin B12 levels than those not receiving metformin (P<.0001; 95% confidence interval [CI] = -220 to -84 pg/mL).

In a Korean study by Ko et al, 2014 a total of 799 T2DM adults using metformin was enrolled. Vitamin B12 levels were measured by chemiluminescent enzyme immunoassay. Vitamin B12 deficiency was defined as vitamin B12 ≤ 300 pg/mL and the prevalence of vitamin B12 deficiency in metformin-treated T2DM adults was 9.5% (n = 76), and the

mean vitamin B12 level was 662.5 ± 246.7 pg/mL. Vitamin B12 deficient patients had longer duration of metformin use (P < 0.001) and higher daily metformin dose (P < 0.001) than non-deficient patients. Compared with daily metformin dose of ≤ 1,000 mg, the adjusted odds ratio for 1,000-2,000 mg, and ≥ 2,000 mg were 2.52 (95% CI, 1.27-4.99, P = 0.008) and 3.80 (95% CI, 1.82-7.92, P < 0.001). Compared with metformin use of < 4 yr, the adjusted odds ratios for 4-10 years, and ≥ 10 years were 4.65 (95% CI, 2.36-9.16, P < 0.001) and 9.21 (95% CI, 3.38-25.11, P < 0.001), respectively. To conclude the study indicated that patients with type 2 diabetes treated with metformin should be screened for vitamin B12 deficiency, especially at higher dosages (> 1,000 mg) and longer durations (≥ 4 yr) of treatment with metformin.

As regards scenario in India the data is limited about the effect of metformin use on serum vitamin B12 levels in type 2 diabetes patients. In a recent study by Raizada et al 2017 in tertiary care center in North India the serum vitamin B12 levels were 267.7 ± 194.4 pmol/l in metformin group and 275.1 ± 197.2 pmol/l in the no metformin group (P = 0.78). When adjusted for duration of diabetes, metformin use was associated with a 87.7 ± 37.7 pmol/l (95% confidence interval [CI], −162.1–−3.3, P = 0.02) lower serum vitamin B12 levels. No significant increase in the prevalence of neuropathy (DNS and DNE scores), anemia, or MCV was found in the vitamin B12 deficient patients (levels <150 pmol/l) as compared to patients with normal vitamin B12. However, serum vitamin B12 levels for the entire cohort were higher by 12.2 ± 3.0 pmol/l (95% CI 6.4–18.0, P < 0.001) for every 1-year increase in the duration of diabetes.

In another Indian study by Singh et al, 2013 at Ranchi, Jharkhand; mean serum B12 levels was significantly lower in metformin exposed group (n=84) compared with non-metformin exposed group(n=52) (410±230.7 versus 549.2±244.7, P=0.0011). Odds ratio for possible B12 deficiency was 4.45 (95% CI 1.24-15.97). There was significant negative correlation between cumulative metformin dose and vitamin B12 level (r=−0.68, P<0.0001).

A mastermind study by Donnelly et al 2020 indicates the risk of anemia with metformin use in T2DM. The mechanism is unknown but the study limitation is that it has not measured serum B12 levels and has no mention of B12 deficiency among metformin users.

A multicenteric centric observational study from seven centers of Pakistan (Sindh, Punjab, Baluchistan and Khyber Pakhtunkhwa) recruited participants with T2DM treated with metformin for >2 years and those not on metformin. Participants were assessed for hb., vitamin B12, homocysteine and diabetic neuropathy (vibration perception threshold (VPT) >15V) and painful diabetic neuropathy (Douleur Neuropathique 4 (DN4) ≥4) and Diabetic Neuropathy Symptom (DNS) score ≥1. This study shows that vitamin B12 insufficiency was associated with neuropathy in subjects on metformin. There was significantly increased prevalence of B12 deficiency in T2DM treated with metformin as compared with non-metformin users. Moreover, we observed that subjects with B12 deficiency have high VPT (>25), DNS score (≥1) and DN4 score (≥4) as compared with non-metformin users (Miyan and Waris, 2020)

NUTRIENT-DRUG INTERACTION: CALCIUM SUPPLEMENTATION IN METFORMIN INDUCED LOW B12 LEVELS

There is scanty of literature about calcium supplementation to improve metformin induced low B12 levels. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor (Andre et al, 2002). Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum. This inhibitory effect is reversed with calcium supplementation (Bauman et al, 2000).

In the study by Bauman et al, 2000 a comparative study design was employed using 2 groups (metformin and control). A total of 21 T2DM adults received sulfonylurea

therapy; 14 of these 21 patients were switched to metformin. Monthly serum total vitamin B12 measurements and holotranscobalamin were performed. After 3 months of metformin therapy, oral calcium supplementation was administered. Serial serum vitamin B12 determinations revealed a similar decline in vitamin B12 and holotranscobalamin. Oral calcium supplementation reversed the metformin-induced serum holoTCII depression. To conclude: patients receiving metformin have diminished B12 absorption and low serum total vitamin B12 and TCII-B12 levels because of a calcium-dependent ileal membrane antagonism, an effect reversed with supplemental calcium.

In the study by Bauman et al 2000 since the results for calcium supplementation are significantly associated with the increase in serum holo TCII, it shows that the transfer of B12-IF complex into holo TCII is independently facilitated by calcium despite the B12 lowering effect of metformin. This concludes that the nutrient calcium is overcoming the inhibitory effect of the drug metformin at the conversion of B12+IF complex, thereby releasing B12-IF complex in the terminal ileum for absorption. Thereby calcium seems to reverse the malabsorbtion of vitamin B12 due to metformin in T2DM males. When calcium is given as dietary supplements in the metformin- treated group it reverses the decreased serum holoTCII levels. The serum holoTCII increased after calcium supplementation from month 3 to month 4 ($P < 0.005$) but the serum total vitamin B12 level did not change significantly. (Bauman et al, 2000)
Till date there are no guidelines of standard care for treating low B12 levels in T2DM given by ADA or IDF.

The study by Kocaçiftçi et al (2013) aimed to compare the effects of metformin versus metformin and calcium treatments on serum vitamin B12 levels in newly diagnosed T2DM and Impaired Fasting Glucose patients. The study patients with a new diagnosis of T2DM and IFG were randomized into two groups. One group received daily metformin of 2000 mg (group 1), while the other group received daily metformin of 2000 mg/d plus oral calcium supplements (group 2). Before and after treatment following parameters were measured: Fasting blood glucose, vitamin B12, lipid parameters, HbA1c and homocysteine levels. The study included 48 patients, 22 women (45.8%) and 26 men

(54.2%). There were 12 men (46,2%) and 14 women (53.8%) in group 1,14 men (63.6%) and 8 women (36.4%) in group 2. Mean ages were 54.77Â±7.59 (36-65) and 53.45Â±9.15 (35-65) years in group 1 and 2, respectively. When pre and post treatment biochemical parameters in group 1 were compared, significant reductions in serum vitamin B12, lipid parameters, HbA1c and fasting blood glucose levels were found following the treatment. In group 2, there were also significant reductions in serum homocysteine vitamin B12, HDL-choesterol, triglyceride and fasting glucose levels after the treatment. When serum vitamin B12 levels were compared before and after the treatment, although the difference was not statistically significant, the decrease in serum vitamin B12 levels in group 2 was found to be 26.60 pg/ml lower than in group 1. To conclude it is found that vitamin B12 levels decreased less with metformin plus calcium therapy compared to only metformin therapy. It may be suggested that additional calcium supplements may prevent B12 deficiency and associated complications in patients on metformin therapy (Kocaçiftçi et al, 2013).

SECONDARY COMPLICATIONS OF T2DM

Broadly, the injurious effects of hyperglycemia are separated into macrovascular complications and microvascular complications. Macrovascular complications are those which are caused due to damage of large vessels whereas microvascular complications are those which manifest due to damage of small blood vessels because of persistent hyperglycemia.

Macrovascular complications include coronary artery disease, peripheral arterial disease, and stroke whereas microvascular complications include diabetic nephropathy, retinopathy and neuropathy. Macrovascular complications include cardiovascular diseases such as heart attacks, strokes and insufficiency in blood flow to legs. There is evidence from large randomized-controlled trials that good metabolic control in both type 1 and 2 diabetes can delay the onset and progression of these complications (WHO, 2016).

DIABETIC RETINOPATHY (EYE DISEASE)

Diabetic retinopathy is a leading cause of blindness and visual disability as stated in WHO 2016 global report on diabetes. The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia (Fowler, 2008). Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hypertension in the U.K. Prospective Diabetes Study (UKPDS), and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis (Keenan et al, 2007) . As stated in global report on Diabetes by WHO 2016 diabetic retinopathy is caused by small blood vessel damage to the back layer of the eye, the retina, leading to progressive loss of vision, even blindness. The common complaints include blurred vision, although other visual symptoms may also be present. Diagnosis of early changes in the blood vessels of the retina can be made through regular eye examinations (Fowler, 2008). Good metabolic control can delay the onset and progression of diabetic retinopathy and an early detection and treatment of vision-threatening retinopathy can prevent or delay blindness. Treatment involves regular eye examinations and timely intervention.

DIABETIC NEPHROPATHY (KIDNEY DISEASE)

Diabetic kidney disease is also caused by damage to small blood vessels in the kidneys. This can cause kidney failure, and eventually lead to death. In developed countries, this is a leading cause of dialysis and kidney transplant (WHO, 2016). Diabetic nephropathy is the leading cause of renal failure in the United States. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or “microalbuminuria.” Microalbuminuria is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes (Fowler, 2008). As stated by Powers, 2012 once macroalbuminuria exists, it is unclear whether improved glycemic control will slow progression of renal disease. During the later

phase of declining renal function, insulin requirements may fall as the kidney is a site of insulin degradation. Furthermore, many glucose lowering medications (sulphonylureas and metformin) are contraindicated in advanced renal insufficiency. If diagnosed at an early stage, several measures can retard the progression to kidney failure. These include control of high blood glucose, control of high blood pressure, intervention with medication in the early stage of kidney damage, and restriction of dietary protein (WHO, 2016)

DIABETIC NEUROPATHY (NERVE DISEASE)

The Global report on diabetes by WHO, 2016 states that diabetes causes nerve damage through different mechanisms, including direct damage by the hyperglycemia and decreased blood flow to nerves by damaging small blood vessels. This nerve damage can lead to sensory loss, damage to limbs, and impotence in diabetic men. It is the most common complication of diabetes. Depending on which nerves are affected, there are many symptoms: for example, numbness in extremities, pain in extremities, and impotence. This decreases sensation to feet which can lead to patients not recognizing cuts and developing foot infections. If not treated early, these can lead to amputation (Powers, 2012). Early diagnosis is made by early recognition of symptoms by patients and health care providers as well as by careful examination by health care providers at regular intervals. If detected early, and blood glucose brought under control, these complications can also be prevented or delayed (WHO, 2016).

Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the costliest complications of diabetes, especially in communities with inadequate footwear. It results from both vascular and neurological disease processes. Regular inspection and good care of the foot can prevent amputations. Comprehensive foot programs can reduce amputation rates by 45-85% (WHO, 2016).

Diabetic neuropathy is the most common microvascular complication of diabetes, and it is a major cause of morbidity and mortality (Fowler, 2008). As diabetic neuropathy

leads to foot ulcers and amputation- major cause of morbidity and disability in people with diabetes, the ADA, 2012 recommends annual foot examination of the people with diabetes so that high risk foot conditions can be identified and treated further. By definition Diabetic peripheral neuropathy (DPN) is somatic and/ or autonomic neuropathy that is attributed solely to diabetes mellitus. Assessment for distal symmetric polyneuropathy should include a careful history and assessment of either temperature or pinprick sensation (small fiber function) and vibration sensation using a 128-Hz tuning fork (for large-fiber function). All patients should have annual 10-g monofilament testing to identify feet at risk for ulceration and amputation. Diabetic neuropathy is a diagnosis of exclusion. Nondiabetic neuropathies may be present in patients with diabetes and may be treatable (ADA, 2020).

Neuropathy prevalence changes (10%-90%) according to diagnostic criteria and patient population. Diabetic peripheral neuropathy is the most common type of diabetic neuropathy, and it is frequently used synonymously with it (Dyck et al, 1993).

Diabetic polyneuropathy (DPN) is one of the most common complications of diabetes mellitus, affecting approximately 50% of patients (Bloomgarden, 2008). The mechanism of DPN is not clear and it is multifactorial (Kennedy and Zochodne, 2005). DPN prevalence increases with age and diabetes duration, with the highest rates found among patients who have had diabetes mellitus for ≥ 25 years (Poncelet, 1998; Yasuda et al, 2003; Boulton et al, 2005).

Suboptimal glycemic control is also associated with higher rates of DPN, although DPN may occur when blood sugar levels are well controlled. Diabetes mellitus is diagnosed as the cause of neuropathy by exclusion of various other conditions, such as trauma or pressure on the nerve, vitamin deficiencies (B1, B6, B12, E and niacin), alcoholism, infections (Lyme disease, varicella zoster, Epstein–Barr, hepatitis C and HIV/AIDS), autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis and Guillain-Barré syndrome), inherited disorders (Charcot– Marie–Tooth disease and amyloid polyneuropathy) and tumours.(Kennedy and Zochodne, 2005).

Symptoms of DPN typically occurs first in the feet and lower limbs then in the hands. This is known as 'stocking and glove' pattern. It affects the longer nerves first before progressing proximally (Boulton et al, 2005). Patients may lose vibration and proprioceptive sensation, temperature sensitivity and, eventually, pain sensation (Maser et al, 1989). The pain of DPN is insidious initially and is usually manifested by burning pain, paraesthesia and numbness.

The diagnosis and classification of DPN can be done on the basis of the history given by the patient and a thorough neurophysiological examination. Diagnosis is based on the presence of at least two of the following three characteristics: abnormal sensory or motor signs, symptoms and decreased tendon reflexes (Trujillo- Hernandez et al, 2005). Electrophysiological testing may be required for diagnosis of DPN in many cases. (Albers et al, 2007) One such test involves measurement of the Hoffmann reflex (H-reflex), which has been found to be more frequently altered in patients with recently diagnosed diabetes mellitus (Maryniak and Yaworski, 1987) Studies have shown that H reflex is apparent before any changes in motor nerve conduction velocity (NCV) so any alterations in the H reflex provide an early indication of DPN. These findings suggest that the H reflex may be useful as a criterion for the diagnosis of DPN (Trujillo et al, 2005; Maryniak and Yaworski, 1987).

As cited in position statement on Diabetic Neuropathy by Pop-Busui et al, 2017 it is said that among the various forms of diabetic neuropathy, distal symmetric polyneuropathy (DSPN) and diabetic autonomic neuropathies, particularly cardiovascular autonomic neuropathy (CAN), are by far the most studied**.**

MECHANISM OF DIABETIC NEUROPATHY

Experimental studies suggest a multifactorial pathogenesis of DSPN (Fig 2.10), but the causes remain unknown. A prevailing view of the pathogenesis is that oxidative and inflammatory stress may, in the context of metabolic dysfunction, damage nerve cells. (Pop-Busui et al, 2017)

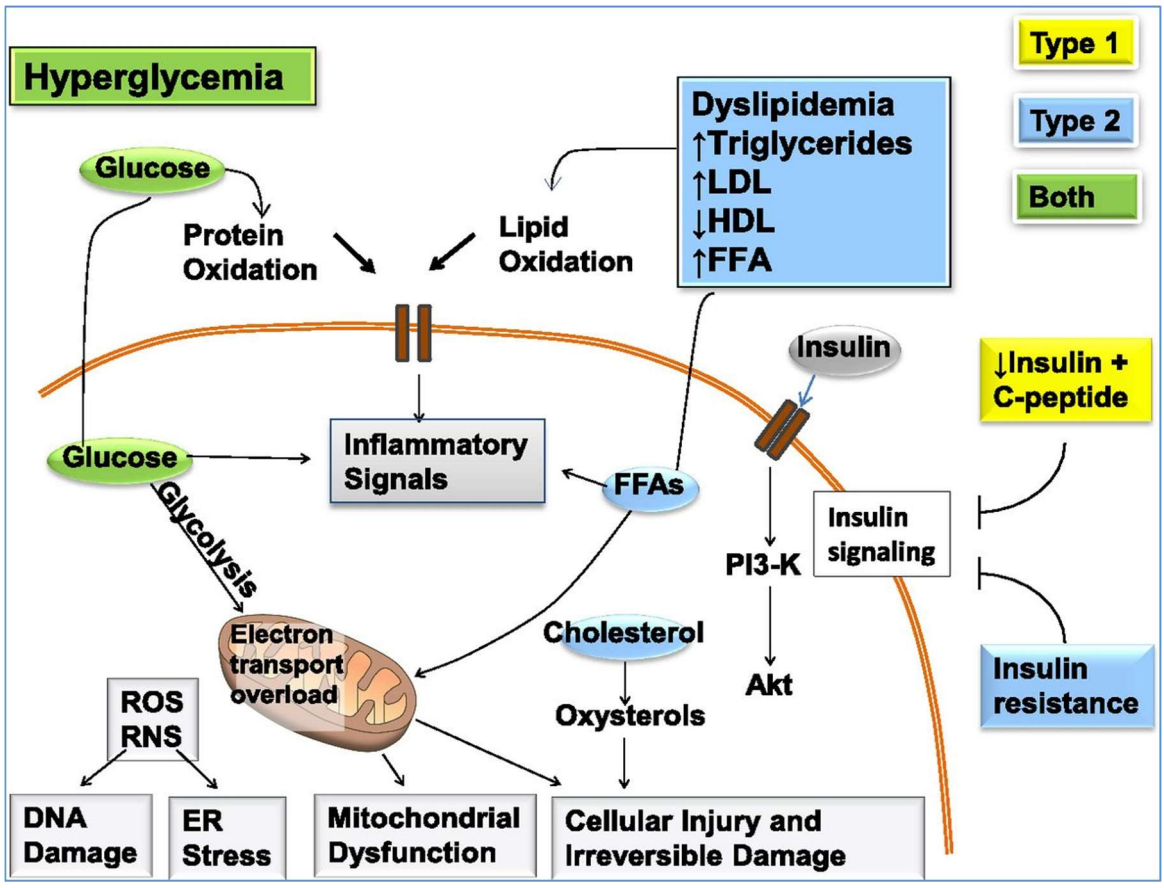
CURRENT GUIDELINES FOR SCREENING OF DPN

Recommendation for screening and management are given in the international Guidelines for Diagnosis and Outpatient Management of Diabetic Peripheral Neuropathy and are summarized in Table 2.4 (Boulton et al, 1998). The Clinical Practice Guidelines of the Canadian Diabetes Association recommend annual screening for neuropathy using 10g Semmes-Winstein monofilament or 128 Hz tuning fork. In type 2 diabetes screening should begin at diagnosis while for type 1 diabetes who are past puberty screening should begin after 5 years (Canadian Diabetes Association, 2003).

Individuals with one or more high risk foot conditions should be evaluated more regularly. Individuals with neuropathy should have their feet inspected visually at every visit with a healthcare professional. According to the latest recommendation of American Diabetes Association people with diabetes should have an annual foot exam to identify high-risk conditions. All patients should be assessed for DPN starting at diagnosis of type 2 diabetes. Assessment for distal symmetric polyneuropathy should include a careful history and assessment of either temperature or pinprick sensation (small fiber function) and vibration sensation using a 128-Hz tuning fork (for large-fiber function). All patients should have annual 10-g monofilament testing to identify feet at risk for ulceration and amputation (ADA, 2020).

To conclude it can be said that DPN is the most common complication associated with both types of diabetes. Diagnosis of DPN is crucial as it can lead to substantial discomfort and pain. In more advanced cases it can lead to non-healing foot ulcerations, amputations and loss of ambulation.

FIG. 2.10: MECHANISM OF DIABETIC NEUROPATHY



(Adapted from Pop-Busui et al, 2017)

Description of figure: (Pop-Busui et al. 2017)

Mechanisms of diabetic neuropathy. Factors linked to type 1 diabetes (yellow), type 2 diabetes (blue), and both (green) cause DNA damage, endoplasmic reticulum stress, mitochondrial dysfunction, cellular injury, and irreversible damage. The relative importance of the pathways in this network will vary with cell type, disease profile, and time. ER, endoplasmic reticulum; FFA, free fatty acids; PI3-K, phosphatidylinositol-3 kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species.

TABLE 2.4: GUIDELINES FOR DIAGNOSIS AND OUTPATIENT MANAGEMENT OF DPN

Patient History	Age, diabetes, physical factors, lifestyle, social circumstances, symptoms, other possible etiological factors
Examination of both feet	Skin status, sweating, infections, ulceration, callusues/ blistering, deformity, muscle wasting, arches, palpitation for temperature, pulse, joint mobility, examination of gait/shoes
Vascular examination	Check foot pulses
Other	Thyroid function to exclude other etiologies for neuropathy. Note the presence or absence of characteristics of the 'at risk foot'

(Adapted from Cornblath, 2004)

In DPN there is nerve damage in early course of diabetes which worsens gradually over time without clinical symptoms until the condition is fairly advanced. The only intervention proven to alter DPN pathogenesis is glycemic control so it is important for clinicians to diagnose DPN early in order to limit its progression owing to DPN association with significant mortality and morbidity. Early intervention strategies can prevent foot ulcers and amputation while preserving the quality of life and ameliorating the social and economic costs of diabetic foot disease. (McCulloch, 2004)

TESTS FOR NEUROPATHY

Several different methods commonly used to screen and assess DPN include:

a. Reflex Testing
b. Superficial Pain testing
c. Light touch perception
d. Vibration testing
e. Sympathetic Skin response
f. Quantitative Sensory testing
g. Nerve Conduction studies

a. REFLEX TESTING

The most common test to assess DPN is to test only ankle reflexes as these are the most sensitive to early DPN. Ankle reflex testing is done at both ankles. The examiner dorsiflexes the foot and gently strikes the Achilles tendon with the reflex hammer while the patient is asked to be in sitting or kneeling position. If no reflex occurs, the test can be repeated with reinforcement. Reflexes are typically scored as 0 (absent), 1 (present but decreased),2 (normal),3 (increased) or 4 (increased with clonus) (Smieja et al, 1999). In a cross sectional study conducted at 10 centers in the United States, Canada and Switzerland, ankle reflex testing had reasonable reproducibility with moderate agreement (ƙ= 0.59) between examiners (Smieja, 1999). Ankle reflex has better reproducibility if

evaluated as normal or abnormal. However, the test is a poor indicator of ulceration. (Boyko et al, 1999).

b. SUPERFICIAL PAIN TESTING

A sterile safety pin can be used to test pain sensation. The site of testing may include the dorsum of the great toe or the planter aspect of the distal first, third and fifth toe of each foot. Pinprick is highly subjective as a means of screening for neuropathy and thus poorly reproducible (Maser et al, 1989)

c. LIGHT TOUCH PERCEPTION

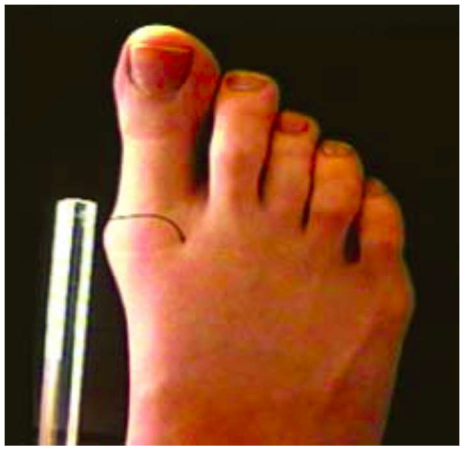
Light touch perception can be evaluated by using a number of methods from a finger, to cotton, to specific calibrated devices. The best known of the calibrated devices is the Semmes- Weinstein 10g- monofilament, a nylon filament embedded in plastic handle. The instrument is calibrated to provide a specific force measured in grams that is 10 times the log of the force in milligrams exerted at the tip of the monofilament (Mayfield and Sugarman, 2000).

Monofilaments have been manufactured in sizes ranging from 1.65 to 6.65. Studies testing various sizes of monofilaments support the utility of the 10g monofilament- the initial study in patients with diabetes found no patient with a neuropathic ulcer could sense the 10g monofilament (Birke and Sims, 1986). Two observational studies and five prospective studies support use of the 10g monofilament as the best correlate to the presence or history of an ulcer, but there are two other observational studies that suggest that the 4.21(1g) is a better discriminator (Boyko et al, 1999).

Till date there is no consensus about the proper testing sites for the Semmes Weinstein monofilament (Fig 2.11) (Mayfield and Sugarman, 2000). Recommendation range from sites distributed over the plantar surfaces of the toes to metatarsal heads, insole, heel and

dorsum of the foot. However, the data show that the sites chosen may affect reproducibility. One study

FIG. 2.11: SEMMES WEINSTEIN MONOFILAMENT (SWM) FOR SENSORY TESTING



(Adapted from Lawrence, 2004)

found that examination of the forefoot had moderate reproducibility (Ƙ= 0.38-0.54), but examination of the arches, heel, and dorsum had only fair reproducibility (Ƙ= 0.22-0.38) (Smieja et al, 1999). Many physicians test the dorsum of the distal toe first, recording yes or no, then test more proximally if there is an abnormality.

Criteria that define an insensate foot according to testing with the Semmes Weinstien monofilament are controversial, but all 5 of the prospective studies validating the use of the 10g monofilament used results from more than one site (Boyko et al, 1999 and Kumar et al, 1991)

d. VIBRATION TESTING

Vibration testing is another measure used to evaluate nerve function. Traditionally, vibration perception has been measured with a 128-Hz tuning fork or less commonly 64 or 256 Hz tunning fork. There are several methods for testing vibration. Many methods rely on "examiner experience", which has a variable correlation with quantitative tests. An analysis of three large cohorts (n=787) found that use of tunning fork overestimated vibration sensation loss as compared with quantitative sensory testing (Burns et al, 2002). Discordance between tests was associated with age and body surface area in one cohort, with age and body surface area in a second cohort and with age in the third cohort. The authors of this analysis recommended that physicians take these factors into account when judging clinical abnormalities. Although vibration testing can be a highly subjective measure of severity of neuropathy and may be poorly reproducible, the absence of vibration sensation at the great toe is significantly associated with development of foot ulcers. (Smieja et al, 1999 and Boyko et al, 1999).

e. SYMPATHETIC SKIN RESPONSE

The sympathetic skin response is a reflex that occurs in response to a change in the electrical potential of the skin. It is transient in nature and can be caused by variety of

stimuli. Measurement requires special equipment that is not typically available in most physician's office. (Bril et al, 2000)

f. QUANTITATIVE SENSORY TESTING

Quantitative sensory testing is the determination of the absolute sensory threshold (ADA 1996) which is useful in assessing the integrity of the axons that form the peripheral nervous system and their distal receptors. Quantitative sensory testing allows differentiation of the relative deficit between small (e.g temperature) and large (e.g. vibration) diameter axons between peripheral neuropathy and mononeuropathy. This test is simple, noninvasive and non-aversive. Different quantitative sensory testing detect different nerve fiber defects. (Cheng et al, 1999)

There are typically 2 types of devices: those that generate specified vibratory or thermal stimuli and those that deliver electrical impulses at certain impulses at certain frequencies (Shy et al, 2003). Using the methods of limits, the patient indicates when he or she first feels an increasingly strong stimulus or when he or she no longer feels a decreasing stimulus. With the method of levels, specific levels are tested and the patient reports whether or not the stimulus is detected. The method of levels is also referred to as the "forced choice" algorithm.

Instruments that quantifies vibration perception include: Bio-Theisometer, Horwell Neurothesiometer and vibratorn II. Biothesimeter (Bio Medical Instrument Company, Newbury Ohio) is the instrument which vibrates at 100 Hz with an amplitude varying from 0 to 50 volts. Studies show that a drop of more than 25 volts in the vibratory threshold is a strong predictor of future ulceration. (Pham et al, 2000) Vibration perception threshold can be measured via Horwell Neurothesiometer (Scientific Laboratory Supplies, Nottingham, UK) and Vibratorn II (Physitemp Instruments, Clifton, NJ). In a comparative head to head trial, Bril and co-workers found that repeated neurothesiometer measurements were less variable than repeated measurements of the

Vibratorn (8% vs 6% for the right and left toes for the Neurothesiometer compared with 31% vs 34% for the right and left toes for the Vibratorn. (Bril et al, 1997)

Although quantitative methods exist to measure both large and small nerve fiber sensory function, measures of the vibratory threshold by different devices is more consistent, providing greater reliability in measuring the function of large sensory nerve fibers. (Perkins and Brill 2003) In 2003, the subcommittee on Therapeutics and Technology Assessment of the American academy of Neurology stated that quantitative sensory testing is an effective tool in documenting sensory abnormalities. But there is no credible prospective evidence that these abnormalities ultimately develop into clinical neuropathy. Thus the utility of quantitative sensory testing as screening tool is unproven (Shy et al, 2003).

g. NERVE CONDUCTION STUDIES (NCS)

Nerve conduction studies assess the presence and severity of peripheral nerve involvement in diabetics. Studies typically are performed on upper and lower limbs on motor and sensory nerves. Nerve conduction studies are used for symptomatic, confusing, unusual or severe neuropathy. There is negative correlation between sensory nerve conduction velocity & glycemic control. There is progressive slowing of sensory nerves in diabetics which is accelerated by poor glycemic control (Farheen & Arif, 2015) NCS can detect peripheral nerve dysfunction even in early-stage disease and allow neuropathy to be quantitatively evaluated (Akaza et al, 2018).

Studies suggest nerve conduction abnormalities are present at diagnosis in 29% to 70% of patients with type 1 diabetes and 45% to 60% of patients with type 2 diabetes (el Bahri-Ben Mrad et al, 2000). Nerve conduction studies show how diabetic sensorimotor peripheral neuropathy progresses. First, sensory and motor amplitudes of distal nerves are lost; then changes occur in the more proximal nerves and in the upper limbs. (Donofrio and Albers, 1990)

Although nerve conduction studies can be used to determine the extent and severity of DPN, recent data suggest measures of sural nerve action potential are useful in identifying patients with early DPN. Sural nerve conduction correlated well to early, mild DPN, potentially allowing patients to be identified and treated at an earlier stage of disease. Dyck and collegues from the Rochester conducted a Cohort study and showed that composite scores tended to be more reproducible than individual attributes and generally correlated better to neurologic impairment. (Dyck et al, 1986)

While the gold standard for diagnosis of DPN continues to be a nerve conduction study, that is time consuming, requires a separate patient visit, and is costly which cannot be recommended for screening. (Al-Geffari, 2012). A simple method that has ease of use for the regular evaluation and diagnosis of DPN in the primary care setting is needed. (McCulloch, 2004)

To compare the above tests it can be said that these tests vary from each other in that they have advantages and disadvantages; they differ in ease of use, required skill levels and costs (Table 2.5). They also differ according to their sensitivity and specificity. (Sensitivity is derived from the threshold of normality; specificity is derived from the threshold of abnormality). (Table 2.6)

TABLE 2.5: TESTS FOR NEUROPATHY:
ADVANTAGE AND DISADVANTAGE AND THE COST

Test	Advantage/ Disadvantage	Ease of use	Level of skill required	Cost
SWMF	Rapid: differentiates nondiabetic controls, diabetic patients with without neuropathy	Easy to use	Requires minimal training, medical or non-medical staff can perform	Inexpensive
SPS	Rapid: less effective than NCS in distinguishing neuropathy in patients with diabetes	Easy to use	Requires minimal training, medical or non-medical staff can perform	Inexpensive
VO/O	Rapid: not universally standardized, many false positives and false negatives	Easy to interpret	Requires minimal training, medical or non-medical staff can perform	Inexpensive
Vibration by Times Method	Takes longer than SWWF,SPS, or VO/O	More complicated than SWWF,SPS, or VO/O	Requires minimal training, medical or non-medical staff can perform	Inexpensive
Vibration Perception Threshold	Cumulative incidence of foot ulceration with VPT<15 =2.9%	Takes 5-10 minutes to use	Requires minimal training, medical or non-medical staff can perform	Instrument costs several hundred dollars
Using Biothesiometer	Cumulative incidence of foot ulceration with VPT>25 =19.8% (OR=7.99% CI=365.175;p<.01)	Time consuming	Requires training	Less expensive

Tip Therm Temperature Discriminator	98.3% of patients with no monofilament sensation had no sensation with tip therm:97.3% of patients with biothesiometery diagnosed neuropathy had no sensation with tip therm	Simple	Requires minimal training medical or nonmedical staff can perform	Inexpensive
Quantitative Sensory testing	Different modalities have measurement errors of>30%.Even when variance is reduced using standardized methods, does not attain the precision of motor or sensory conduction velocity measures of NCS	Varied approaches	Requires extensive training	Expensive equipment
Electrophysiologic studies	Highly sensitive; poor sensitivity for ulceration, amputation and overall neuropathic impairment; may or may not correlate with symptoms; limited availability; Insensitive in identifying small fiber neuropathy	Complicated	Requires extensive training	Expensive equipment

SWMF= Semmes Weinstein 10-g monofilament; DPN-Diabetic polyneuropathy; SPS- Superfecial pain sensation; NCS- Nerve conduction studies; VO/O-Vibration by on/off; VPT-vibration perception threshold; OR-odds ratio; CI-Confidence Interval

(Adapted from Cornblath, 2004)

TABLE 2.6: SENSITIVITY AND SPECIFICITY FOR POSITIVE AND NEGATIVE LIKELIHOOD RATIOS FOR 4 SIMPLE SCREENING TESTS

Test	Abnormal Test Likelihood Ratio >1 of 8 Attempts insensate	Specificity	Normal Test Likelihood Ratio <1 of 8 Attempts insensate	Sensitivity
Vibration (VO/O)	26.6	99	0.51	53
Monofilament	10.2	96	0.34	77
Superficial pain	9.2	97	0.50	59
Vibration	18.5	98	0.33	80

VO/O- vibration by on off

(Adapted from Perkins et al, 2001)

A recent systematic review with meta-analysis to quantitatively evaluate the currently available evidence regarding the diagnostic accuracy of monofilament tests for DPN detection concluded that monofilament tests had limited sensitivity for screening diabetic peripheral neuropathy. The clinical use of the monofilament test in the evaluation of diabetic peripheral neuropathy cannot be encouraged based on currently available evidence. At present, monofilament tests have been already widely used and advocated for in many clinical guidelines. However, there is no consensus on the optimal location, number of sites, and threshold values for DPN diagnosis and the use of monofilament testing alone cannot be considered as an optimal practice for the diagnosis of DPN. Therefore, further research is needed to standardize the method for clinical practice because heterogeneity exists among studies (Wang et al, 2017).

DPN SCREENING INSTRUMENTS

Majority of screening instruments for DPN are noninvasive, inexpensive, sensitive-specific and highly predictive of clinical endpoints. To evaluate a patient for neuropathy, clinicians needs to ask patients about signs and symptoms, perform a thorough physical exam, including deep tendon reflexes, motor strength and vibration; as well as perform diagnostic studies such as nerve conduction velocities (NCV). (Lawrence et al, 2004)

A single instrument may not be sufficient for sensory testing (Armstrong, 2000 and Young et al, 1994) Clinicians should use a history-symptom questionnaire, a physical exam, Semmes-Weinstein monofilaments (SWM), vibration perception threshold (VPT) evaluation and NCV. Establishing a true sensitivity and specificity for VPT, SWM and nerve conduction is difficult because there is no true gold standard among these instruments however few studies assume NCV to be gold standard (Al-Geffari, 2012). Several studies have reported a strong correlation between VPT, NCV results and subjective symptoms of neuropathy (Lawrence et al, 2004).

One study by Brown et. al, 2017 examined the effectiveness of the 128 Hz tuning fork, two monofilaments: 1g and 10g, and Norfolk Quality of Life Diabetic Neuropathy (QOL-

DN) questionnaire as tools for the early detection of diabetic peripheral neuropathy (DPN) in overweight, obese, and inactive adults or those who have prediabetes or type 2 diabetes. The study concluded that1 g monofilament and total QOL-DN are effective, low-cost tools for the early detection of DPN. The 128 Hz tuning fork and 10 g monofilament may assist DPN screening as a tandem, but not primary, early DPN detection screening tools.

Several questionnaires are helpful in screening patients for DPN. These are the Michigan Neuropathy Screening Instrument (MNSI) (Feldman et al, 1994), Neuropathy Symptom Profile, Neuropathy Symptom Score, Diabetic Neuropathy Symptom Score (Meijer et al, 2002) and the UT Abbreviated Neuropathy Questionnaire. (Armstrong et al, 1998).

The most commonly used screening instruments are MNSI (discussed further) and Diabetic Neuropathy Symptom Score (Lawrence et al 2004) which is a four-item symptom score where 1 point is given for each positive response including

- unsteadiness in gait
- pain, burning or aching of the feet or legs
- prickling sensation of the feet or legs
- numbness in the feet or legs

The Diabetic Neuropathy Symptom Score has been evaluated in 73 diabetic studies, comparing it with clinical testing with SWM and VPT ((Lawrence et al, 2004). It was found to have a reliability of 0.64, a sensitivity of 79% and a specificity of 78%. (Meijer et al, 2002)

A physical exam of the patient should include close inspection, with shoes and socks removed. The physician should perform deep tendon reflexes, vibration with a128-Hz tuning fork; sharp, dull and light touch; and motor strength. (Lawrence et al, 2004). Such a provision is found in a set of questionnaire known as Michigan Neuropathy Screening Instrument (MNSI) which is discussed further.

As cited in position statement on Diabetic Neuropathy, it was said that the use of validated clinical instruments such as MNSI (most widely used in large cohorts of patients with type 1 and type 2 diabetes), the modified Toronto Clinical Neuropathy Scale (mTCNS), the Utah Early Neuropathy Scale (UENS), or the Neuropathy Disability Score (NDS) are recommended. (Pop-Busui et al, 2017)

These screening instruments may be combined with electrophysiology which measures the small-fiber damage and repair, such as intraepidermal nerve fiber density (219–221) to assess the microvascular complications- DPN. (Pop-Busui et al, 2017)

MICHIGAN NEUROPATHY SCREENING INSTRUMENT (MNSI)

MNSI includes two separate assessments: a 15-item self-administered questionnaire and a lower extremity examination that includes inspection and assessment of vibratory sensation and ankle reflexes. (Herman et al, 2012) Responses are added to obtain a total score: 'Yes' responses to questions 1–3, 5–6, 8–9, 11–12, 14–15 are each counted as one point. 'No' responses to questions 7 and 13 each count as one point. Question 4 was considered to be a measure of impaired circulation and question 10 a measure of general asthenia and were not included in the published scoring algorithm. A score of ≥ 7 was considered abnormal (Feldman et al, 1994)

The self-administered questionnaire answers the following yes or no questions based on how you feel in your legs and feet.
(*Source: a practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care 1994; 17: 1281-9.)*

1. Are your legs and/or feet numb?
2. Have you ever had burning sensation in your legs and/or feet?
3. Are your feet too sensitive to touch?
4. Do you get muscle cramps in your legs and/or feet?
5. Have you ever had any prickling feelings in your legs or feet?

6. Does it hurt when the bed covers touch your skin?
7. When you get into the tub or shower, are you able to distinguish the hot water from the cold water?
8. Have you ever had an open sore on your foot?
9. Has your doctor ever told you that you have diabetic neuropathy?
10. Do you feel weak all over most of the time?
11. Are your symptoms worse at night?
12. Do your legs hurt when you walk?
13. Are you able to sense your feet when you walk?
14. Is the skin on your feet so dry that it cracks open?
15. Have you ever had an amputation?

After the questionnaire, patients are evaluated neurologically in physical examination where a trained health professional inspects each foot for the following signs and symptoms:

- ✓ deformities,
- ✓ dryskin,
- ✓ calluses,
- ✓ infections
- ✓ fissures.

Foot deformities included prominent metatarsal heads, hallux valgus, joint subluxation, and Charcot joint. Each foot with any abnormality receives a score of 1. Each foot is also inspected for ulcers and each foot with an ulcer receives a score of 1.

Vibration sense was evaluated using 128 Hz vibration fork (Mete et al, 2013 and Herman et al, 2012). Vibrating fork was located on the interphalangeal joint of the right great toe. If the patient could not perceive vibration, two points were given. If the patient perceived vibration on the great toe, diaposone was located over ankle (inner malleolus) while it was still vibrating and the patient was asked to compare vibrations from two locations. If vibration was perceived better in ankle, 1 point was given. If no difference could be

found, no point was given. Zero point was accepted as normal, 1 point showed mild-moderate deficit, and 2 points showed a severe deficit (Herman et al 2012).

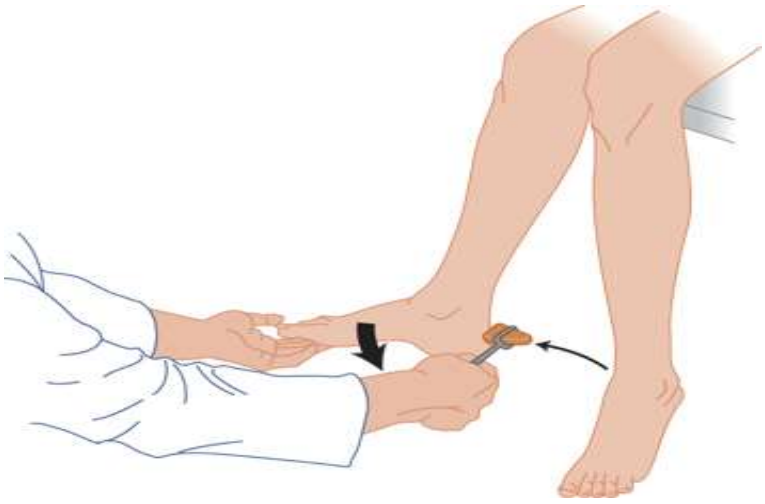
In yet another study by Mete et al, 2013 it was said that in general, the examiner should be able to feel vibration in his or her hand for 5 s longer than a normal subject can at the great toe. Vibration is scored as present if the examiner senses the vibration on his or her finger for < 10 s longer than the subject feels it in the great toe, decreased if sensed for ≥ 10 s (scored as 0.5) or absent (scored as 1).

Achilles reflex (The ankle jerk reflex, also known as the **Achilles reflex**, occurs when the **Achilles** tendon is tapped while the foot is dorsiflexed. It is a type of stretch reflex that tests the function of the gastrocnemius muscle and the nerve that supplies it) (Figure 2.11) was observed and reported as absent, decreased, or normal. Patients with normal Achilles reflex were given 0 point while patients with decreased Achilles reflex got 0.5 point and patients with no reflex got 1 point (Mete et al, 2012).

In another study by Mete et al, 2013 the ankle reflexes are elicited with Jendrassic Manoeuver (JM) (Fig 2.13). The JM has been used to reinforce lower limb reflexes since the 19th century and despite its common use, the application of the maneuver has not been standardized (Ertuglu et al, 2018). While in most research the JM was performed by pulling clenched hands and without teeth clenching (Khanal et al, 2007, Nardone and Schieppati, 2008), other studies included teeth clenching in the maneuver as well (Zehr and Stein, 1999).If the Achillies reflex is absent, the patient is asked to perform the Jendrassic manoeuver and if present, the reflex is designated as present with reinforcement and is scored as 0.5. If the reflex is absent with the JM, the reflex is designated as absent and is scored as 1.

The total possible score is 8 points and, in the published scoring algorithm, a score ≥ 2.5 is considered abnormal (Feldman et al, 1994).

FIGURE 2.12 ACHILLES REFLEX



(Adapted from neurology.mhmedical.com)

FIG 2.13 JENDRASSIC MANOEUVER

(Adapted from http://www.intranet.tdmu.edu.ua)

DPN MEASUREMENT THROUGH ELECTRONIC DEVICES: BIOTHESIOMETER AND NEUROTHESIOMETER

For past many years it has been known that biothesiometer is an electronic device which provides a quick and reliable assessment of vibration thresholds, which when related to the centile charts gives an objective measure of the progress of diabetic peripheral neuropathy. (Bloom et al, 1984).

Biothesiometer has been used as a noninvasive tool for the detection of subclinical neuropathy in children and adolescents. The use of height-related reference ranges may make screening for neuropathy more feasible in younger patients and allow large-scale longitudinal analysis of its development. (Davis et al, 1997)

The Neurothesiometer (Fig 2.14) is a new device designed to replace the Biothesiometer in screening for diabetic peripheral neuropathy by measuring vibration perception threshold (VPT). (Young et al, 1993). It gives results that are in close agreement with the Biothesiometer and has a good coefficient of variation in routine clinical use. It is a self-contained, battery operated device, which, although more expensive than biothesiometer, is well suited to diabetes screening programmes. As it is handy so it would be particularly useful to epidemiological surveys in the community.

There have been evidence which compared the effectiveness of the graduated tuning fork (128 Hz) and the neurothesiometer in assessing vibration sensation perception in type 2 diabetes mellitus patients. There was a positive correlation between the results for the two methods of assessment for both investigators, and also between the results for both tools at three individual sites. Overall, there was 66.2% agreement between the results obtained from the two investigators using the tuning fork at each site; however, Kappa values only reached statistical significance at one site, indicating variability between the results from the two tools. This study suggests that assessment of vibration sensation with the tuning fork may be unreliable. (O'Neill et al, 2005)

FIG 2.14 NEUROTHESIOMETER

(Adapted from http://www.drmohans.com)

PREVALENCE OF DPN: WORLD AND INDIA

Owing to the use of different tools and various screening instruments to study DPN the comparison of prevalence of DPN has become difficult and it varies greatly in different studies. Worldwide the various studies showed that the prevalence of DPN ranged from as low as 8% to as high as 59% (Young et al 1993, Partanen et al, 1995, Deli et al, 2013, Dyck et al, 1993).

There is limited evidence for the prevalence of DPN in Indian context. There are only few studies in Indian patients which have reported the prevalence of DPN to be 26% (Pradeepa et al, 2008), 34.9 per cent with VPT (Jayaprakash et al, 2011) and 31% by Paul et. al, 2012 and 39.3% in rural south Indian population by Darivemula et al, 2019.

In one study by Gill et. al, 2014 where DPN was assessed by Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) as well as the vibration perception threshold using a biothesiometer, the prevalence of DPN was 29.2% and the prevalence was similar in males (26.1%) and females (33.8%). An abnormal NDS was present in 44.5%, with 20% having a moderate - severe grade score. An abnormal NSS was found in 50%, with moderate - severe symptoms in 35%.

QUALITY OF LIFE (QOL)

It has been challenging to define QoL and many approaches to defining quality of life exist (Brazier et al, 2014). There are approaches based on human needs, subjective well-being, expectations, and phenomenological viewpoints. A related literature on well-being distinguishes between approaches based on objective lists, preference satisfaction, hedonism, flourishing, and life satisfaction (Karimi and Brazier, 2016).

Examples of definitions of QoL are: “a conscious cognitive judgment of satisfaction with one's life” (Rejeski and Mihalko, 2001 as cited in Karimi and Brazier 2016) and “an individuals' perception of their position in life in the context of the culture and value

systems in which they live and in relation to their goals, expectations, standards and concerns" (Kuyken and Group, 1995).

Recently, quality of life tools has been crucial in the evaluation of health care (WHOQOL, 1993). Different definitions of QOL have been proposed by different researchers or Organizations. The above definition reflects the view that quality of life refers to a subjective evaluation, which is embedded in a cultural, social and environmental context. (As such, quality of life cannot be equated simply with the terms "health status", "life style", "life satisfaction", "mental state" or "well-being") (WHOQOL, 1998). The study by Carr, Higginson and Robinson (as cited in Vahedi, S. 2010) states that quality of life is a broad-ranging concept affected in a complex way by the individual's physical health, psychological state, level of independence, social relationships, and their relationships to salient features of their environment. (Carr, Higginson and Robinson , 2003).

There are numerous tools available to measure quality of life. Some of the popular ones are: Health Related Quality of Life (HRQoL) which is a multi-dimensional concept that includes domains related to physical, mental, emotional, and social functioning and HRQoL is commonly used to examine the impact of health status on quality of life (Yin et al, 2016), Nottingham Health Profile(NHP) which consist of six domains assessing energy, sleep, pain, physical domain, emotional reactions and social isolation (Benbow, 1998), Modified Diabetes Quality of life (MDQoL) which consist of 17 diabetic specific questions and 8 concepts for physical, social functioning, role limitations due to personal and emotional problems, psychological impact, energy/fatigue, bodily pain, and general health perceptions. (Acharya et. al, 2014).

In our study WHO QOL Bref is used to assess QoL which is described in detail in the following text.

WHO has developed a quality of life tool, the WHOQOL, which captures several subjective aspects of quality of life. (WHOQOL, 1994 and WHOQOL, 1998).

The WHOQOL-BREF is one of the best tools that has been developed for cross-cultural comparisons of quality of life and is available in more than 40 languages (Vahedi, 2010).

It has been adopted in the United State of America, Netherlands, Poland, Bangladesh, Thailand, India, Australia, Japan, Croatia, Zimbabwe and many other countries (WHOQOL, 1992, Nejat et al, 2006).

Noerholm and coworkers (as cited in Vahedi, 2010) states that during the development of the WHOQOL, it was emphasized that quality of life is a multidimensional concept and the WHOQOL is under cross-cultural validation by the WHOQOL group.

In Brinbaum's study (as cited in Vahedi, 2010) it was stated that an abbreviated version of the WHOQOL-BREF that contains 26 items is applicable in clinical trials in which brief measures are needed, and also in epidemiological studies in which quality of life might be one of several outcome variables. The WHOQOL BREF covers four different domains of quality of life (WHOQOL, 1998).

RATIONALE FOR THE DEVELOPMENT OF THE WHOQOL

As stated in WHO QOL-BREF 1996 and WHO QOL 2012 user manual there are several reasons for which WHO took initiative to develop a quality of life assessment. Recently there has been a focus in the measurement of health, beyond usual health indicators such as mortality and morbidity, to include measures of the impact of disease and impairment on daily activities and behaviour, perceived health measures and disability / functional status measures. These measures, whilst beginning to provide a measure of the impact of disease, do not assess quality of life per se, which has been aptly described as the missing measurement in health. Second, most measures of health status have been developed in North America and the UK, and the translation of these measures for use in other settings is time-consuming and unsatisfactory for a number of reasons. Third, the increasingly mechanistic model of medicine, concerned only with the eradication of disease and symptoms, reinforces the need for the introduction of a humanistic element into health care. (WHO QOL-Bref 1996).

DEVELOPMENT OF THE WHOQOL-100

The WHOQOL-100 contains 100 questions. This is based on four questions per facet, for 24 facets of quality of life. In addition, four questions address *Overall quality of life and general health*. To date, around 30 language versions of the WHOQOL-100 have been developed (Table 2.7). Development work is ongoing on several further language versions of the WHOQOL-100. The required language version of the WHOQOL-100 (instructions, headers, questions and response scales) can be obtained from principal investigators in the respective country / region or failing that, from the Programme on Mental Health, World Health Organization, CH-1211 Geneva 27, Switzerland. (WHO QOL BREF, 1996).

The WHOQOL-100 development process consisted of several stages. In the first stage, concept clarification involved establishing an agreed upon definition of quality of life and an approach to international quality of life assessment which is stated above. This definition reflects the view that quality of life refers to a subjective evaluation which is embedded in a cultural, social and environmental context. (WHO QOL BREF, 1996)

In the second stage of development, exploration of the quality of life construct within 15 culturally diverse field centres was carried out to establish a list of areas/facets that participating centres considered relevant to the assessment of quality of life. This involved a series in meetings of focus groups which included health professionals, patients and well subjects. A maximum of six specific items for exploring each proposed facet were generated by each centers focus group. In the third stage of development, questions from each centre were assembled into a global pool. (WHOQOL-BREF, 1996)

Following field testing in these 15 centres, 100 items were selected for inclusion in the WHOQOL-100 Field Trial Version. These included four items for each of 24 facets of quality of life, and four items relating to the overall quality of life and general health facet (Table 2.8). The method by which these 100 items were selected is fully documented elsewhere (The WHOQOL Group, in preparation). The WHOQOL-100

TABLE 2.7 - EXISTING LANGUAGE VERSIONS OF THE WHOQOL

Language	Originating field centre
Bulgarian	Sofia, Bulgaria
Chinese	Guang Zhou, China
Chinese (Australian)	Melbourne, Australia
Chinese (Hong Kong)	Hong Kong
Croatian	Zagreb , Croatia
Czech	Bohnice,Czech Republic
Danish Copenhagen	Denmark
Dutch Tilburg	the Netherlands
English (Australian)	Melbourne, Australia
English (Canadian)	Victoria BC , Canada
English (UK) Bath	United Kingdom
English (USA) Seattle	USA
Estonian Tallinn	Estonia
French Paris	France
French (Canadian)	Rimouski PQ, Canada
German	Mannheim & Leipzig, Germany
Hebrew	Beer-Sheva, Israel
Hindi	Delhi India
Hungarian	Hungary
Italian Bologna	Italy
Japanese	Tokyo
Korean	Seoul
Malay	Kelantan
Norwegian	Bergin
Polish	Poznan
Portuguese (Brazil)	Porto Alegre
Russian	St Petersburg,Russia
Shona	Harare, Zimbabwae
Slovakian	Bratislava, Slovakia
Spanish (Argentina)	La Plata, Argentina
Spanish (Panama)	Panama City, Panama;
Spanish	Barcelona, Spain
Swedish	Molndal, Sweden
Tamil	Madras, India
Thai	Bangkok, Thailand
Turkish	Izmir, Turkey
Urdu (Pakistan)	Rawalpindi, Pakistan
Lozi	Lusaka, Zambia

Field Trial Version is currently being tested in new centres world-wide (these centres are outlined on page 6 of this document). The initial conceptual framework for the WHOQOL-100 proposed that the 24 facets relating to quality of life should be grouped into 6 domains. Recent analysis of available data, using structural equation modelling, has shown a four domain solution to be more appropriate. For a more detailed explanation of this, the reader is referred to The WHOQOL Group (in preparation). The WHOQOL-BREF is therefore based on a four domain structure (Table 2.9).

DEVELOPMENT OF THE WHOQOL-BREF

The WHOQOL group reports on the development of the WHOQOL-BREF, an abbreviated version of the WHOQOL-100 quality of life assessment. The WHOQOL-BREF was derived from data collected using the WHOQOL-100. It produces scores for **four domains related to quality of life: physical health, psychological, social relationships and environment.** It also includes one facet on overall quality of life and general health. (WHOQOL-BREF, 1996)

Domain scores produced by the WHOQOL-BREF correlate highly (0.89 or above) with WHOQOL-100 domain scores (calculated on a four domain structure). WHOQOL-BREF domain scores demonstrated good discriminant validity, content validity, internal consistency and test-retest reliability. This data suggest that the WHOQOL-BREF provides a valid and reliable alternative to the assessment of domain profiles using the WHOQOL-100. It is envisaged that the WHOQOL-BREF will be most useful in studies that require a brief assessment of quality of life, for example, in large epidemiological studies and clinical trials where quality of life is of interest. In addition, the WHOQOL-BREF may be of use to health professionals in the assessment and evaluation of treatment efficacy. (WHOQOL-BREF, 1996)

The WHOQOL-100 allows detailed assessment of each individual facet relating to quality of life. But the use of WHOQOL-100 may be too lengthy for practical use. The

WHOQOL BREF Field Trial Version has therefore been developed to provide a short form quality of life assessment that looks at Domain level profiles, using data from the pilot WHOQOL assessment and all available data from the Field Trial Version of the WHOQOL-100. Twenty field centres situated within eighteen countries have included data for these purposes (see Table 2.8). The WHOQOL-BREF contains a total of 26 questions. To provide a broad and comprehensive assessment, one item from each of the 24 facets contained in the WHOQOL-100 has been included. In addition, two items from the Overall quality of Life and General Health facet have been included. The WHOQOL-BREF is therefore based on a four domain structure as shown in Table 2.9. (WHOQOL-BREF, 1996)

The WHOQOL-BREF is available in 19 different languages. The WHOQOL in hindi has been tried in India. The WHOQOL-100, Hindi appears to be a suitable instrument for comprehensively assessing quality of life in health care settings and WHOQOL-Bref, Hindi can be used for intervention studies including drug trials (Saxena S. 1998). The appropriate language version and permission for using it, can be obtained from the WHOQOL Group, Programme on Mental Health, World Health Organisation. A methodology has been developed for new centres wishing to develop a further language version of the WHOQOL-100 or the WHOQOL-BREF. This can be obtained from The WHOQOL Group, Programme on Mental Health, World Health Organisation, CH-1211, Geneva 27, Switzerland.

TABLE 2.8: CENTERS INCLUDED IN DEVELOPMENT OF THE WHOQOL-BREF

Centers in the pilot version of the WHOQOL	Centers in the field trial of the WHOQOL-100
Bangkok, Thailand Beer Sheva, Israel Madras, India Melbourne, Australia **New Delhi, India** Panama City, Panama Seattle, USA Tilburg, The Netherlands Zagreb, Croatia Tokyo, Japan Harare, Zimbabwe Barcelona, Spain Bath, UK St Petersburg, Russia Paris, France	Bangkok, Thailand Beer Sheva, Israel Madras, India Melbourne, Australia **New Delhi, India** Panama City, Panama Seattle, USA Tilburg, The Netherlands Zagreb, Croatia Tokyo, Japan Harare, Zimbabwe Barcelona, Spain Bath, UK Hong Kong Leipzig, Germany Mannheim, Germany La Plata, Argentina Port Alegre, Brazil

(Adapted from WHO QOL BREF, 1996)

TABLE 2.9: WHO QOL BREF DOMAINS

DOMAIN	FACETS INCORPORATED WITHIN DOMAINS
1. Physical health	Activities of daily living Dependence on medicinal substances and medical aids Energy and fatigue Mobility Pain and discomfort Sleep and rest Work Capacity
2. Psychological Bodily image and appearance	Negative feelings Positive feelings Self-esteem Spirituality / Religion / Personal beliefs Thinking, learning, memory and concentration
3. Social relationships Personal relationships	Social support Sexual activity
4. Environment Financial resources	Freedom, physical safety and security Health and social care: accessibility and quality Home environment Opportunities for acquiring new information and skills Participation in and opportunities for recreation / leisure activities Physical environment (pollution / noise / traffic / climate) Transport

(Adapted from WHOQOL-BREF, 1996)

SCORING THE WHOQOL-BREF

The WHOQOL-BREF (Field Trial Version) produces a quality of life profile. It is possible to derive four domain scores. There are also two items that are examined separately: question 1 asks about an individual s overall perception of quality of life and question 2 asks about an individual's overall perception of their health. The four domain scores denote an individual's perception of quality of life in each particular domain. Domain scores are scaled in a positive direction (i.e. higher scores denote higher quality of life). The mean score of items within each domain is used to calculate the domain score. Mean scores are then multiplied by 4 in order to make domain scores comparable with the scores used in the WHOQOL-100. (WHO QOL BREF, 1996)

OVERALL SUMMARY:

- Metformin, a biguanide drug derived from a herb has been used as first line therapies in treatment of type 2 diabetes since 1958 in United Kingdom, in 1972 in Canada and 1995 in the United States and till date continues to be so by most of the bodies across World due to its superior hypoglycemic role (ADA, 2020).

- Metformin has been recognized to cause B12 malabsorption since 1969 by Berchtold et al for short term duration of 3 months of metformin administration. Then B12 lowering effect by long term metformin use of metformin for 2yrs was studied by Tomkin, 1973. In 2003 Wulffelé et. al carried out the first randomized, placebo-controlled trial that found the short term metformin treatment of 16 weeks in T2DM was associated with a decrease in vitamin B12.

- Thereafter there have been several studies (Bell 2010; Herman et al, 2012; Kumthekar et al, 2012; Liu et al, 2011; Nervo et al, 2011; Pflipsen et al, 2009;) from several countries in past four-five decades but the data regarding the same is sparse in Indian context and it has gained impetus since last decade. However, there are only few RCTs addressing this issue across the globe (Wulffele et al, 2003, de Jager et al, 2010).

- Studies assessing type 2 diabetic patients on metformin have reported the prevalence of vitamin B12 deficiency to range from 5.8% to 33% (Kibirige and Mwebaze, 2013)

- Several mechanisms for B12 malabsorption due to metformin have been suggested. B12-IF complex needs ionic calcium for being absorbed in the intestine and metformin competes with it.This form of malabsorption is reversible by calcium supplementation (Bauman et al, 2000).

- B12 deficiency presents with symptoms indistinguishable from that caused by DPN, a very common micro vascular complication of diabetes which has gained attention in recent past where physician, diabetologist, endocrinologist and others in allied field has gained impetus in foot work.

- Worldwide it is challenging to assess and compare DPN because of various tools and questionnaires available. Several questionnaires are helpful in screening patients for DPN. These are the Michigan Neuropathy Screening Instrument (MNSI) (Feldman et al, 1994), Neuropathy Symptom Profile, Neuropathy Symptom Score, Diabetic Neuropathy Symptom Score (Meijer et al, 2002) and the UT Abbreviated Neuropathy Questionnaire. (Armstrong et al, 1998).The most commonly used screening instruments are MNSI and Diabetic Neuropathy Symptom Score (Lawrence et al, 2004).

- Worldwide the prevalence of DPN ranged from as low as 8% to as high as 59% (Young et al, 1993, Partanen et al, 1995, Deli et al, 2013, Dyck et al, 1993).

- There is limited evidence for the prevalence of DPN in Indian context. There are only few studies in Indian patients which have reported the prevalence of DPN to be 26% by Pradeepa et al, 2008, 34.9 % by Jayprakash et al, 2011 and 31% by Paul et. al, 2012 and 39.3% in rural south Indian population by Darivemula et al, 2019.

- There is scanty literature to bridge the link between the two diseased conditions: B12 deficiency and DPN, in T2DM adults on metformin and there is limited evidence for prevalence of DPN in Indian setting.

- The coexistence of B12 deficiency and DPN would further deteriorate the quality of life in T2DM adults. However, it has been challenging to define QoL and many approaches to defining quality of life exist.

- WHO QOL BREF captures several subjective aspects of quality of life grouped in four domains: Physical Health, Psychological Health, Social Relationship and Environment domain.

- For holistic wellbeing of T2DM adults on metformin the three aspects: Metformin induced low B12, DPN and quality of life should be studied comprehensively.