"PLASMA HOMOCYSTEINE LEVELS IN TYPE 2 DIABETES MELLITUS-ITS CORRELATION WITH MICROVASCULAR COMPLICATIONS."

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Under The Guidance Of

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LIST OF ABBREVIATIONS USED

DM – Diabetes Mellitus

Hcy – Homocysteine

tHcy – plasma homocysteine

SAM – S-adenosyl Methionine

SAH – *S*-Adenosyl-L-homocysteine

MTHFR – Methylene tetra hydrofolate reductase

CBS - Cystathionine-β-synthase

MS – Methionine synthase

AGE – Advanced Glycation end product

AdoMet – S-Adenosyl methionine

THF – Tetrahydrofolate

CAD – Coronary Artery Disease

LDL – Low Density Lipoprotein

VCAM – Vascular cell adhesion molecule

MCP – Monocyte chemo attractant protein

NO – nitric oxide

NOS – nitric oxide synthase

PECAM **–** Platelet/endothelial cell adhesion molecule

HMGCoAR - hydroxyl methylglutaryl co-enzyme A reductase

GFR – Glomerular filtration rate

ERG – Electroretinography

ECM – Extracellular membrane

LOX – Lysyl oxidase

HPLC – High-Performance Liquid Chromatography

NADP – Nicotinamide adenine dinucleotide

NADPH **–** Nicotinamide adenine dinucleotide phosphate

GADPH – Glyceraldehyde phosphate dehydrogenase

DAG – diacyl-glycerol

PKC – Protein kinase C

RAGE – Receptor for Advanced Glycation end product

FADH – Flavin adenine dinucleotide dehydrogenase

ROS – Reactive Oxygen Species

UAE – Urinary Albumin excretion

AER – Albumin excretion rate

UKPDS – United Kingdom prospective diabetes study

PN – Peripheral Neuropathy

DPN – Diabetic peripheral neuropathy

PVD – Peripheral vascular disease

PDR – Proliferative diabetic retinopathy

NPDR – Non proliferative diabetic retinopathy

UACR – Urine albumin creatinine ratio

MDNS – Michigan Diabetic Neuropathy score

ABSTRACT

BACKGROUND/AIMS: .

Vasculopathy in DM is not dependent only on hyperglycemia and related metabolic events. There are several other factors which influence the development of vascular complications. Homocysteine is one of these possible factors. Elevated homocysteine levels increase the risk of nearly every complication associated with Type 2 diabetes. Hyperhomocysteinemia is associated with premature vascular disease . Homocysteine has been definitely shown to be associated with increased risk of diabetic cardiovascular disease, but its association with nephropathy, retinopathy and neuropathy has been inconclusive. Aim of this study was to determine the plasma homocysteine (tHcy) concentrations in type 2 diabetic patients with microvascular complications and to find correlation between homocysteine (tHcy) values & microvascular complications.

METHODS:

It is a Cross sectional study involving 150 subjects divided into three groups. Group 1 is non diabetics, group 2 is diabetics without microvascular complications and group is diabetics with microvascular complications. Study was conducted in RLJH medicine OPD during the period of march 2016 to August 2017 who are fitting into the inclusion criteria.

RESULTS:

A cross sectional study of 150 subjects were divided into three groups.

Mean age of group 1, 2 and 3 was 42.41±4.5 years, 59.57±2.7 years and 63.75±10.3 years respectively. The difference in homocysteine, HbA1c, FBS, PPBS among the three groups

were found statistically significant (<0.001). Homocysteine levels had a positive correlation with FBS, PPBS and HbA1c. Mean difference in homocysteine levels between those with neuropathy, nephropathy and retinopathy was statistically significant.

CONCLUSION:

There is significant association between elevated homocysteine levels with microvascular complications in type 2 diabetes mellitus patients, more so with increasing age. Hyperhomocysteinemia could be a risk factor for developing microvascular complications in type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia.. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future. Progressive disease of large and small vessels is an integral part of DM.

Micro and macrovascular complications of DM have a complex pathogenesis involving dysfunction and damage of vascular endothelial cells. Vasculopathy in DM is not dependent only on hyperglycemia and related metabolic events. There are several other factors which influence the development of vascular complications. Homocysteine is one of these possible factors. Elevated homocysteine levels increase the risk of nearly every complication associated with Type 2 diabetes. Hyperhomocysteinemia is associated with premature vascular disease. Homocysteine acts as an atherogenic and thrombophilic agent. Homocysteine induces endothelial cell dysfunction which includes induction of oxidative stress, impaired generation of nitric oxide and decrease in anticoagulant endothelial cell properties. An increase in homocysteine represents an independent risk factor for coronary cerebrovascular and peripheral artery disease[1,2] Homocysteine has been definitely shown to be associated with increased risk of diabetic cardiovascular disease, but its association with nephropathy, retinopathy and neuropathy has been inconclusive and not many Indian studies have dealt with the association between homocysteine levels and microvascular complications in DM patients. Hence there is a need for a study to evaluate the association of

plasma levels of homocysteine with microvascular complications in type 2 DM in Indian population.

OBJECTIVES

1. To estimate plasma homocystiene levels in type 2 DM.
2. To record the microvascular complications.
3. To correlate plasma homocystiene levels with micro vascular complications.

REVIEW OF LITERATURE

HOMOCYSTEINE

Butz and du Vigneaudn bb at the University of Illinois ,discovered the molecule Homocysteine 70 years ago. Homocysteine is a key branch point intermediate in the ubiquitous four step methionine cycle, the function of which is to generate one carbon methyl groups for transmethylation reactions essential to all life forms.[3]

Homocysteine has a formula of HSCH2CH2CH(NH2)CO2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. It is biosynthesized from methionine by the removal of its terminal Cε methyl group.

Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins.[4] Homocysteine is not dietary in origin.[3] t is biosynthesized from methionine through a multi-step mechanism. Methionine obtained from nutrition is changed to S-adenosyl-methionine (SAM), a universal methyl donor . A by-product of this reaction, S-adenosyl-homocysteine (SAH) is hydrolysed subsequently to homocysteine.

Homocysteine metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration. In remethylation, homocysteine acquires a methyl group from N-5-methyltetrahydrofolate or from betaine to form methionine. The reaction with N-5-methyltetrahydrofolate occurs in all tissues and is vitamin B12 dependent, whereas the

reaction with betaine is confined mainly to the liver and is vitamin B12 independent. A considerable proportion of methionine is then activated by ATP to form S-adenosylmethionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors. S-adenosylhomocysteine (SAH), the by-product of these methylation reactions, is subsequently hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer. This hydrolysis is a reversible reaction that favors the synthesis of SAH, and that elevated cellular concentrations of this metabolite are likely to precede and accompany all forms of hyperhomocysteinemia.

In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-50- phosphate (PLP)– containing enzyme, cystathionine β-synthase. Cystathionine is hydrolyzed by a second PLP-containing enzyme, γ-cystathionase, to form cysteine and α-ketobutyrate. Excess cysteine is oxidized to taurine or inorganic sulfates or is excreted in the urine. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes excess homocysteine, which is not required for methyl transfer.[3,4,5]

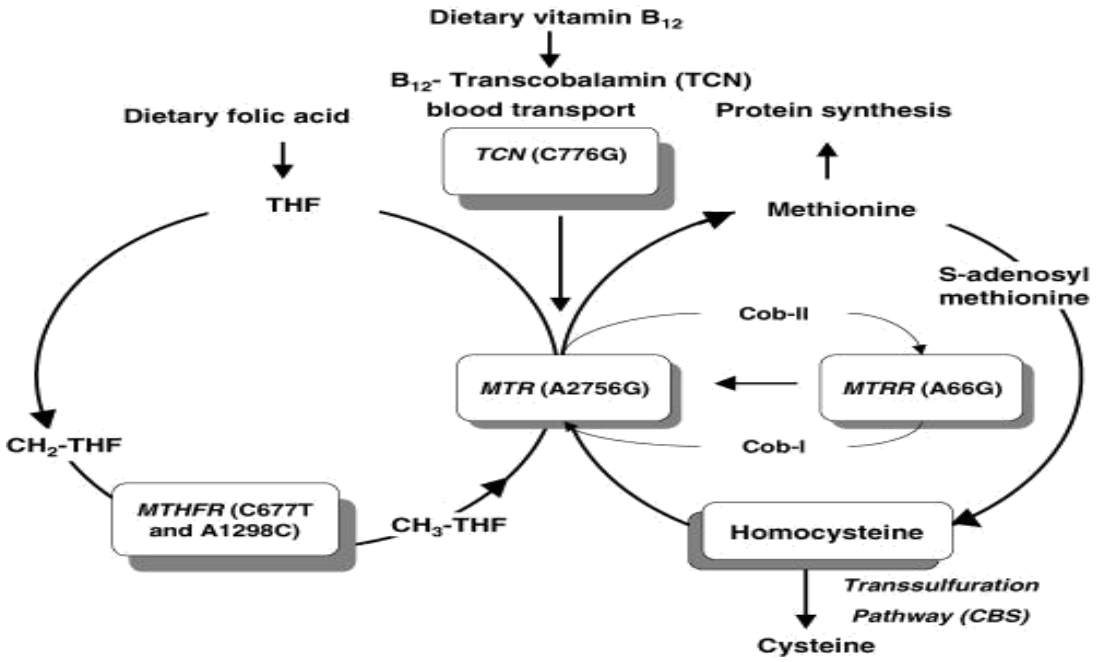


Fig 1: Homocysteine metabolism

Either a genetic defect in one of the enzymes of homocysteine metabolism or a nutritional deficiency of one or more of the vitamins that participate in homocysteine metabolism can lead to metabolic disruption and potentially to hyperhomocysteinemia. The severity and type of the resulting hyperhomocysteinemia is dependent on the extent to which the particular disturbance affects the coordination of the two pathways of homocysteine metabolism.[3]

Defective Synthesis of N-5-Methyltetrahydrofolate

Synthesis of N-5-methyltetrahydrofolate is the first step specifically concerned with the synthesis of methionine. An immediate consequence of impaired synthesis of this folate, either because of folate deficiency or because of a defect in MTHFR, is a depressed synthesis of methionine. This leads to the diversion of homocysteine, which was destined for remethylation, toward the transsulfuration pathway. This latter pathway, however, is incapable of handling the additional homocysteine

Defective Homocysteine Remethylation

In cases of impaired homocysteine remethylation, as in vitamin B12 deficiency or defects in any of the methyl-cobalamin synthesis enzymes, conditions and consequences are somewhat different from those of impaired N-5-methyltetrahydrofolate synthesis. The methyl trap hypothesis predicts that N-5-methyltetrahydrofolate will actually accumulate when remethylation is impaired. Therefore, despite the decrease in SAM synthesis due to the B12 deficiency or enzyme defect, intracellular SAM concentrations may be less affected because the accumulated N-5-methyltetrahydrofolate will inhibit the utilization of SAM in glycine methylation. As a consequence, less homocysteine will be synthesized from SAM and there will be at least some activation of cystathionine β-synthase. homocysteinemia that results from impaired homocysteine remethylation may not be as severe as that observed in impaired N-5-methyltetrahydrofolate synthesis because transsulfuration will be somewhat more active in the catabolism of homocysteine.[3]

Defective Homocysteine Transsulfuration

When the latter pathway is severely impaired, as in homozygous cystathionine β-synthase defect, there is a diversion of homocysteine toward the remethylation pathway. Therefore, the rate of methionine synthesis is increased, leading to a temporal increase in intracellular SAM concentration. This increase in SAM concentration will continue until the level of this metabolite is sufficient for a feedback inhibition of MTHFR, and at this point the remethylation system is inhibited. Consequently, both pathways of homocysteine metabolism are impaired and severe hyperhomocysteinemia results. When transsulfuration is only mildly

impaired, as in vitamin B6 deficiency or in a heterozygous defect of cystathionine β-synthase, the fully active remethylation pathway and the residual activity of the transsulfuration pathway are sufficient to prevent the precipitation of hyperhomocysteinemia provided the homocysteine burden is low as in fasting state.[3]

Other factors

Other determinants of plasma homocysteine levels include increasing age, male gender and renal failure When a person consumes high protein diet 50% of homocysteine metabolism will be via trans-sulphuration and 50% via remethylation. Seventy to ninety per cent of total homocysteine is bound to protein ;one to two per cent of total homocysteine circulates freely in the blood in the reduced form and the remainder circulates as disulphides homocysteine and the mixed disulphide homocysteine-cysteine. Normal values after an overnight fast lie between 5 and 15 mmol/L.

Compounds that contain a free sulphydryl group are called thiols. The sulphydryl containing amino acids or amino thiols are a part of the antioxidant defense mechanisms. They are also intermediates of various metabolic pathways, examples include glutathione, coenzyme A, dithiol, glycine, cysteine and homocysteine. Homocysteine is found in relatively low concentrations within the cells (<1mcmol/l) and in the serum (5 - 15 mcmol/l). A general property of thiols is their ability to oxidise in the presence of an electron acceptor such as molecular oxygen to form disulfides. Thus homocysteine will auto oxidize to form oxidized homocysteine. Homocysteine can oxidize with other thiols as cysteine and glutathione to form disulfides, these compounds are referred to as "homocysteine - cysteine mixed disulfide".

The percent distribution of reduced and oxidized homocysteine in human plasma.

1. Reduced homocysteine <1%
2. Oxidized Homocystine 5-15%

As Mixed Disulfides :

- Cysteine - homocysteine 5-15%
- Protein bound homocysteine >70%

Significance Of Protein- Bound Homocysteine

In normal circumstances and in mild hyperhomocysteinemic state, 70% of the homocysteine in the plasma circulates as a mixed disulfide. When plasma or serum samples are freeze stored for prolonged periods, the fractions of free reduced homocysteine and free oxidized species decrease and become protein bound via disulphide linkages. Because current assays for plasma total homocysteine use reducing agents to break disulfide bonds, all forms of homocysteine except possible trace amounts of homocystenide are detected.

Hyperhomocysteinemia

A variety of unstable homocysteine forms exist in human plasma. These forms in descending relative concentration are : protein (albumin)-bound, free circulating disulfide, and sulfhydryl forms. Current laboratory methods detect the presence of all three forms and report this as total homocysteine concentration, but reference intervals published for clinical practice may be misleading since they are generally not corrected for factors known to influence circulating homocysteine levels (e.g., ethnicity, gender, etc.)[7]

According to the American Heart Association (AHA) advisory statement, normal homocysteine concentrations range from 5-15 μmol/L, although the reference ranges vary from centre to centre, depending on the method of estimation. Intermediately elevated homocysteine levels are between 31-100 μmol/L, while severely elevated levels are >100 μmol/L, and are essentially pathognomonic for the presence of an inborn error of homocysteine metabolism causing homocystinuria.[7]

Causes of Hyperhomocysteinemia

Genetic:

- Mutation or defects of the enzymes, methylenetetrahydrofolate reductase (MTHFR), cystathionine beta-synthase (CBS) and methionine synthase (MS).

Nutritional:

- Deficiency of folic acid
- Deficiency of Vitamin B12
- Deficiency of Vitamin B6

Drugs:

- Methotrexate
- Sulfzalazine.
- Isoniazid
- Antiepileptic agents.
- Nitrous oxide
- Others

Primary disease associated etiologies

- End stage renal disease
- Hypothyroidism
- Psoriasis
- Systemic lupus erythematosus
- Acute lymphoblastic leukemia
- Other factors which may contribute to hyperhomocysteinemia are smoking, alcoholism, menopause.

Homocysteine in Health

The amino acid homocysteine (Hcy), formed from methionine has profound importance in health and diseases. In normal circumstances, it is converted to cysteine and partly remethylated to methionine with the help of vit B12 and folate. The main purpose is production of methionine and cysteine.

It is a key substrate in three additional essential reactions or sequences:

(1) The recycling of intracellular folates;

(2) The catabolism of choline and betaine;

(3) The transsulfuration pathway that leads to the formation of cystathionine, cysteine, glutathione, and other metabolically important metabolites.[8]

Under normal conditions, methionine and homocysteine can be interchanged. The interchangeability of the two amino acids allows the assumption that a shared set of mechanisms governs the regulation of the metabolism of each. However, any disruption of

the methionine cycle may interfere with this coordinate control, and a dissociation may be characteristic of the metabolic disturbances.

Failure of metabolic regulation[8]

Given the capacity of the transmethylation -transsulfuration sequence to adapt to excessive methionine, neither excessive ingestion nor excessive production of homocysteine is likely to be the basis for hyperhomocysteinemia.

In the absence of renal impairment, failure of homocysteine catabolism is the usual etiology and can result from impairment of either cystathionine synthesis or homocysteine remethylation. The suggestion that homocysteine accumulation requires limitation of both pathways, based on the "switch" function of *S*-Adenosyl methionine (AdoMet), is a simplification that fails to account for either tissue specific differences in metabolism or the metabolic effects of AdoHcy and methylTHF [13]. Furthermore, it is inconsistent with the increased levels of cystathionine in some patients with genetic disorders of homocysteine remethylation.

Based on the changes in other metabolites ,they may allow us to distinguish important differences. The groups would be:

(a) Failure of cystathionine synthase;

(b) Impaired homocysteine methylation with low methylTHF (folate deficiency, methyleneTHF reductase deficiency); and

(c) Impaired homocysteine methylation with normal or increased methyl THF (cobalamin deficiency, nitrous oxide)

Homocysteine in disease

When normal metabolism is disturbed, Hcy is accumulated in the blood. The unique biochemical profile of homocysteine is characterized by chemical reactivity supporting a wide range of molecular effects, and a tendency to promote oxidant stress-induced cellular toxicity[9].

The harmful effects of homocysteinemias are due to

- Production of oxidants (reactive oxygen species) generated during oxidation of Hcy to homocystine and disulphides in the blood. These could oxidize membrane lipids and proteins
- Hcy can react with proteins with their thiols and form disulphides (thiolation)
- It can also be converted to highly reactive thiolactone which could react with the proteins forming -NH-CO- adducts, thus affecting the body proteins and enzyme.[9]

Because of oxidation of Hcy, urine in such cases contains homocystine referred to as homocystinuria. Thus, for abnormal metabolism of Hcy, the blood can be analyzed for Hcy or urine for homocystine or both. Blood total Hcy levels can also be estimated by reducing the disulphides.

Homocysteine and diseases

Homocysteine and vascular disease.

The relationship between hyperhomocysteinemia and atherosclerosis was suggested by McCully way back in 1969. In one study Asian Indians were found to have significantly

higher homocysteine levels than Europeans, which was believed to cause twice as many CAD deaths in Asian Indians as compared to Europeans. This study concluded that homocysteine was an independent risk factor in Asian Indians, which probably contributed to the increased CAD risk.[10]

Homocysteine is an unstable amino acid, which undergoes autooxidation to produce free oxygen radicals. Hyperhomocysteinemia, causes increased production of free oxygen radicals and an oxidative stress. This is believed to contribute to atherosclerosis in the following ways.

The free oxygen radicals convert LDLc deposited in the sub-endothelial tissue to oxidized LDLc (oxLDLc). OxLDLc then acts as the key mediator of the inflammatory process in atherosclerosis. OxLDLc causes the release of vascular cell adhesion molecule (VCAM) and monocyte chemoattractant protein (MCP1), which in turn causes monocyte adhesion and penetration respectively. The monocytes then get converted to macrophages, which take up oxLDLc to get converted to foam cells. The foam cells get deposited below the endothelium to form a fatty streak, the first lesion in atherosclerosis. The free oxygen radicals also combine with nitric oxide (NO), inactivating it to peroxynitrite. The resulting endothelial dysfunction, also contributes significantly to atherosclerosis.[11]

Hyperhomocysteinemia and neurological diseases

Hyperhomocysteinemia has been shown to be associated with a number of neurological conditions like stroke, silent brain infarct, dementia, movement disorders, etc.

Hyperhomocysteinemia and Stroke

At the Ruby Hall Clinic, Pune, serum homocysteine, B12 and folate were estimated in

consecutive cases of ischemic stroke, arterial or venous infarction. A raised homocysteine was the commonest risk factor for stroke in this population.[12]

The striking features are that in 461 cases of stroke, homocysteine was elevated in 370 (80.26%). In the raised homocysteine group, B12 was low in 213 (57.5%) and folic acid was low in 63 (17.0%). B12 was borderline in an additional 9.5%. A borderline B12 level with elevated homocysteine (or methylmalonic acid) implies metabolic deficiency of vitamin B12.[12]The Ruby Hall Study is by far the largest Indian study in stroke. However, several other neurologists in Pune, Mumbai, Guwahati and Hyderabad have reported similar findings.

Homocysteine and Dementia

The topic of homocysteine and dementia was opened up by a report of the association from the Framingham study, in which 1092 elderly persons without dementia were followed up for 8 years. The serum was available for estimation of serum homocysteine at the onset and 8 years before the start of the study. In follow up 111 persons developed dementia. It was found that those in the highest quintile (top 1/5) of serum homocysteine had an increased risk of developing dementia. The highest quintile, compared to all other quintiles at the end of 8 years, had a 1.9 times greater chance of developing dementia and this risk was not only for vascular dementia but also for Alzheimer's dementia.

Potential mechanisms of elevated homocysteine causing dementia

- Hyperhomocysteinemia has been related to Cerebral microangiopathy, endothelial dysfunction, impaired nitric Oxide activity, and increased oxidative stress.
- Increased concentrations of homocysteic acid, an *N*-methyl-D-aspartate receptor agonist and a metabolite of homocysteine, may result in excitotoxic damage to neurons.[13]

- In addition, disturbed homocysteine metabolism may indicate altered turnover of another folate dependent cofactor in neurotransmitter metabolism, tetrahydrobiopterin, which may compromise nerve function.
- Induces apoptosis in hippocampal neurons in rats.
- Homocysteine promotes copper-mediated and β-amyloid-peptide– mediated toxic effects in neuronal cell cultures.
- Elevated homocysteine can impact on SAM, the only methyl donor in the CNS, required for the synthesis of neurotransmitters. Impaired methylation also affects synthesis of phospholipids and myelin.[13]

Other neurologic conditions

Besides stroke and dementia, hyperhomocysteinemia is known to cause abnormal movement and dystonia. There are several reports of patients who had progressive increase of movement disorders while various treatments were being tried. Subsequently the detection of hyperhomocysteinemia led to treatment and significant recovery. The exact mechanism of which remains unclear.

Homocysteine and Marfan syndrome

The two disorders both have similar ocular abnormalities and other similar clinical manifestations, such as skeletal deformabilities, cardiovascular problems and generalized osteoporosis.

This may be linked to the collagen abnormality, as HHcy has been reported to have effect on the copper containing lysyl oxidase, a major enzyme involved in the collagen modification. There might also be defect in the metabolism of copper, which is a cofactor required for the

enzyme activity. Thus, further studies are needed to trace out the link between the two disorders.[9]

Homocysteine and Osteoporosis

Two observational studies done by McLean et al. and Van Meurs et al. suggested that increased homocysteine levels are a risk factor for osteoporosis in older men andwomen.[14,15]

If homocysteine concentration truly is a causal mechanism for the risk of fracture, the public-health implications could be substantial, because total homocysteine concentrations can be easily modified by dietary intake of folic acid and vitamins B6 and B12.

The association between elevated homocysteine levels and the risk of fracture needs to be confirmed in large population studies.[14, 15]

Homocysteine and Diabetes mellitus

Effects of Hyperglycemia and Insulin on Homocysteine Metabolism.

High Glucose decreases MTHFR activity when concentrations are from 100 to 300mg/dl in the HepG2 cells. Such inhibitory effect of high glucose on homocysteine remethylation, has an effect on the homocysteine degradation.

Insulin increases homocysteine concentration primarily by inhibition of transsulfuration of homocysteine. Some studies have shown that moderate hyperhomocysteinemia (15–30 μmol/l) has been observed in some studies of patients with diabetes, although the findings are inconsistent.[16]

It has been suggested that homocysteine could contribute to microvascular disease of the eyes or kidneys in diabetes. This is supported by in vitro studies of the effects of homocysteine on human venous endothelial cells. After exposure to advanced glycation end product (AGE) albumin, these endothelial cells release increased amounts of thrombomodulin, an endothelial marker, when exposed to homocysteine. This suggests that homocysteine may produce endothelial damage in vessels exposed to AGEs and, by this mechanism, could contribute to microvascular disease.[17]

Potential Mechanisms of Homocysteine-Induced Vascular Damage in diabetes

In patients with diabetes, endothelial dysfunction which may be caused by hyperhomocysteinaemia is an early manifestation of atherosclerosis.[18] Even In healthy subjects following an acute increase in plasma homocysteine after an oral methionine load, endothelial dysfunction has been demonstrated.[19] Plasma homocysteine rose by 2-3-fold from a fasting baseline level of 13-15 μmol/L. This suggests that homocysteine has detrimental effect on vascular cells even when the circulating levels are low. Homocysteine adversely affects endothelial function by reduced production and bioavailability of nitric oxide by increasing oxidant stress.[20] Increased homocysteine levels can cause oxidant stress through a variety of mechanisms. In-vitro studies using cultured endothelial cells have demonstrated auto-oxidation of homocysteine to form reactive oxygen species,[21] including superoxide anion and hydrogen peroxide, increased lipid peroxidation [22] and impaired production of the antioxidant glutathione peroxidase.[15]

A clinical study involving patients with inherited defects of homocysteine metabolism found a significant increase in plasma glutathione peroxidase activity and a non-significant increase in red blood cell super-oxide dismutase activity in those with hyperhomocysteinaemia.[23] This indirectly indicates up-regulation of antioxidant activity as a response to oxidant insult caused by homocysteine-mediated vascular damage.

In patients with diabetes there is in vitro evidence of reduced platelet nitric oxide synthase (NOS) activity.[24]Incubation of platelet-rich plasma with homocysteine leads to a further reduction in platelet nitric oxide production in patients with diabetes compared to healthy controls. This reduced platelet-derived NOS leads to increased platelet activation and aggregation and contributes to reduced nitric oxide bioavailability, which provides an additional potential mechanism for the atherogenic action of homocysteine in diabetic patients.

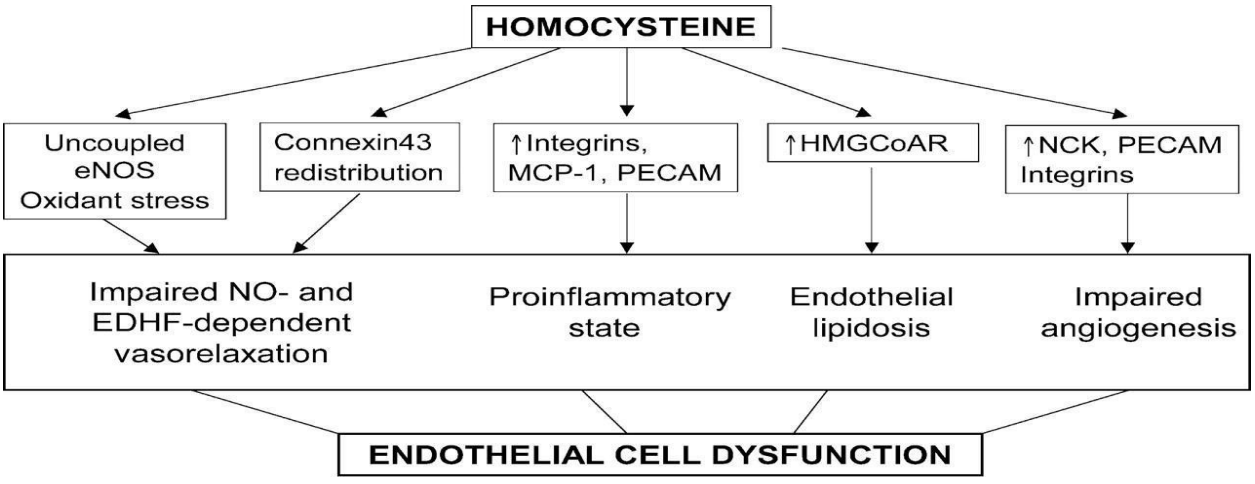


Fig 2: Mechanism of endothelial dysfunction by homocysteine (Source: Michael S. Goligorsky American Journal of Physiology - Renal PhysiologyPublished 1 May 2005Vol. 288)

Homocysteine in diabetic nephropathy

Younger diabetics with no complications have significantly lower homocysteine levels than non-diabetic controls[24,25]. The proposed mechanism for this is renal hyperperfusion. Homocysteine is ultrafiltrated in glomeruli.Renal metabolism of homocysteine accounts for a large fraction of total renal clearance of homocysteine.

Role of GFR

After filtration, homocysteine is almost completely reabsorbed in the renal tubules and degraded in the renal parenchyma via transmethylation and transsulphuration.[30]

In the early stages of diabetes in those with normoalbuminuria, glomerular filtration rate (GFR) is increased due to hyperfiltration. Wollesen et al. showed that in type 1 and type 2 diabetic patients without nephropathy GFR is a strong determinant of plasma homocysteine concentration independent of age, serum creatinine and serum vitamins. Hence, diabetic patients with relative hyperfiltration and normal serum creatinine have lower plasma homocysteine levels.[31]

In contrast, hyperhomocysteinaemia is well described in patients with diabetes and established renal failure.[32] Patients with microalbuminuria may have increased, normal or reduced GFR as the microalbuminuria progresses. Some studies report a calculated GFR, however this may be misleading as the Cockcroft-Gault formula can overestimate he measured GFR in patients with diabetes.[33] With increasing urinary excretion of albumin, typical structural changes are seen in the glomerulus, including mesangial expansion,

thickening of the basement membrane and glomerulosclerosis which could potentially interfere with renal metabolism of homocysteine.

Podocyte injury and change in expression of podocyte associated proteins

In a study done in Department of Pharmology in Medical College of Virginia, it was observed that urinary albumin excretion increased in hyperhomocysteinemic rats who did not have diabetes. Morphological examination of glomeruli showed that mesangial expansion occurred at the 2nd week of hyperhomocysteinemia and podocyte effacement was also observed which were seen as signs of glomerular damage. Furthur, Immunofluorescence analyses demonstrated that podocin and nephrin expressions were reduced, while α-actinin-4 increased during hyperhomocysteinemia. So it was concluded that Increased plasma Hcys level is an important pathogenic factor resulting in glomerular injury and such pathogenic effects of Hcys are associated with podocyte injury and changed expression and distribution of podocyte-associated proteins.[34]

However, further studies are required to confirm these evidences, to support a causative role of homocysteine in the development of diabetic nephropathy in humans Prospective studies confirm that hyperhomocysteinaemia is an independent risk factor for cardiovascular morbidity and mortality in end-stage renal disease.[35] Therefore, hyperhomocysteinaemia in diabetic patients with both incipient and clinical nephropathy may partly contribute to the increased risk of vascular disease in this group of patients.

Homocysteine in diabetic retinopathy

Homocysteine has been associated with vaso-occlusive diseases in the eye.[36] There is no explanation for the variation in the severity of diabetic retinopathy among diabetic patients with similar duration and control of the disease, which raises the possibility of other risk factors being involved in the pathogenesis of diabetic retinopathy In the development and progression of diabetic retinopathy , numerous factors were described as having an effect on, such as puberty,[37-38] hypertension[39], and pregnancy.[40]

Since diabetes is a microvascular occlusive disease, an adjuvant risk factor contributing to a hypercoagulability state, such as increased levels of plasma homocysteine, may accelerate or aggravate the development or progression of diabetic retinopathy. Ocular complications associated with Homocysteine include ectopia lentis, secondary glaucoma, optic atrophy, age-related macular degeneration (ARMD), central retinal vein occlusion (CRVO), and diabetic retinopathy. The consequences of elevated levels of Homocysteine on retinal function in in vitro and in vivo models, has shown that Homocysteine induces apoptotic retinal ganglion cell (RGC) death.

Poloschek et al. reported in a case study that hyperhomocysteinemia caused by methionine synthase deficiency showed decreased rod response and RGC loss, which were analyzed by ERG and visual evoked potential. However, not much is known about the effects of Homocysteine on retinal function. Plasma total Homocysteine concentration has been suggested to be a useful biomarker and a risk factor for diabetic retinopathy in people with type 2 diabetes.

Homocysteine has also been shown to have a role in collagen synthesis and crosslinking.. Extensive ECM disruption has been demonstrated in vitreoretinal diseases, like proliferative diabetic retinopathy (PDR) and rhegmatogenous retinal detachment (RRD). PDR is a common complication of diabetes mellitus characterized by preretinal neovascularization and development of epiretinal fibrovascular traction and retinal detachment. Collagen turnover in the vitreous has been shown to be associated with ageing and vitreoretinal diseases, which predisposes to posterior vitreous detachment RRD is a complication of proliferative vitreoretinopathy (PVR), that involves inflammation, ECM deposition, and tissue remodelling.[41] The covalent cross-linking of collagens and elastin in the ECM is performed by LOX, a copper-dependent amine oxidase enzyme. Inhibition of LOX activity has been related with hyperhomocysteinemia and has been studied in terms of its molecular mechanisms in vascular diseases.[41]

Homocysteine and Diabetic Peripheral Neuropathy

In vitro studies suggest that the pathogenesis of vascular diseases associated with Hcys is related to endothelial dysfunction, smooth muscle proliferation, and abnormalities of coagulation. Hence it is suggested that due to the resultant circulation impairment and thus nutrient deficit, neuropathy may occur.

In addition, increasing Hcy levels could enhance the vulnerability of neurons to excitotoxic and oxidative injury in vitro and in vivo. Studies indicate that indicating that Hcy may serve as an excitatory amino acid. A study by Maler JM et al, found that Administration of a very high concentration of Hcys to cell cultures caused astroglial cell death .[42, 43] However the

exact cellular mechanism of Hcys in causing PN remains to be

Elucidated

Methods for Measuring Homocysteine Concentrations

There are ever increasing number of methods to measure Homocysteine. Many research studies have measured Homocysteine levels using the following methods.

Simple colorimetric enzyme assays: Allows analysis to be performed on routine clinical chemistry analyzers. These assays are based on either an enzymatic cycling assay[45] or on enzymatic release of hydrogen sulfide which reacts to form a chromogen.[46] High-Performance Liquid Chromatography (HPLC).

Evolution of advanced immunoassays for homocysteine on a variety of instruments has enabled more laboratories to perform this test . Homocysteine can be detected during HPLC as a fluorescent derivative or by direct electrochemical detection of Homocysteine. These assays are based on quantitative enzymatic conversion of Homocysteine to S-adenosylhomocysteine. Antibodies that can recognize this compound specifically have been produced .[46]

Tandem mass spectrometry has become another option for analysis of Homocysteine.[47] Comparisons between different methods show relatively small biases between them.[44-48]
All methods seem to provide adequate analytical performance for routine clinical use. However, the choice of method therefore usually depends on practical considerations of cost,

labor efficiency, and the types of analyzers are already available in the laboratory. There is a trend for an increasing number of clinical laboratories to use routine chemistry analyzers rather than immunoassay or HPLC methods.

Treatment of Hyperhomocysteinemia

The internationally accepted treatment for hyperhomocysteinemia involves the use of three homocysteine lowering vitamins viz. folic acid, vitamin B12 and pyridoxine. Folic acid and B12 act predominantly under fasting conditions and pyridoxine acts after meals. Pyridoxine probably does not add to the effect of folate and B12 in the fasting state. Pyridoxine has been shown to cause a reduction in the post methionine loading homocysteine levels by 22%.

DIABETES MELLITUS

The first complete clinical description of Diabetes was given by a physician named Aretaeus of Cappadocia (1st century AD) in ancient Greece. Information written on ancient Egyptian papyrus describes diabetes as a condition that causes a person to melt in the loins and the resultant urine to attract ants (due to high sugar content). Aretaeus gave diabetes its name (from the Greek word for "siphon," indicative of the diabetic's intense thirst and excessive emission of fluids. Mellitus, a Latin word which means "honey sweet" was later added by Thomas Willis (Britain) in 1675. Great Indian physician Sushruta (6th century BC) identified the disease and called it *Madhumeha*. He also associated it with obesity and sedentary lifestyle, advising exercises to help "cure" it.

Criteria for the Diagnosis of Diabetes Mellitus

- Symptoms of diabetes plus random blood glucose concentration 11.1 mmol/L (200 mg/dL) or
- Fasting plasma glucose 7.0 mmol/L (126 mg/dL) or
- A1C > 6.5%c or
- Two-hour plasma glucose 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (Source: American Diabetes Association, 2011)

COMPLICATIONS OF DIABETES [1]

Diabetes has both acute and chronic complications. Acute complications are:

• Diabetic ketoacidosis

• Hyperglycemic hyperosmolar state

• Hypoglycemia

CHRONIC COMPLICATIONS

The chronic complications of DM affect various organ systems & are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications are divided into vascular and nonvascular complications. Nonvascular complications include problems such as gastroparesis, uropathy/sexual dysfunction, infections, cataract, glaucoma, periodontal disease, and skin changes. Long-standing diabetes may be associated with hearing loss.

Vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications [coronary heart disease (CHD), peripheral arterial disease (PAD), cerebrovascular disease]. Diagnosis of diabetes increases the risk of developing various complications that are largely irreversible.

Duration of diabetes is an important factor in the pathogenesis of complications, but other risk factors such as hypertension, cigarette smoking and dyslipidemia interact to affect the clinical course of microangiopathy and macroangiopathy.[50]

The vascular complications are :

Microvascular

1. Diabetic retinopathy
2. Diabetic nephropathy
3. Diabetic neuropathy

Macrovascular

1. Coronary artery disease

2. Cerebro vascular disease

3. Peripheral vascular disease

Vascular complications are serious consequences of diabetes which are responsible for most of the morbidity and mortality observed in DM patients. It is likely that all blood vessels both small & large are abnormal in diabetic patients with long standing disease.

In a study done in Diabetes Care and Research Centre, S.P. Medical College, Bikaner, Rajasthan in 2012 comprising 11157 subjects of type-2 DM , retinopathy was diagnosed in 32.5%, nephropathy was present in 30.2%, peripheral neuropathy was present in 26.8%, coronary heart disease (CHD) was present in 25.8% and peripheral vascular disease (PVD) was present in 28% of the subjects.[4] The risk of CAD or stroke is increased 2-4 folds compared to general population, and the risk of PVD increased four times.[49-51]

Pathophysiology of vascular complications

Macroangiopathy in DM constitutes an accelerated form of atherosclerosis affecting carotid , coronary , and peripheral arteries, which leads to the risk of developing stroke, myocardial infarction, and diabetic foot.[0-53]

Clinical trials in both Type I and Type II diabetes have shown that increased glucose level has a vital role in the pathogenesis of microvascular complications. It has been showed in

studies that microvascular complications are mainly because of hyperglycemia, whereas insulin resistance is the major determinant in macrovascular disease[50]

Various mechanisms leading to the development of microvascular complications

Most cells are able to regulate the transport of glucose inside the cell when they are exposed to hyperglycemia, so that they maintain a constant glucose concentration. However, cells affected by hyperglycemia are those that are not efficient in doing such a thing. So DM selectively affects cells such as mesangial cells and endothelial cells. Four major hypotheses about how hyperglycemia causes diabetic complications have generated a large amount of data as well as several clinical trials based on specific inhibitors of these mechanisms.

Increased Polyol Pathway Flux

Aldose reductase is a cytosolic, monomeric oxidoreductase that catalyzes the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of a wide variety of carbonyl compounds, including glucose. NADPH is the cofactor in this reaction and in the regeneration of glutathione by glutathione reductase.

Aldose reductase has a low-affinity for glucose, and at the normal glucose concentrations found in nondiabetic patients, the metabolism of glucose by this pathway constitutes a small percentage of total glucose utilization. In hyperglycemia, however, there is increased enzymatic conversion to the polyalcohol sorbitol, with decreases in NADPH. Flux through this pathway during hyperglycemia varies from 33% of total glucose utilization in the rabbit lens to 11% in human erythrocytes. Therefore, the contribution of this pathway to diabetic

complications may be very much species, site, and tissue dependent.

NADPH is required for regeneration of reduced glutathione, By reducing the amount of reduced glutathione, the polyol pathway could induce or exacerbate intracellular oxidative stress.

It has been proposed that oxidation of sorbitol by NAD+ increases the cytosolic NADH/NAD+ ratio, thereby inhibiting activity of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GADPH) and increasing the concentrations of triose phosphate. Elevated triose phosphate concentrations increase the formation of both methylglyoxal a precursor of AGEs, and diacylglycerol (DAG), and activates protein kinase C(PKC).

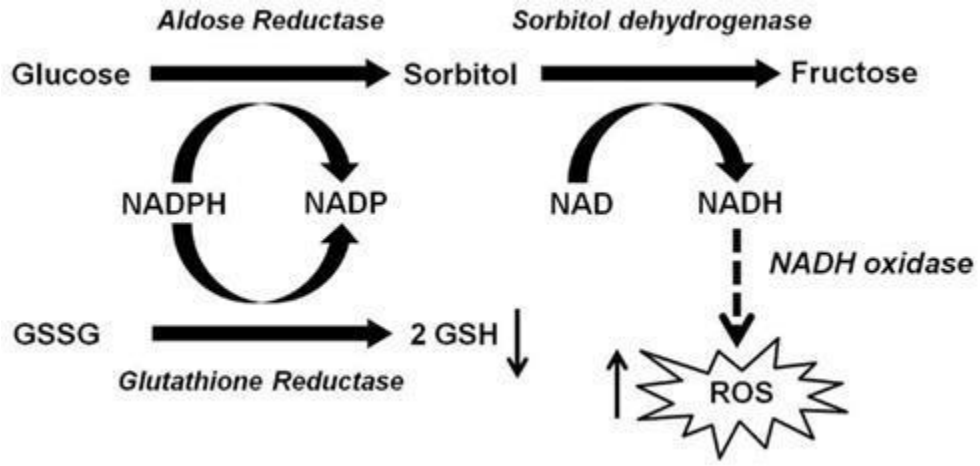


FIG 3: POLYOL PATHWAY

Intracellular production of advanced glycation endproducts (AGE)

AGEs form when proteins or lipids interact with aldose sugars for an extended period of time, subsequently undergoing molecular transformations that glycate the protein or lipid.

Early glycation and oxidation processes result in the formation of Schiff bases and Amadori products. Further glycation of proteins and lipids causes molecular rearrangements that lead to the generation of AGEs. This is a non-enzymatic reaction.

General mechanisms through which AGEs contribute to diabetic complications include the following: (1) formation of cross-links between key molecules in the basement membrane of the ECM, permanently altering cellular structure; and (2) interaction of AGEs with RAGE on cell surfaces, altering cellular function.

AGEs alter properties of the large matrix proteins collagen, vitronectin, and laminin, through AGE-AGE intermolecular covalent bonds, or cross-linking. AGE cross-linking on type I collagen and elastin causes an increase in the area of ECM, resulting in increased stiffness of the vasculature. AGEs also form on intracellular proteins. Intracellular AGEs change cellular properties that are critical in vascular homeostasis.

Plasma proteins modified by AGE precursors bind to AGE receptors on cells such as macrophages, inducing receptor-mediated ROS production. This AGE-receptor ligation activates the pleiotropic transcription factor nuclear factor-κB (NFκB) causing the production of inflammatory cytokines and growth factors like including IL-1α, IL-6, tumor necrosis factor-α, endothelin-1, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, tissue factor, thrombomodulin, and vascular endothelial growth factor (VEGF) [57]

Activation of Protein Kinase C(PKC)

Intracellular hyperglycemia increases Diacyl Glycerol(DAG) content primarily by increasing its de novo synthesis from the glycolytic intermediate glyceraldehyde-3-phosphate via reduction to glycerol-3-phosphate and stepwise acylation. Increased de novo synthesis of DAG activates PKC in vascular cells and in retina and glomeruli [58]

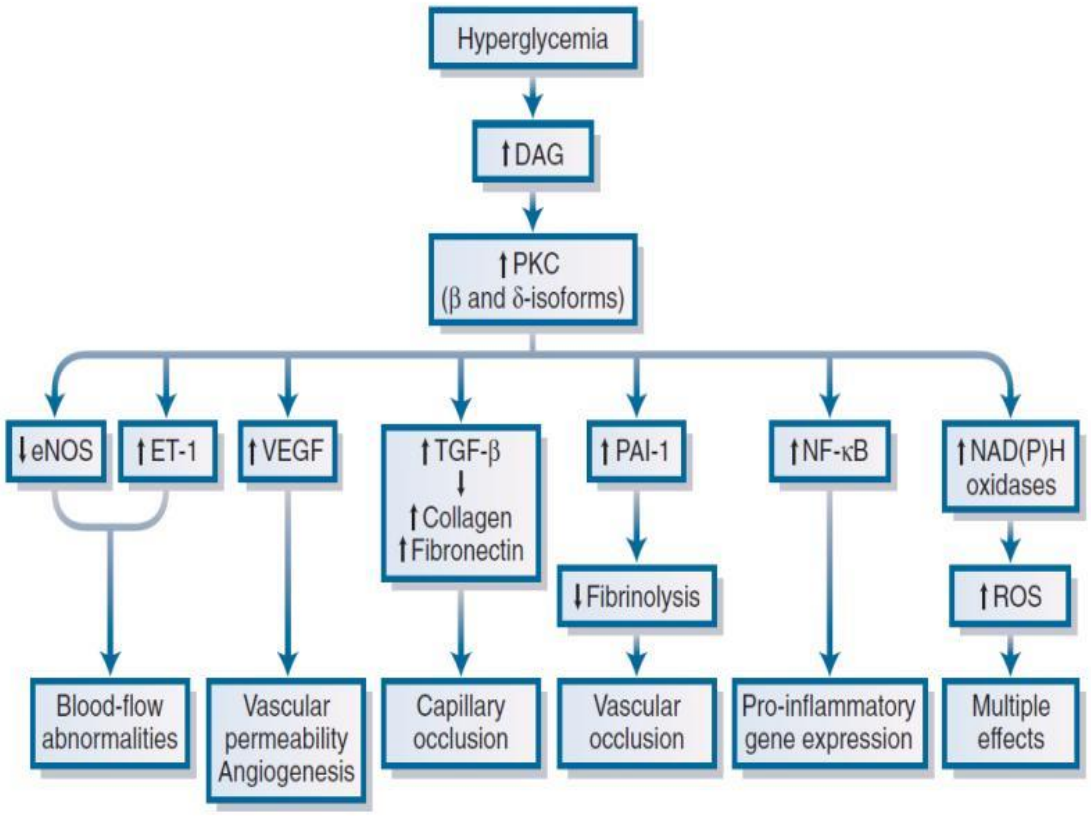


Fig 4: Effect of PKC on the micro vasculature

Source: Adapted from Koya D, Jirousek MR, Lin YW, et al. Characterization of proteinkinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. J Clin Invest. 1997; 100:115-126.)

When PKC is activated by intracellular hyperglycemia, it has a variety of effects on gene expression. In such cases, the factors that support normal functioning of cells are reduced and the things that are detrimental are increased. Activation of PKC-β isoforms mediate retinal and renal blood flow abnormalities,by decreasing NO production and increasing endothelin-1 activity. PKC activation also mediates glucose enhanced extracellular matrix accumulation in glomerular mesangial cells. Activation of PKC also induces expression of the permeability enhancing factor VEGF in smooth muscle cells [58]

Increased hexosamine pathway activity

A fourth hypothesis about how the excessive glucose is shunted into the hexosamine pathway. When glucose is high inside a cell, fructose-6-phosphate from glycolysis gets diverted into signaling pathway in which an enzyme called GFAT (glutamine fructose-6 phosphate aminotransferase) converts the fructose-6 phosphate to glucosamine-6 phosphate and finally to UDP (uridine diphosphate) N-acetyl glucosamine.

Increased attachment of N-acetylglucosamine moieties to serine and threonine residues of transcription factors such as Sp1 increases production of such complication-promoting factors as plasminogen activator inhibitor 1 (PAI -1) and transforming growth factor-β1(TGF-β1).Both of which are harmful to the blood vessels.

Production of superoxide by the mitochondrial electron transport chain

It has been discovered that each of the four different pathogenic mechanisms reflects a single hyperglycemia-induced process: overproduction of superoxide by the mitochondrial electron

transport chain. In diabetic cells with high glucose inside, there is more glucose being oxidized in the TCA cycle, which, in effect, pushes more electron donors (NADH and FADH2) into the electron transport chain. As a result of this, the voltage gradient across the mitochondrial membrane increases until a critical threshold is reached. At this point, electron transfer inside complex III is blocked, causing the electrons to back up to coenzyme Q, which donates the electrons one at a time to molecular oxygen, thereby generating superoxide. Hyperglycemia induced intracellular ROS are produced by the proton electrochemical gradient generated by the mitochondrial electron transport chain. Hyperglycemia-induced ROS activates AGE formation, PKC, the hexosamine pathway, and the polyol pathway by inhibiting activity of the key glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) .When GAPDH activity is inhibited, the levels of all the glycolytic intermediates that are upstream of GAPDH increase. An increased level of glycolytic metabolite glyceraldehyde-3 phosphate activates two of the four pathways, because the major intracellular AGE precursor, methylglyoxal, and the activator of PKC, DAG, are both formed from glyceraldehyde-3 phosphate. Furthur raised levels of the glycolytic metabolite fructose-6-phosphate increase flux through the hexosamine pathway, in which fructose-6 phosphate is converted by the enzyme GFAT to UDP-GlcNAc. Finally, inhibition of GAPDH increases intracellular levels of the first glycolytic metabolite, glucose. This increases flux through the polyol pathway, where the enzyme aldose reductase reduces it, consuming NADPH in the process. Therefore inhibition of GAPDH using activates each of the four pathways. This is the key process, which activates all other pathways thus it is the unifying the mechanism.[58]

DIABETIC NEPHROPATHY

Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality.

Earlier, Diabetic nephropathy had been classically defined by the presence of proteinuria >0.5 g/24 h. This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria, or macroalbuminuria. Later in 1980s, seminal studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional methods, were predictive of the later occurring overt proteinuria in type 1 and type 2 diabetic patients. This stage of renal involvement was termed micro albuminuria or incipient nephropathy.[62]

Epidemiology

Both type 1 and type 2 diabetes cause renal disease. Compared to type 1, a slightly smaller and imperfectly defined proportion of type 2 patients progress to ESRD, but they represent more than 90% of those receiving renal replacement therapy with the diagnosis of diabetes. The distribution of renal disease due to type 2 diabetes is uneven among racial groups. American Indians, African Americans, and Mexican Americans have a greater incidence than non-Hispanic whites. Genetic predisposition, environmental factors, delayed diagnosis of type 2 diabetes, and sub adequate medical care in minority groups contribute in undefined amounts to such disparity.

SCREENING AND DIAGNOSIS

Screening for diabetic nephropathy must be initiated at the time of diagnosis in patients with

type 2 diabetes , since it has been found that 7% of them already have microalbuminuria at that time For patients with type 1 diabetes, the first screening has been recommended at 5 years after diagnosis However, the prevalence of microalbuminuria before 5 years in type 1 diabetics can reach 18%, especially in patients with poor glycemic and lipid control and high normal blood pressure levels .Therefore , in case of poor glycemic and metabolic control in type 1 DM, screening for microalbuminuria might be performed 1 year after diabetes diagnosis. If microalbuminuria is absent, the screening must be repeated annually for both type 1 and 2 diabetic patients.[62]

The first step in the screening and diagnosis of diabetic nephropathy is to measure albumin in a spot urine sample, collected either as the first urine in the morning or at random, for example, at the medical visit. This method is accurate, easy to perform, and recommended by American Diabetes Association guidelines. Twenty-four hour and timed urine collections are cumbersome and prone to errors related to collecting samples or recording of time. The results of albumin measurements in spot collections may be expressed as urinary albumin concentration (mg/l) or as urinary albumin-to-creatinine ratio.[62]

Stages	Albuminuria cutoff values (ref. 14)	Clinical characteristics (ref. no.)
Microalbuminuria	20–199 μg/min	Abnormal nocturnal decrease of blood pressure and increased blood pressure levels (163)
	30–299 mg/24 h	Increased triglycerides, total and LDL cholesterol, and saturated fatty acids (164, 165)
	30–299 mg/g*	Increased frequency of metabolic syndrome components (166)
		Endothelial dysfunction (167)
		Association with diabetic retinopathy, amputation, and cardiovascular disease (168)
		Increased cardiovascular mortality (2, 169)
		Stable GFR (82)
Macroalbuminuria†	≥200 μg/min	Hypertension (99)
	≥300 mg/24 h	Increased triglycerides and total and LDL cholesterol (170)
	>300 mg/g*	Asymptomatic myocardial ischemia (171, 172)
		Progressive GFR decline (83, 84)

*Spot urine sample. †Measurement of total proteinuria (≥500 mg/24 h or ≥430 mg/l in a spot urine sample) can also be used to define this stage.

Fig 5: Diagnostic criteria for Diabetic Nephropathy[62]

All abnormal tests must be confirmed in two out of three samples collected over a 3- to 6-month period, due to the known day-to-day variability in UAE.

Clinical features

Symptoms suggestive of renal impairment:

1. Oliguria

2. Anuria

3. Puffiness of face

4. Distension of abdomen

5. Pedal edema

The course of diabetic nephropathy can be followed by two main variables, Proteinuria and GFR.

There are five distinct stages: [63]

Stage 1: Glomerular hyper filtration and renal enlargement.

Stage 2: Early glomerular lesions or silent stage with normal albumin excretion.

> Early glomerular lesions, consisting of glomerular basement membrane thickening and mesangial matrix expansion, characterize the second stage. Those structural changes appear 18 to 36 months after onset of type 1 diabetes and may become prominent after 3.5 to 5 years.

Stage 3: Incipient diabetic nephropathy or micro albuminuric stage Microalbuminuria, defined as urinary AER greater than 30 mg/24 hours or 20μg/minute and less than 300 mg/24 hours or 200 μg/minute, represents the first evidence of diabetic renal disease. AER varies greatly and is increased by hypertension, strenuous exercise, fever, poor glycemic control, and congestive heart failure. Therefore, a diagnosis of incipient diabetic nephropathy is made only when Microalbuminuria is detected in at least two of three urine specimens over several months.

Stage 4: Clinical or Overt diabetic nephropathy: proteinuria and falling GFR Albuminuria greater than 300 mg/24 hours, relentless decline of renal function, and hypertension define the fourth stage of diabetic nephropathy. This stage, though variable, usually occurs 15 to 20 years after the onset of type 1 diabetes and after 5 or more years of diagnosed type 2 diabetes. The amount of urinary protein can be as little as 500 mg, but it can reach massive

proportions, such as 20 to 40 g/24 hours.

Stage 5: End-stage renal disease.

Pathological features of Diabetic nephropathy

The consensus classification of DN developed by Renal Pathology Society is as follows

CLASS ONE

- Mild or nonspecific LM changes and EM-proven GBM thickening
- Biopsy does not meet any of the criteria mentioned below for class II, III, or IV
- GBM > 395 nm in female and >430 nm in male individuals 9 years of age and older

CLASS 2A

- Mild mesangial expansion
- Mild mesangial expansion in >25% of the observed mesangium
- Biopsy does not meet criteria for class III or

IV CLASS 2B

- Severe mesangial expansion in >25% of the observed mesangium
- Biopsy does not meet criteria for class III or IV

CLASS 3

- Nodular sclerosis (Kimmelstiel–Wilson lesion)
- At least one convincing Kimmelstiel–Wilson lesion

- Biopsy does not meet criteria for class IV

CLASS 4

- Advanced diabetic glomerulosclerosis
- Global glomerular sclerosis in >50% of glomeruli
- Lesions from classes I through III

DIABETIC RETINOPATHY

Diabetic retinopathy is a well-characterized, sight-threatening, chronic micro vascular complication that eventually afflicts, virtually all patients with diabetes mellitus Diabetic retinopathy is characterized by gradually progressive alterations in the retinal microvasculature that lead to areas of retinal nonperfusion, increased vascular permeability, and pathologic intraocular proliferation of retinal vessels[58]

Epidemiology

All patients with type 1 diabetes and more than 60% of patients with type 2 diabetes develop some degree of retinopathy after 20 years. In patients with type 2 diabetes, approximately 20% have retinopathy at the time of diabetes diagnosis. 58 Blindness has been estimated to be 25 times more common in persons with diabetes than in those without the disease.

Pathophysiology

The pathophysiologic mechanisms underlying diabetic retinopathy and other diabetes-related complications have been discussed earlier.

The earliest histologic effects of diabetes mellitus in the eye include loss of retinal vascular pericytes (supporting cells for retinal endothelial cells), thickening of vascular endothelium basement membrane, and alterations in retinal blood flow. With increasing loss of retinal pericytes, the retinal vessel wall develops outpouchings (micro aneurysms) and becomes fragile. With increasing sclerosis and narrowing of vessels, perfusion is reduced which results in retinal ischemia which induces angiogenic growth factors. These factors promote the development of new vessel growth and retinal vascular permeability. Proliferating new vessels in diabetic retinopathy have a tendency to bleed, which results in preretinal and vitreous hemorrhages and later macular edema. Retinal neovascularization given sufficient time eventually becomes quiescent, as with most scarring processes there is progressive fibrosis of the new vessel complexes that is associated with contraction. Such forces can exert traction on the retina, leading to tractional retinal detachment and retinal tears.[58]

Clinical findings

Clinical findings associated with early and progressing diabetic retinopathy include hemorrhages or microaneurysms (H/Ma), cotton-wool spots (CWSs), hard exudates, intraretinal microvascular abnormalities (IRMAs), and venous caliber abnormalities (VCABs), such as venous loops, venous tortuosity, and venous beading. Commonly, IRMAs are found adjacent to CWSs, which are caused by micro infarcts in the nerve fiber layer. In some cases of extensive vascular loss, the retina can actually appear free of non-proliferative lesions. Such areas are termed “featureless retina” and are a sign of severe retinal hypoxia.[58]

Classification of Diabetic retinopathy

Patients with diabetic retinopathy can be classified based on Early Treatment Diabetic Retinopathy Study (**ETDRS)** classification of Diabetic Retinopathy

1. Nonproliferative Diabetic Retinopathy (NPDR)

    A. Mild NPDR

        • At least one micro aneursym

    B. Moderate NPDR

        • Hemorrhages or micro aneurysms (H/Ma)

        • Soft exudates, Venous beading (VB), and intra retinal micro vascular abnormalities (IRMAs) definitely present.

    C. Severe NPDR

        • Hemorrhages or microaneurysms in all 4 quadrants

        • Venous beading in 2 or more quadrants

        • IRMA in at least 1 quadrant

    D. Very Severe NPDR

        • Any two or more of C

2. Proliferative Diabetic Retinopathy

    E. Early PDR

        • New vessels on the retina

        • Definition not met for F

F. High-Risk PDR

• New vessels on the disc (NVD) of 1/4 to 1/3 or more of the disc area or

• Any NV and vitreous or preretinal or vitreous hemorrhage

3. Clinically Significant Macular Edema (Any ONE of the following)

• Thickening of the retina located 500 µm or less from the center of the macula

• Hard exudates at 500 µm or less from the centre of the macula with thickening of the adjacent retina

• A zone of retinal thickening, one disc area or larger in size, any portion of which is one disc diameter or less from the centre of macula.

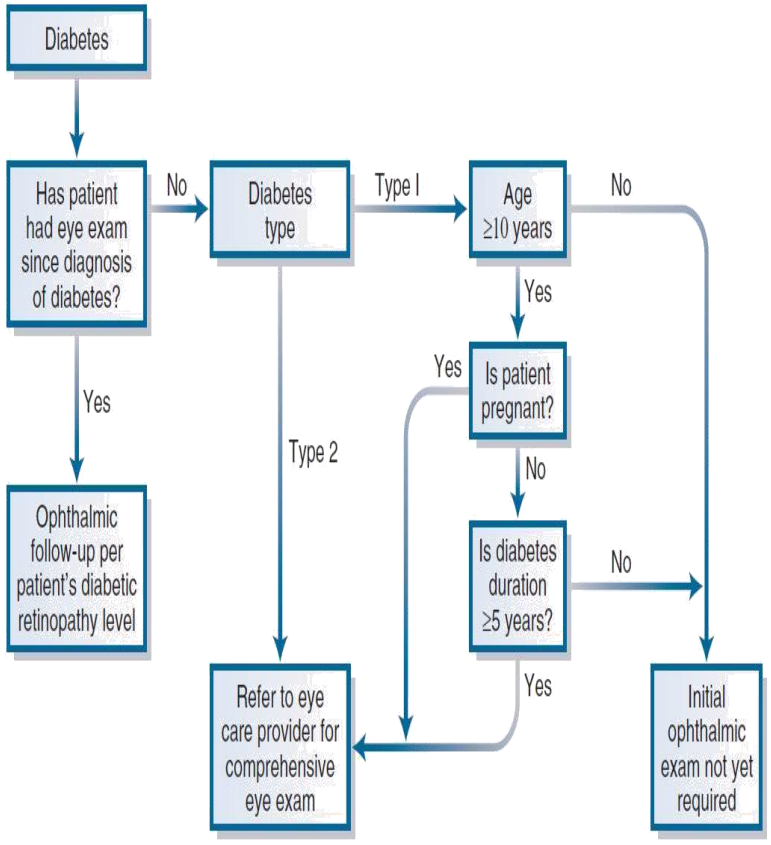


Fig 6: Initial Ophthalmic examination flow chart.

DIABETIC NEUROPATHY

Diabetic neuropathies are a heterogeneous group of disorders that cause a wide range of abnormalities. They are among the most common long-term complications of diabetes and are a significant source of morbidity and mortality.[58]

Diabetic neuropathy is a set of clinical syndromes that affect distinct regions of the nervous system, singly or combined. Clinical signs and symptoms can be nonspecific and insidious, and progression can be slow.

The major morbidity associated with somatic neuropathy is foot ulceration, the precursor of gangrene and limb loss. Neuropathy increases the risk of amputation 1.7-fold overall, 12-fold if there is deformity (itself a consequence of neuropathy), and 36-fold if there is a history of previous ulceration.

The natural history of diabetic neuropathy separates patients into two very distinctive entities:

(1) those who progress gradually with increasing duration of diabetes mellitus and

(2) those who have a relatively explosive onset and experience remission almost completely. Sensory and autonomic neuropathies generally progress, whereas mononeuropathies, radiculopathies, and acute painful neuropathies, although symptoms are severe, are short-lived and tend to recover. [58,68]

Classification of diabetic neuropathy

1. Symmetrical

- Distal symmetric polyneuropathy
  - Early, reversible sensory neuropathy
  - Chronic progressive polyneuropathy
- Predominantly sensory (large / small fibre) Painful neuropathy
- Chronic sensorimotor

2. Focal and multifocal

- Mononeuropathy: cranial/spinal
- Mononeuritis multiplex
- CIDP

3.Radiculopathy

- Miscellaneous, or mixed
- Proximal motor neuropathy Asymmetric (amyotropic), symmetric
- Entrapment neuropathy
- Atypical claudication

Distal Polyneuropathy

The distal, symmetrical, primarily sensory form of polyneuropathy is the most common type. The Clinical manifestations are predominately sensory with minor motor involvement or sensorimotor with obvious atropy and weakness of the muscles of the feet, legs and the hands

The main complaints are persistent and often distressing numbness and tingling, usually confined to the feet and lower legs, and worse at night. The ankle jerks are absent, and sometimes the patellar reflexes as well. As a rule, sensory loss is confined to the distal parts of the lower extremities, but in severe cases the hands are involved and the sensory loss may even spread to the anterior trunk, giving rise to confusion in diagnosis. Trophic changes in the form of deep ulcerations and neuropathic degeneration of the joints (Charcot joints) are encountered in the most severe and long-standing cases, presumably due to sensory analgesia, trophic changes, and repetitive injury. [69]

Small fiber neuropathy

This occurs as an early complication of diabetes mellitus with varying degrees of pain and sensory loss. It is clinically characterized by pain there is dissociated pattern of pain and temperature deficit with preserved vibration senses, tendon reflexes and power [69]

Autonomic Neuropathy

Symptoms of autonomic involvement include any combination of pupillary and lacrimal dysfunction, impairment of sweating and vascular reflexes, nocturnal diarrhea, atonicity of the gastrointestinal tract (gastroparesis) and bladder dilation, sexual impotence, and postural hypotension. The most striking examples in our experience have included severe abdominal and limb pain, reminiscent of tabetic crises. The basis of this type of involvement is not well understood. [69]

Diagnosis

Diabetes as the cause of neuropathy is diagnosed by excluding other causes of neuropathy.

A number of relatively inexpensive devices allow suitable assessment of somatosensory function, including vibration, thermal, light touch, and pain perception. These types of instruments allow cutaneous sensory functions to be assessed noninvasively, and their measurements are correlated with specific neural fiber function.

The most widely used device in clinical practice is the Semmes-Weinstein monofilament. The filament assesses pressure perception when gentle pressure is applied to the handle sufficient to buckle the nylon filament. The one that exerts 10 g of pressure is most commonly used to assess pressure sensation in the diabetic foot. A number of cross-sectional studies have assessed the sensitivity of the 10-g monofilament to identify feet at risk for ulceration. The sensitivities of testing vary from 86% to 100%. [58]

MATERIALS AND METHODS

SOURCE OF DATA:

The study was conducted on 150 subjects during the study period from March 2016 to August 2017.

TYPE OF STUDY: A Cross-sectional study.

ETHICAL CLEARANCE:

This study was approved by the Ethical Committee of Sri Devaraj Urs Medical college, Bangalore.

METHOD OF COLLECTION OF DATA:

Informed consent was obtained from all patients enrolled for the study. The data of the patients was collected in a preformed proforma. All the subjects meeting the inclusion and exclusion criteria visiting the medicine OPD of R L Jalappa Hospital. The patient's demographics, presenting complaints, past medical history and detailed examination findings were recorded soon after admission.

INCLUSION CRITERIA FOR CASES:

- Patients diagnosed with diabetic microvascular complications.
- Adults more than 18 years of ag

EXCLUSION CRITERIA FOR CASES:

- Liver diseases.
- Thyroid disorders.
- Patients on anti epileptics, anticancer drugs, metformin.
- Pregnant and lactating mothers.
- History of alcohol consumption.
- Patients with proven deficiency of vitamin B6, folic acid, Vitamin B12
- Vasculitis syndromes.

INCLUSION CRITERIA FOR CONTROLS:

- Non diabetic healthy individuals without any chronic microvascular complications.

EXCLUSION CRITERIA FOR CONTROLS:

- Liver diseases.
- Thyroid disorders.
- Patients on anti epileptics, anticancer drugs, metformin.
- Pregnant and lactating mothers.
- History of alcohol consumption.
- Patient with proven deficiency of vitamin B6, folic acid, Vitamin B12
- Vasculitis syndrome.

- Detailed history was taken including family history of diabetes and hypertension and its duration. Venous blood samples from brachial vein was collected and sent for homocysteine levels in both diabetics and non-diabetic healthy individuals. Renal parameters investigation such as blood urea, serum creatinine, urine microalbumin and urea albumin/serum creatinine was done. Patients with microalbuminuria was accepted as having diabetic nephropathy. Microalbuminuria is defined as excretion of 30-300mg of albumin per 24 hours on 2 of 3 urine collections. Fundoscopy to look for retinopathy changes. At least two microaneurysms and/or retinal hemorrhages and/or other signs of retinal damage will be as recognized as diabetic retinopathy. Biothesiometry will be done for assessment of neuropathy.

- The study population was divided into three groups:

1. Group A: Normal healthy non diabetic
2. Group B: Diabetics without microvascular complications.
3. Group C: Diabetics with microvascular complications.

Investigations conducted on patients

- HbA1c
- FBS, PPBS
- TSH level
- B12 & Folate levels
- Plasma Homocysteine levels

- Blood Urea
- Serum Creatinine
- Urine Routine
- Urine for microalbumin
- Fundoscopy
- Lipid Profile

Serum homocysteine level was estimated by chemiluminescence immunoassay using Immulite 1000 immunoassay system (Siemens, Germany)

STATISTICAL METHODS

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analysed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package

The results were averaged (mean + standard deviation) for continuous data and number and percentage for dichotomous data are presented in Table and Figure.

Proportions were compared using Chi-square (χ^2) test of significance. Proportion of Cases belonging to specific group of parameter or having a particular problem was expressed in absolute number and percentage**.**

RESULTS

A cross – sectional study consisting of 150 subjects were divided into three groups:

Group 1 → Non-diabetics

Group 2 → Diabetics without microvascular complications

Group 3 → Diabetics with microvascular complications

Normal range of plasma homocysteine:

- In males - 5.5 to 16.2 µmol/L
- In females- 4.4 to 13.6 µmol/L

Table 1:- Distribution of subject according to age group among the groups

AGE Group	GROUP			Total
	1	2	3	
26-40yrs	6	5	5	16
	12.0%	10.0%	10.0%	10.7%
41-55yrs	14	21	16	51
	28.0%	42.0%	32.0%	34.0%
56-70yrs	21	21	22	64
	42.0%	42.0%	44.0%	42.7%
>70yrs	9	3	7	19
	18.0%	6.0%	14.0%	12.7%
Total	50	50	50	150
	100.0%	100.0%	100.0%	100.0%

Mean age of the Group1 was 42.41 $\pm$ 4.5 yrs. Mean age of the Group2 was 59.57 $\pm$ 2.7 yrs. Mean age of the Group 3 was 63.75 $\pm$ 10.3 yrs. P Value =0.592, There was no statistically significant difference between the Age groups among the groups.

Graph 1:- Graph showing distribution of subject according to age group

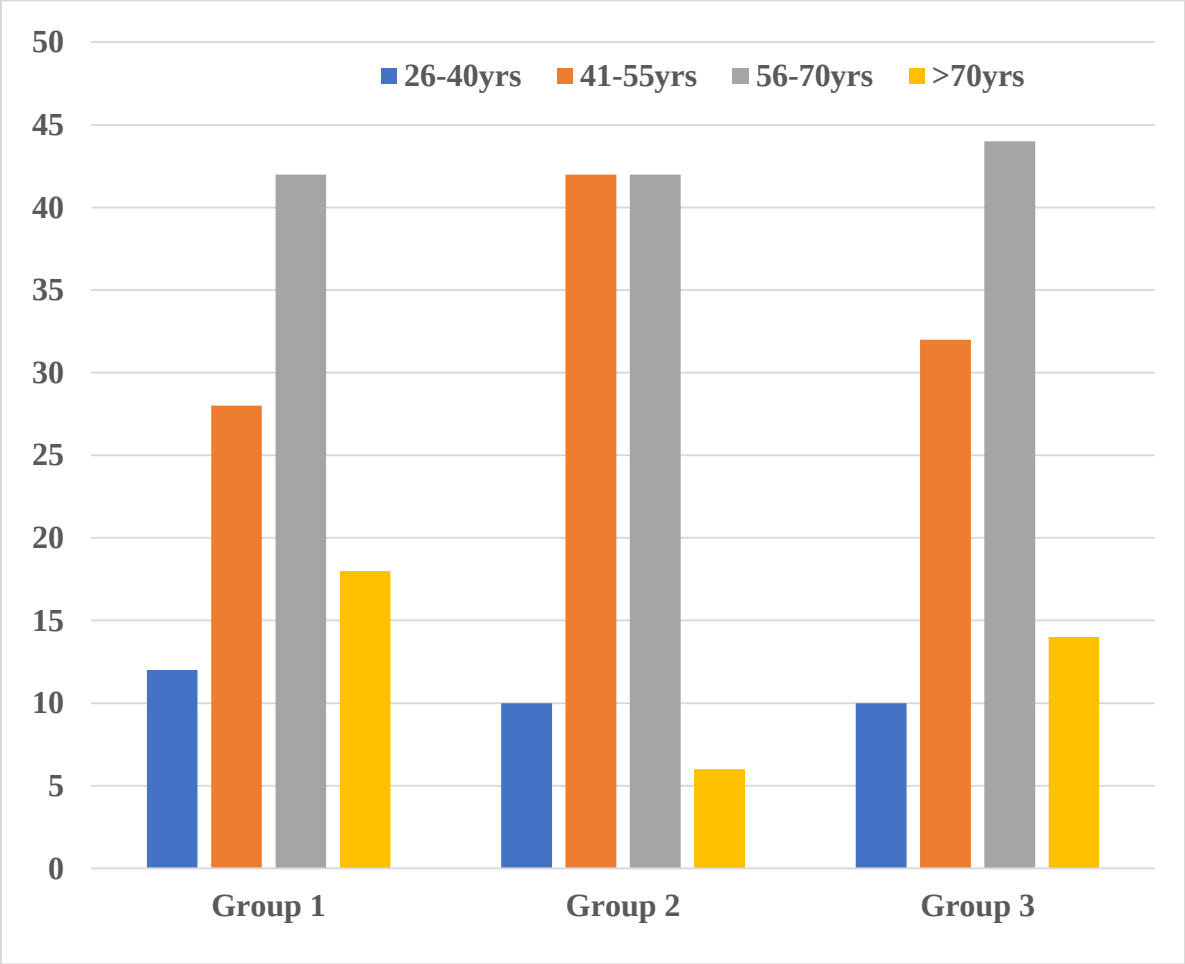


Table 2:- Distribution of subject according to sex among the groups

SEX	Group			Total
	1	2	3	
Female	24	26	19	69
	48.0%	52.0%	38.0%	46.0%
Male	26	24	31	81
	52.0%	48.0%	62.0%	54.0%
Total	50	50	50	150
	100.0%	100.0%	100.0%	100.0%

P value = 0.351, There was no statistically significant difference between sex among the groups.

Graph 2:- Graph showing distribution of subject according to sex among the groups

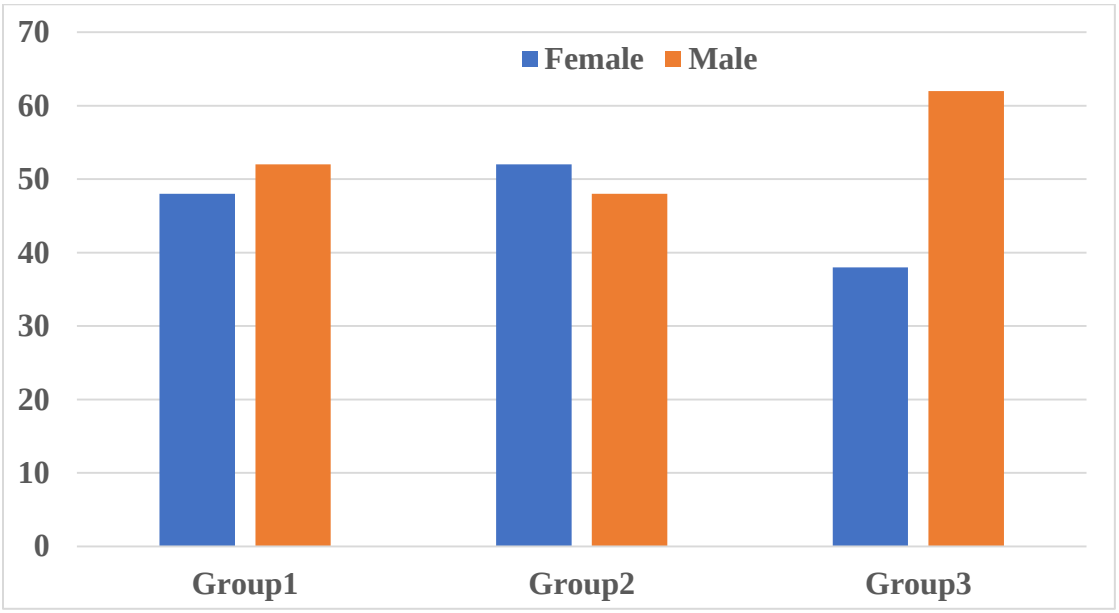


Table 3:- Comparison of mean Homocysteine levels between the age groups

Age Group	Homocysteine		P Value
	Mean	Std. Deviation	
26-40yrs	9.806250	4.7654967	0.811
41-55yrs	10.388235	4.0044299	
56-70yrs	9.989063	4.1758610	
71yrs	9.315789	4.5391217	

There was no statistically significant difference of Mean Homocysteine levels between the age groups.

Graph 3:- Graph showing Comparison of mean Homocysteine levels among the groups

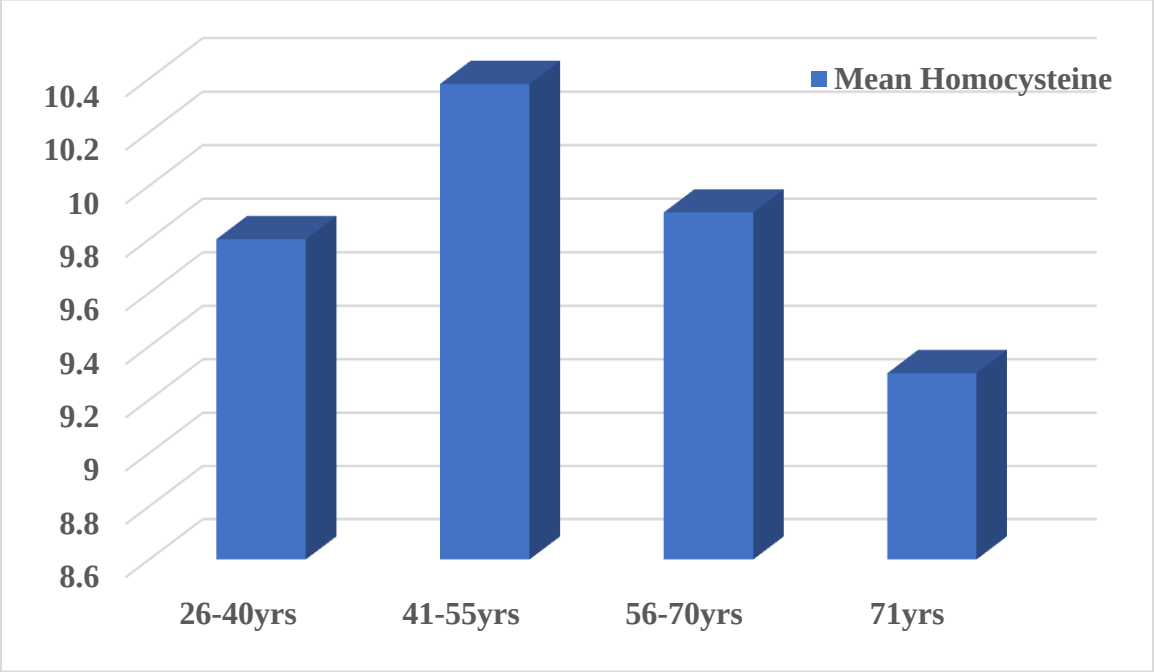


Table 4:- Comparison of mean Homocysteine levels among sex

Sex	Homocysteine		P Value
	Mean	Std. Deviation	
Female	9.611594	3.9924022	0.273
Male	10.367901	4.3643679	

There was no statistically significant difference of Mean Homocysteine levels among sex

Graph 4:- Graph showing Comparison of mean Homocysteine levels among sex

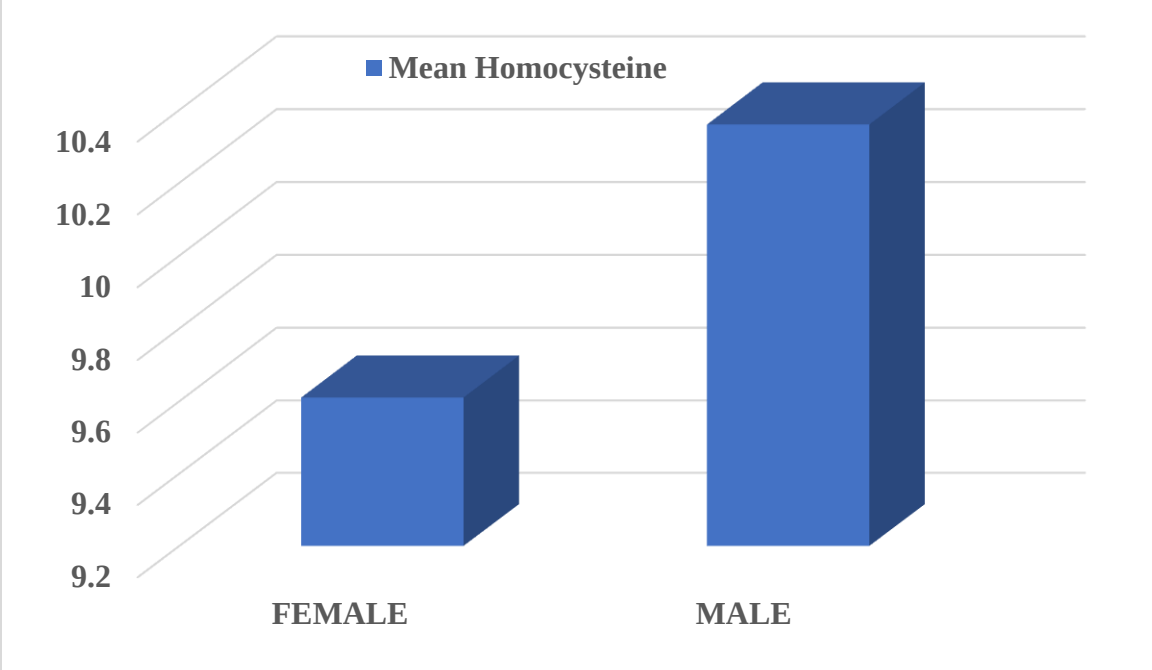
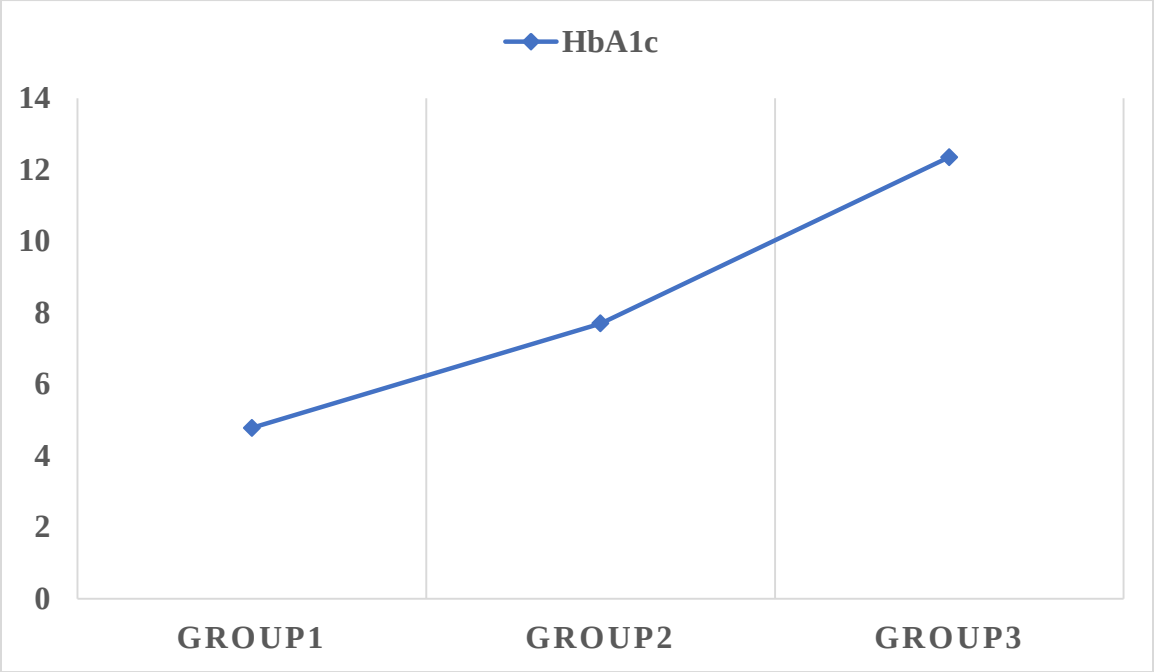


Table 5:- Comparison of HbA1c, FBS, PPBS, Homocysteine among the groups

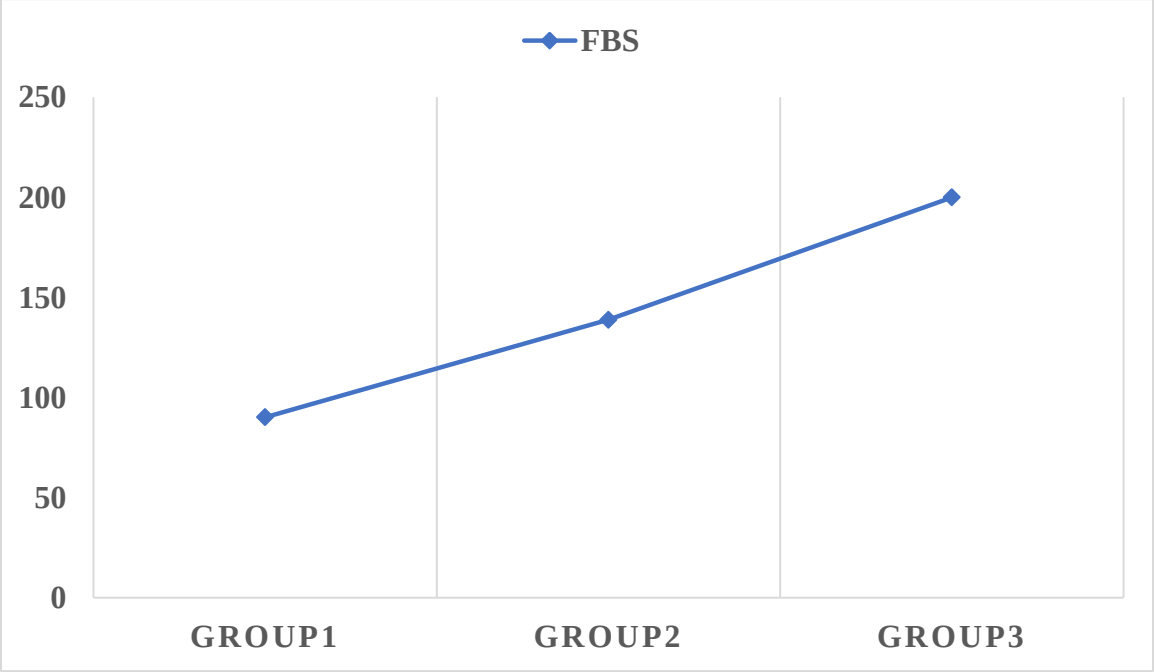
Group		HbA1C (%)	FBS(mg/dl)	PPBS(mg/dl)	Homocysteine (mol/L)
1	Mean	4.796000	90.24	128.20	5.268000
	Std. Deviation	.4194068	5.637	5.014	.5060612
2	Mean	7.740000	138.98	230.00	9.740000
	Std. Deviation	.8882452	8.707	17.341	.8882452
3	Mean	12.352000	200.12	324.80	15.052000
	Std. Deviation	1.5749623	12.462	47.089	1.9099354
Overall	Mean	8.296000	143.11	227.67	10.020000
	Std. Deviation	3.2966707	46.052	85.582	4.2005113
	P value	<0.001	<0.001	<0.001	<0.001

The difference in Homocysteine, HbA1c, FBS, and PPBS among the groups were found statistically significant (<0.001)

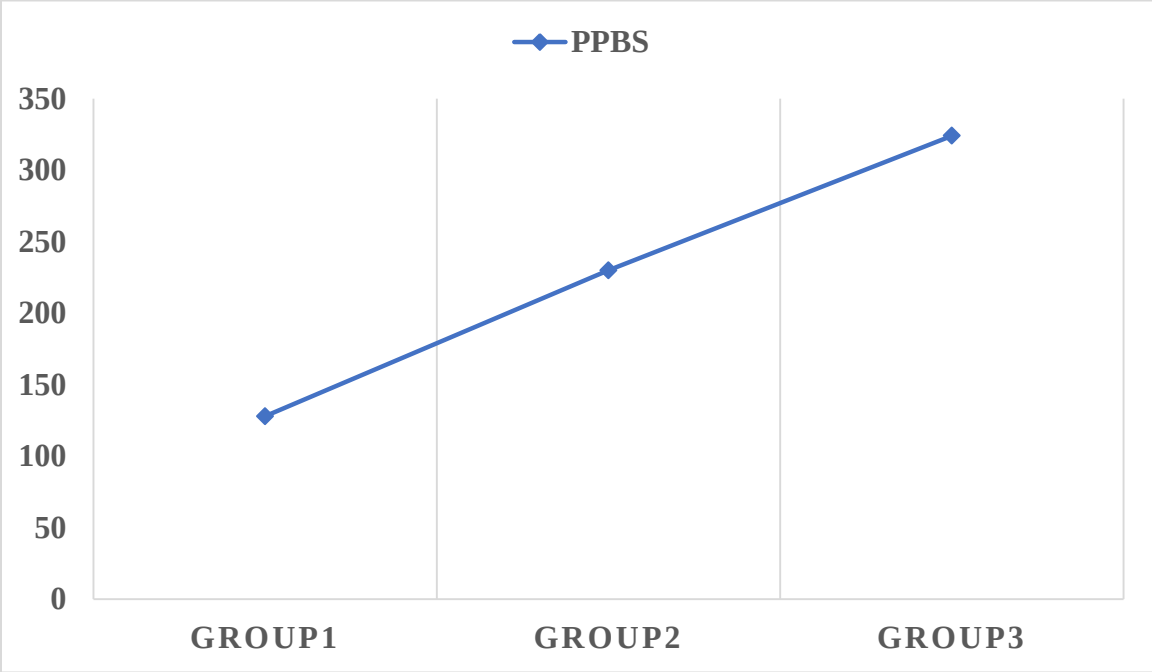
Graph 5:- Graph showing comparison of HbA1c among the groups



Graph 6:- Graph showing comparison of FBS among the groups



Graph 7:- Graph showing comparison of PPBS among the groups



Graph 8: - Graph showing comparison of Homocysteine among the groups

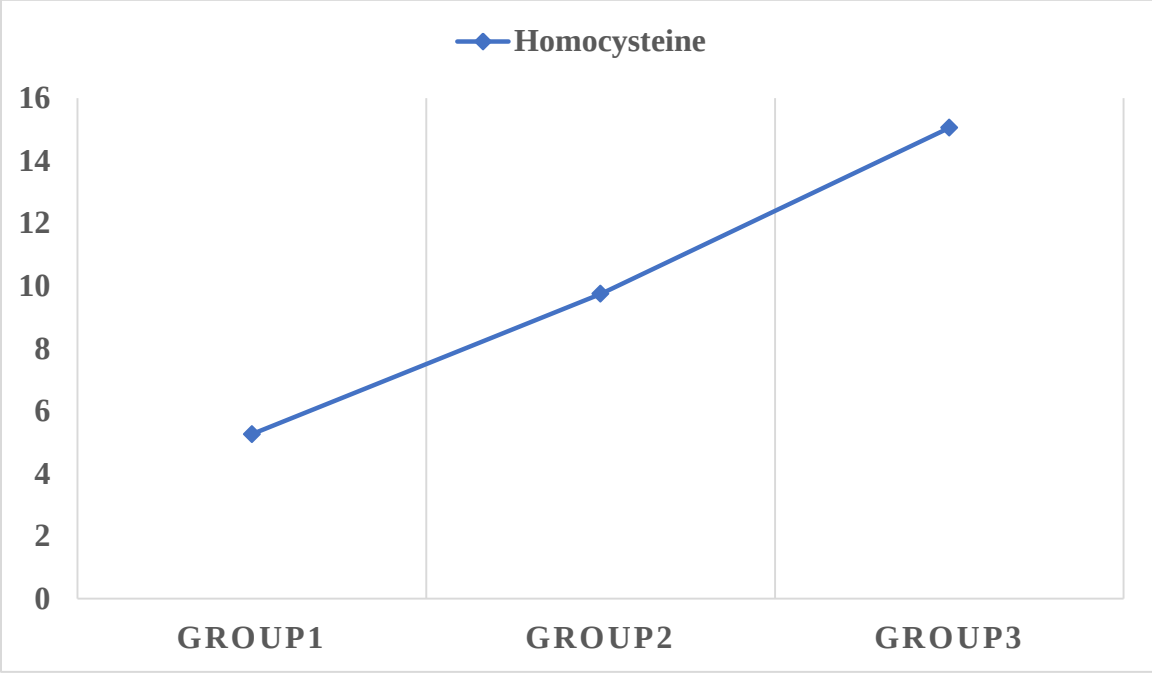


Table 6:- Correlation of Homocysteine with FBS, PPBS and HbA1c.

		Homocysteine
FBS(mg_dl)	Pearson Correlation	.952**
	Sig. (2-tailed)	.000
PPBS(mg_dl)	Pearson Correlation	.913**
	Sig. (2-tailed)	.000
HBA1c	Pearson Correlation	.991**
	Sig. (2-tailed)	.000

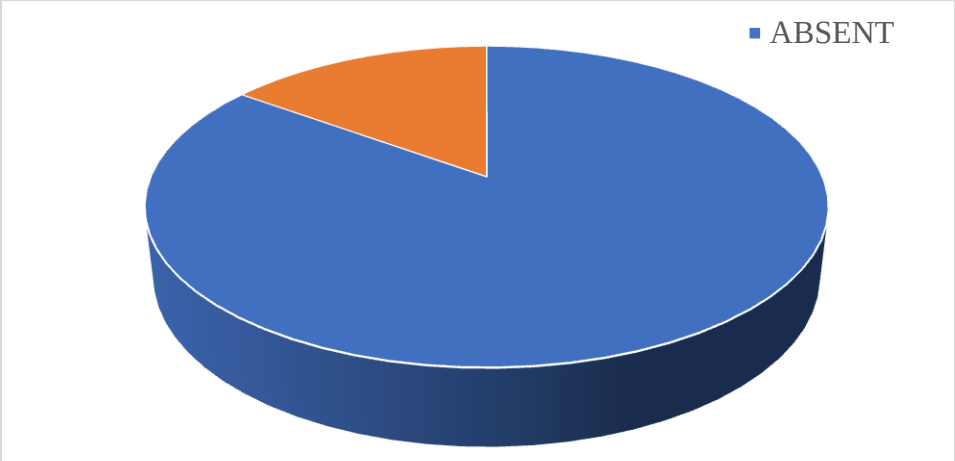
Homocysteine levels had a positive correlation with FBS, PPBS and HbA1c. These was found to be statistical significant.

Table 7:- Mean homocysteine levels and Nephropathy

	Nephropathy	Number	Mean	Std. Deviation	P value
Homocysteine	Absent	85	11.918824	2.9760750	<0.001
	Present	15	15.100000	1.8864176	

The Mean difference in Homocysteine levels between those with nephropathy and those without nephropathy was statistically significant.

Graph 9:- Graph showing distribution of diabetic subjects according to nephropathy



Graph 10:- Graph showing Homocysteine levels between those with nephropathy and those without nephropathy

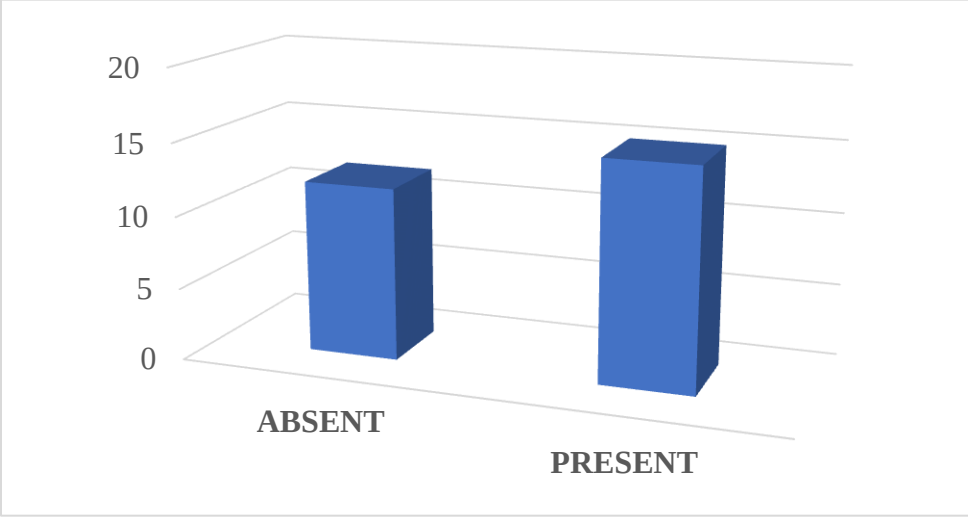
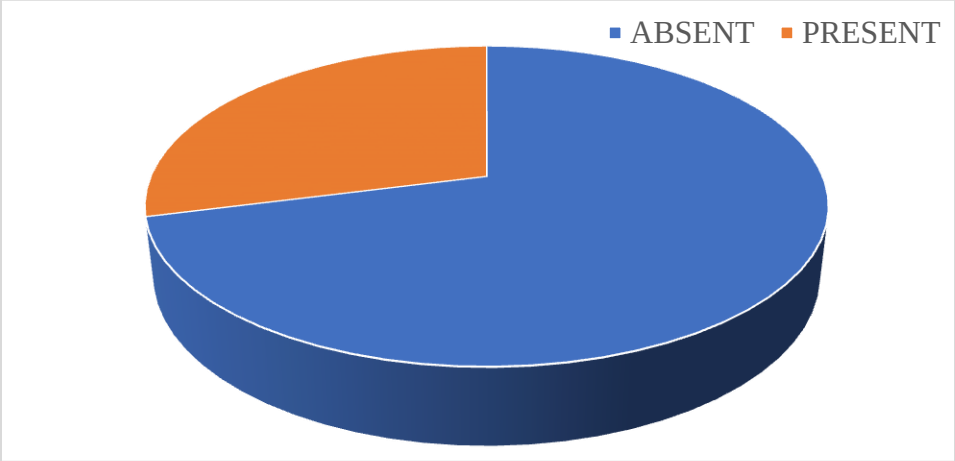


Table 8:- Mean homocysteine levels and Retinopathy

	Retinopathy	Number	Mean	Std. Deviation	P value
Homocysteine	Absent	71	11.333803	2.7840845	<0.001
	Present	29	14.996552	1.9275230	

The Mean difference in Homocysteine levels between those with Retinopathy and those without Retinopathy was statistically significant

Graph 11:- Graph showing distribution of diabetic subjects according to Retinopathy



Graph 12:- Graph showing Homocysteine levels between those with Retinopathy and those without Retinopathy

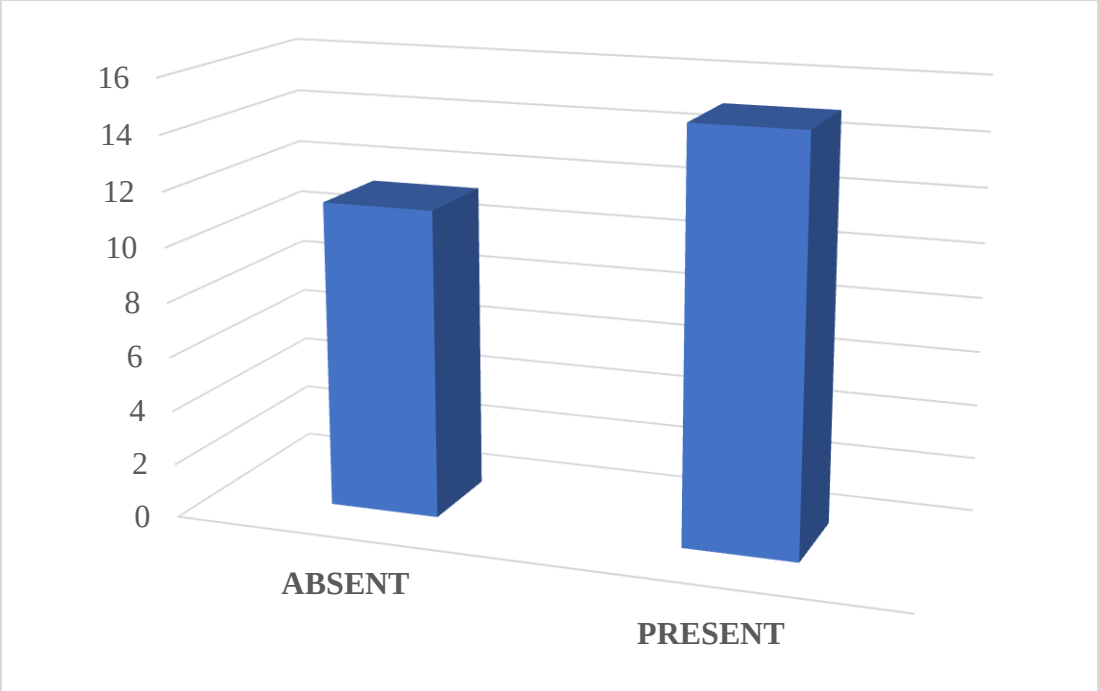
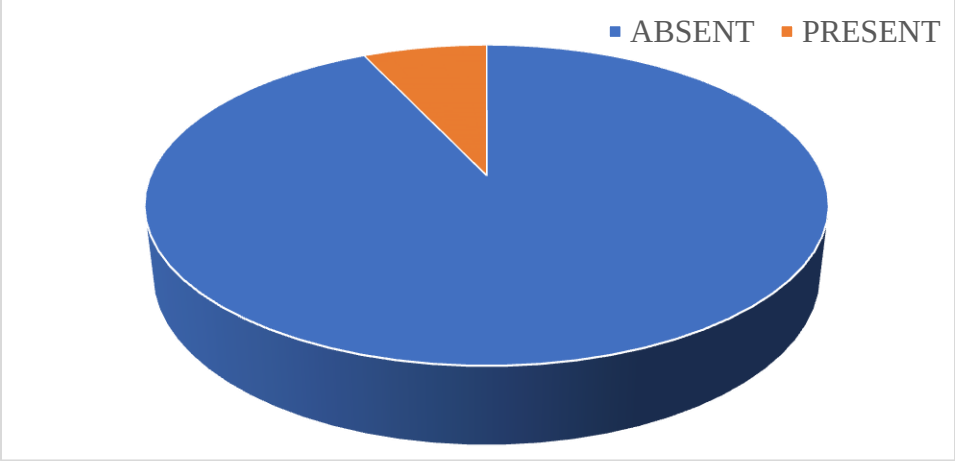


Table 9:- Mean homocysteine levels and Neuropathy

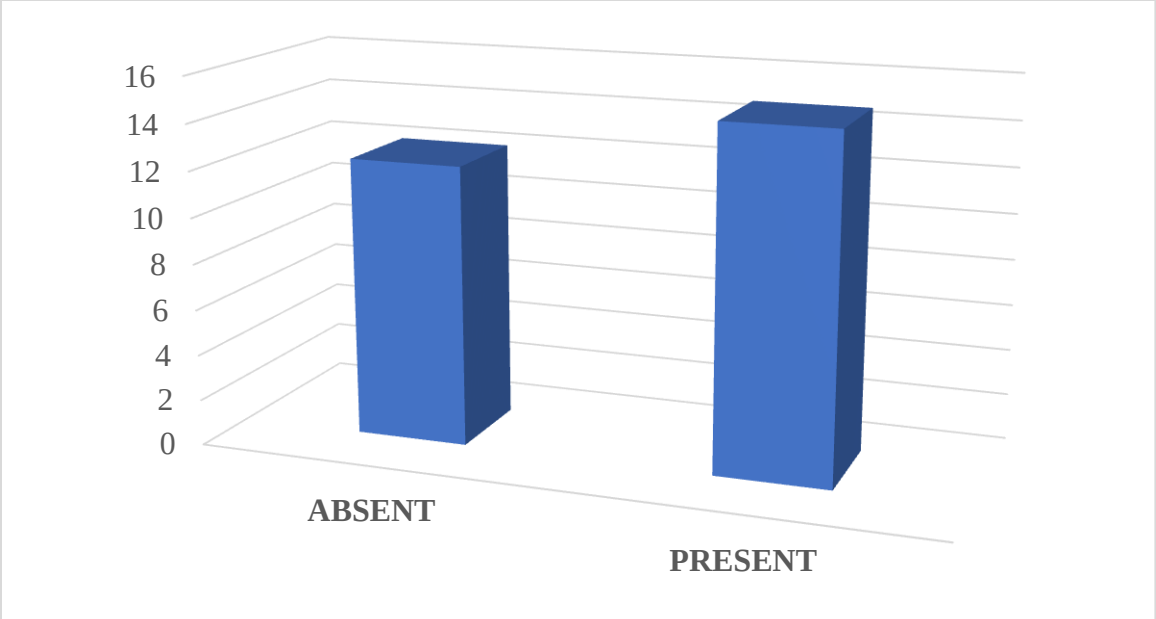
	Neuropathy	Number	Mean	Std. Deviation	P value
Homocysteine	Absent	93	12.212903	3.0357732	<0.001
	Present	7	14.828571	2.2521947	

The Mean difference in Homocysteine levels between those with Neuropathy and those without Neuropathy was statistically significant

Graph 13:- Graph showing distribution of diabetic subjects according to Neuropathy



Graph 14:- Graph showing Homocysteine levels between those with Neuropathy and those without Neuropathy



DISCUSSION

In the present study, mean age group of the study population of group 1 was 42.41±4.5 years, group 2 was 59.57±2.7 years and group3 was 63.75±10.3 years. Overall mean age of the population was 56.24±5.88. This was comparable with the results of a study done by Masanori Emoto et. al., in Japan where the mean age group of the patients with diabetic nephropathy was 56.06±9.9yrs. In another study done by Chico et. al. the mean age was 60±11 yrs among type 2 diabetic patients with microvascular complications.

In the present study, group 1,2 and 3 had 34%, 37% and 27.5% of females respectively. Males were predominant with 32%, 30% and 38% in group 1,2 and 3 respectively. Overall, they were 46% females and 54% males. But there was no statistical significant difference of mean homocysteine levels among sex (p=0.273). In the study conducted by Buysschaert et al[73], males were predominate (67%) and females were 33%.

There was no statistically significant difference of mean homocysteine levels between the age groups. But in similar longitudinal studies[78], there was significant difference ie in subjects >60 years had higher mean homocysteine compared to other age groups.

In the present study, difference in HbA1c, FBS and PPBS were found to be statistically significant (<0.001). These findings are similar to the study done by Eun-Hee et al[77], mean HbA1c between diabetic with nephropathy and without nephropathy was significant.

In the present study, nephropathy was found in 33% of group 3. Homocysteine levels in diabetics with nephropathy was 15.1±1.88 and in diabetics without nephropathy was 11.9±2.97 and differences was statistically significant. These findings are similar to previous

cross sectional and longitudinal studies[78-79] which showed between plasma homocysteine levels and nephropathy. In study done by Chico A et al., nephropathy was found in 80% of patients with hyperhomocysteinemia.

In present study, neuropathy was seen in 14% of group 3. Homocysteine levels in diabetics with neuropathy was 14.828±2.25 and without neuropathy was 12.21±3.03 and the difference was significant statistical difference. In study done by Jun Luo et al. the percentage of patients with diabetic peripheral neuropathy in hyper homocysteinemic group was **21.1% vs 5%** in normal Hcy group with p value of 0.05.

In present study, retinopathy was seen in 58% of group 3. Homocysteine levels in diabetics with retinopathy was 14.99±1.92 and without retinopathy was 11.33±2.78 and difference was significant. But in study conducted by Buysschaert et al[73], reported no significant relationship between Hcys levels and diabetic retinopathy.

CONCLUSION

- Type 2 diabetic patients have a higher homocyetine, compared to non-diabetic controls.

- Higher homocysteine is known to cause endothelial damage, which in turn predisposes the diabetic patients to vascular complications

- Microvascular complications of diabetes are associated with a higher homocysteine, as compared to diabetic patients without vascular complications.

- There is positive correlation between Plasma homocysteine and FBS, PPBS and HbA1c

- Homocyteine can be used as a simple tool to monitor complications in diabetic patients. It would be feasible even in a tertiary care hospital. However, its usefulness and sensitivity, when used on a larger scale, requires further
  large-scale studies and analysis.

LIMITATIONS OF THIS STUDY

- This study population may not be representative of the general population, as all our subjects were patients visiting the hospital

- There were confounding variables like hypertension and dyslipidemia which may interfere with some variables in the study

- This was a small scale study, hence, further studies are required to assess the utility of Plasma homocysteine in diabetes

SUMMARY

- This study was done in R L Jalappa Hospital, Tamaka, Kolar. The main aim of the study was to understand the association between homocysteine and microvascular complications of type 2 diabetes mellitus. 150 subjects were divided into three groups: Group 1: non diabetics, group 2: diabetics without microvascular complication and group 3: diabetics with microvascular complications.
- Homocysteine was higher in diabetic patients with microvacular complications (15.05 ± 0.1.9 mol/LL), when compared to diabetic patients without microvascular complications (9.74±0.88 mol/L) and non-diabetics (5.26±0.506) and this difference was statistically significant (p value<0.0001)
- FBS, PPBS and HbA1c were significantly higher in diabetics with microvascular complications, when compared to other two groups. FBS, PPBS and HbA1c were found to have a positive correlation with MPV (p<0.0001 for all three parameters)
- Among the diabetic cases, patients with microvascular complications had a higher homocysteine than diabetic patients without microvascular complications (p<0.001).
- When individual microvascular complications were assessed, the following were the results obtained:

1. Neuropathy: homocysteine was significantly higher in diabetic patients with neuropathy compared to diabetic cases without neuropathy, (p value<0.001)
2. Retinopathy: homocysteine was higher in diabetic patients with retinopathy compared to diabetics without retinopathy , this difference was statistically significant (p value<0.001)

3. Nephropathy: homocysteine was higher in diabetic cases with nephropathy compared to diabetic cases without nephropathy, however, this difference was of statistical significance (p value<0.001)

- There was a significant association between the duration of diabetes and microvascular complications. Longer the duration, more the likelihood to develop complications.

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ANNEXURE

PROFORMA

Name:

Age:

Sex:

OP/IP Number:

Current symptoms:

Past history:

Duration of diabetes:

Treatment taken:

Other Co-morbidities, if any:

Other medications:

Personal history:

- Smoker : Y/N
- Alcoholic: Y/N
- Tobacco chewer: Y/N

Family history of diabetes:

- Date of admission:
- Date of discharge:

CLINICAL EXAMINATION

VITALS:

- PULSE
- BP
- TEMPERATURE
- RR

GENERAL EXAMINATION

- Pallor: Y/N
- Icterus: Y/N
- Lymphadenopathy: Y/N
- Pedal edema: Y/N
- Cyanosis: Y/N
- Clubbing: Y/N

- Any other significant finding:

SYSTEMIC EXAMINATION:

CVS:

RS:

PER ABDOMEN:

CNS:

SIGNS OF PERIPHERAL NEUROPATHY: Y/N

FUNDOSCOPY:

E/O DIABETIC RETINOPATHY: Y/N

INVESTIGATIONS:

1. Hb-
2. Platelet count-
3. Mean Platelet volume-
4. FBS-
5. PPBS-
6. HbA1c -
7. B. Urea -

8. S. Creatinine-

9. Urine R/E –

10. U P:Cr ratio

11. ECG

12. ANY OTHER RELEVANT INVESTIGATION:

INFORMED CONSENT FORM

STUDY NUMBER:

SUBJECT'S NAME: **HOSPITAL NUMBER:**

AGE:

If you agree to participate in the study we will collect information (as per proforma) from you or a person responsible for you or both. We will collect the treatment and relevant details from your hospital record. This information collected will be used for only dissertation and publication. This study has been reviewed by the institutional ethical committee. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

I understand that I remain free to withdraw from the study at any time and this will not change my future care. I have read or have been read to me and understood the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information that will be collected and disclosed during the study. I have had the opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction. I, the undersigned agree to participate in this study and authorize the collection and disclosure of my personal information for dissertation.

Subject name

(Parents / Guardians name)

DATE: SIGNATURE /THUMB IMPRESSION

KEY TO MASTER-CHART

A1C	Glycosylated Hemoglobin
B.UREA	Blood Urea
FBS	Fasting Blood Sugar
HbA1c	Glycosylated Hemoglobin
Hcy	Homocysteine
NPDR	Non-Proliferative Diabetic Retinopathy
NRP	Nephrotic Range Proteinuria
PDR	Proliferative Diabetic Retinopathy
PPBS	Post-Prandial Blood Sugar
S.Cr	Serum Creatinine

DIABETES WITH MICROVASCULAR COMPLICATIONS

S.no	Hsptl no.	Age(yrs)	Sex	HbA1C(%)	FBS(mg/dl)	PPBS(mg/dl)	Homocysteine(mol/L)	Nephropathy	Retinopathy	Neuropathy
1	228398	62	M	10.5	210	326	12.6	absent	mild npdr	absent
2	287251	56	F	13.3	218	284	15.4	absent	moderate npdr	absent
3	287339	52	M	12.8	196	266	15.6	present	absent	absent
4	287304	86	F	10	188	360	12.8	absent	moderate npdr	absent
5	12834	62	M	14.3	216	258	16.4	present	absent	absent
6	286319	60	M	12.2	182	348	14.9	absent	absent	present
7	286327	55	M	11.9	220	389	13.7	absent	mild npdr	absent
8	286305	54	F	13.6	194	400	16.8	absent	severe npdr	absent
9	279214	60	F	10.4	185	336	12.3	present	absent	absent
10	286486	60	M	12	192	268	14.8	present	absent	absent
11	286869	60	M	10.8	200	294	12.6	absent	mild npdr	absent
12	286653	70	M	13.7	188	339	16.9	absent	absent	present
13	286436	58	F	11.8	198	282	14.5	absent	severe npdr	absent
14	286099	60	M	14	184	338	16.9	present	absent	absent
15	287334	58	M	15.2	216	387	17.8	absent	mild npdr	absent
16	336308	56	F	10.2	208	269	12.3	present	absent	absent
17	157705	72	F	14.8	192	332	17.1	absent	absent	present
18	94690	38	F	13.2	188	284	16.6	absent	moderate npdr	absent
19	318023	47	F	11.9	220	398	14.3	absent	severe npdr	absent
20	327708	38	M	12.5	207	283	16.2	present	absent	absent
21	163232	78	M	10.8	213	335	12.8	absent	moderate npdr	absent
22	174194	79	F	13.6	204	368	15.7	absent	severe npdr	absent
23	336255	26	F	10.4	188	400	12.8	present	absent	absent
24	336610	50	M	11.8	195	332	16.2	absent	mild npdr	absent
25	174881	67	F	10.2	212	285	12.6	absent	absent	present
26	110802	80	M	14.1	192	297	18	absent	mild npdr	absent
27	336620	41	F	12.9	186	263	14.8	present	absent	absent
28	174329	73	F	10.9	217	339	12.7	absent	moderate npdr	absent
29	174719	62	F	11.2	182	287	14.3	present	absent	absent
30	336628	65	F	13	197	352	16.2	absent	severe npdr	absent
31	41046	50	M	10.6	180	389	13.4	absent	mild npdr	absent
32	336610	50	M	10.3	220	333	12.8	absent	severe npdr	absent
33	336510	75	M	10.8	214	279	12.6	present	absent	present
34	315823	70	M	10.5	188	261	12.2	absent	moderate npdr	absent
35	336602	47	M	14	196	384	16.6	absent	mild npdr	absent
36	336593	67	F	15.2	210	319	17.2	absent	mild npdr	absent
37	336559	38	M	14.6	188	288	17.8	present	absent	absent
38	336365	65	M	12.1	192	400	16.4	absent	severe npdr	absent
39	336361	48	M	13.8	212	325	15.9	absent	moderate npdr	absent
40	336568	43	F	13.4	198	244	17.2	absent	absent	present
41	327708	65	M	12.9	182	382	16.2	absent	mild npdr	absent
42	327415	49	M	11.6	208	296	14.4	absent	moderate npdr	absent
43	336328	49	M	12.4	216	301	16.2	present	absent	absent
44	336388	40	M	11.9	204	380	15.9	present	absent	absent
45	93080	60	M	10.8	197	263	12.5	absent	absent	present
46	94690	58	M	10.3	183	289	12	absent	severe npdr	absent
47	336047	58	F	14.4	199	344	17.6	present	absent	absent
48	164858	50	M	13.9	206	376	16.8	absent	mild npdr	absent
49	329150	50	M	15	217	294	18	absent	moderate npdr	absent
50	335546	48	M	11.1	208	394	14.3	absent	mild npdr	absent

DIABESTES WITHOUT COMPLICATIONS

S.no	Hsptl no.	Age(yrs)	Sex	HbA1C(%)	FBS(mg/dl)	PPBS(mg/dl)	Homocysteine(mol/L)	Nephropathy	Retinopathy	Neuropathy
1	336545	56	F	6.6	128	202	8.6	absent	absent	absent
2	248696	65	F	7.2	132	210	9.2	absent	absent	absent
3	329199	60	M	6.4	126	200	8.4	absent	absent	absent
4	337417	64	F	8.2	143	224	10.2	absent	absent	absent
5	53921	63	F	7.6	138	216	9.6	absent	absent	absent
6	329922	56	F	9.1	152	242	11.1	absent	absent	absent
7	336682	65	M	6.7	129	206	8.7	absent	absent	absent
8	337290	59	M	8.8	148	232	10.8	absent	absent	absent
9	336447	60	F	9.3	156	248	11.3	absent	absent	absent
10	337358	65	M	7.4	136	214	9.4	absent	absent	absent
11	337456	55	F	8.1	142	230	10.1	absent	absent	absent
12	337353	50	F	7.3	133	212	9.3	absent	absent	absent
13	336208	60	F	6.7	129	203	8.7	absent	absent	absent
14	337407	40	F	7.8	140	228	9.8	absent	absent	absent
15	204351	78	M	7.4	136	214	9.4	absent	absent	absent
16	337431	48	M	6.6	128	202	8.6	absent	absent	absent
17	311374	50	M	8.3	144	226	10.3	absent	absent	absent
18	337437	48	M	9.1	152	236	11.1	absent	absent	absent
19	336161	54	F	6.5	127	243	8.5	absent	absent	absent
20	337011	66	M	7.6	138	234	9.6	absent	absent	absent
21	337363	60	F	7.8	140	256	9.8	absent	absent	absent
22	337401	48	M	6.6	128	223	8.6	absent	absent	absent
23	337484	60	F	9.5	158	246	11.5	absent	absent	absent
24	181853	50	F	8	141	218	10	absent	absent	absent
25	331557	46	M	7.3	135	220	9.3	absent	absent	absent
26	337372	85	F	6.5	127	234	8.5	absent	absent	absent
27	337102	49	M	8.3	144	258	10.3	absent	absent	absent
28	290562	90	M	7.6	138	236	9.6	absent	absent	absent
29	337339	55	M	9	150	253	11	absent	absent	absent
30	337113	40	F	6.6	128	226	8.6	absent	absent	absent
31	337113	45	F	8.5	146	248	10.5	absent	absent	absent
32	335546	45	M	7.6	138	260	9.6	absent	absent	absent
33	337429	45	M	7.8	140	229	9.8	absent	absent	absent
34	337504	60	F	8.3	144	243	10.3	absent	absent	absent
35	337883	65	F	6.7	129	216	8.7	absent	absent	absent
36	336554	45	F	7.4	136	249	9.4	absent	absent	absent
37	336697	40	M	7.9	140	254	9.9	absent	absent	absent
38	333778	48	F	9.2	152	218	11.2	absent	absent	absent
39	339351	64	F	9.5	158	206	11.5	absent	absent	absent
40	225472	50	F	8.5	146	225	10.5	absent	absent	absent
41	315880	26	M	7.6	138	238	9.6	absent	absent	absent
42	339354	60	F	6.7	129	260	8.7	absent	absent	absent
43	338955	34	M	7.9	140	247	9.9	absent	absent	absent
44	41675	58	M	7.1	133	223	9.1	absent	absent	absent
45	339297	45	F	7.6	138	236	9.6	absent	absent	absent
46	324858	55	M	8.4	146	214	10.4	absent	absent	absent
47	339288	48	F	6.6	128	208	8.6	absent	absent	absent
48	328785	65	M	7.9	140	248	9.9	absent	absent	absent
49	339288	48	M	7.2	134	234	9.2	absent	absent	absent
50	328785	65	M	8.7	148	252	10.7	absent	absent	absent

NON DIABETES

S.no	Hsptl no.	Age(yrs)	Sex	HbA1C(%)	FBS(mg/dl)	PPBS(mg/dl)	Homocysteine(mol/L)	Nephropathy	Retinopathy	Neuropathy
1	358528	55	M	5.2	96	132	5.9	absent	absent	absent
2	358294	46	M	4.8	90	128	5.2	absent	absent	absent
3	87462	40	F	5	92	130	5.4	absent	absent	absent
4	349865	52	M	5.3	97	134	6.1	absent	absent	absent
5	259898	50	F	5.4	99	138	6.3	absent	absent	absent
6	352770	70	F	4.6	88	126	5.1	absent	absent	absent
7	358299	48	F	4.2	84	122	4.8	absent	absent	absent
8	322019	60	M	5	92	130	5.4	absent	absent	absent
9	358535	38	F	4.4	85	124	4.9	absent	absent	absent
10	358555	48	M	4.8	90	128	5.2	absent	absent	absent
11	358530	49	F	5.1	95	131	5.5	absent	absent	absent
12	359138	68	F	5.2	96	132	5.9	absent	absent	absent
13	358957	32	M	4.7	89	127	5.1	absent	absent	absent
14	344000	80	M	5	92	130	5.4	absent	absent	absent
15	358947	30	F	4.2	84	122	4.8	absent	absent	absent
16	230318	68	F	4.4	85	123	4.9	absent	absent	absent
17	355755	59	M	4.8	90	128	5.2	absent	absent	absent
18	355791	88	F	5.1	95	131	5.8	absent	absent	absent
19	355682	72	F	5.2	96	132	5.6	absent	absent	absent
20	355680	55	F	4.6	88	126	5	absent	absent	absent
21	355655	78	F	4.4	85	124	4.9	absent	absent	absent
22	355619	67	F	4	78	118	4	absent	absent	absent
23	355756	82	F	5.2	96	132	5.6	absent	absent	absent
24	355721	58	F	5	92	130	5.4	absent	absent	absent
25	355886	87	F	5.3	97	133	5.7	absent	absent	absent
26	355834	58	M	4.1	81	120	4.4	absent	absent	absent
27	355813	70	F	4.2	83	122	4.8	absent	absent	absent
28	295535	57	M	5.1	95	131	5.5	absent	absent	absent
29	184161	63	M	5	92	130	5.4	absent	absent	absent
30	196550	56	M	5.3	97	136	5.9	absent	absent	absent
31	13215	56	M	4.5	86	124	5	absent	absent	absent
32	119432	60	F	4.2	84	122	4.8	absent	absent	absent
33	354367	62	M	4.9	90	128	5.2	absent	absent	absent
34	359797	32	M	4	78	118	4	absent	absent	absent
35	359801	44	M	4.6	88	126	5	absent	absent	absent
36	227502	63	M	5	92	130	5.4	absent	absent	absent
37	359860	27	M	5.2	96	132	5.6	absent	absent	absent
38	359404	42	M	5.4	98	137	6	absent	absent	absent
39	358255	50	F	5.1	95	131	5.6	absent	absent	absent
40	359156	72	M	5	92	130	5.5	absent	absent	absent
41	358665	50	F	5	92	130	5.5	absent	absent	absent
42	359428	45	F	4.6	88	126	5.2	absent	absent	absent
43	359808	70	M	4.2	83	123	4.9	absent	absent	absent
44	356789	72	M	4.7	89	127	5.3	absent	absent	absent
45	187797	60	F	4.3	85	124	5.1	absent	absent	absent
46	359812	75	M	4	78	118	4	absent	absent	absent
47	359801	55	M	5.2	95	134	5.6	absent	absent	absent
48	314480	60	F	5.1	95	134	5.6	absent	absent	absent
49	287792	57	M	5.2	97	136	5.8	absent	absent	absent
50	287355	60	M	5	92	130	5.2	absent	absent	absent

INTRODUCTION

OBJECTIVES

REVIEW OF LITERATURE

METHODOLOGY

RESULTS

DISCUSSION

SUMMARY

CONCLUSION

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ANNEXURE

MASTER CHART