INTRODUCTION
1.1. DIABETES – A BRIEF OVERVIEW

Diabetes mellitus (DM) refers to a group of metabolic disorder characterized by chronic hyperglycemia. These disorders usually result from defects in insulin secretion, insulin action or both. Sustained hyperglycemia is associated with complications in microvasculature, microvasculature in nerves, causing protracted morbidity. Macrovascular complications, particularly coronary artery disease and stroke, are increased two- to four-fold, and diabetic patients have a higher prevalence of peripheral vascular disease. Microvascular complications such as retinopathy and nephropathy, and peripheral and automatic neuropathy, are long term complications of diabetes.

In 1997 the American Diabetes Association (ADA) had classified diabetes according to aetiology, which was subsequently endorsed by WHO, recognizes four main categories.

Type 1- formerly known as insulin dependent diabetes mellitus (IDDM) or juvenile-onset diabetes-usually manifests before adulthood and accounts for about 5% of all cases. Type 1 diabetes arises mainly through autoimmune destruction of pancreatic β cells, which leaves the patients with severe insulinopenia and extreme hyperglycemia.

Type 2 diabetes – formerly known as non-insulin dependent diabetes mellitus (NIDDM)- usually manifests in later adult life and accounts for about 95% of all cases. Type 2 diabetes develops mostly through a combination of insulin resistance and defective β cell function. The progressive and heterogeneous nature of type 2 diabetes adds to the complexity of treatment.

Other specific forms- like MODY (Maturity-Onset Diabetes of the Young) [ Genetic defects on β cell function ];
Leprechaunism [Genetic defects in insulin action] ; other genetic
conditions like Turner Syndrome.

Infections, eg congenital rubella, etc.

Chronic pancreatic disease, other endocrine disease.

*Gestational Diabetes* - is the fourth main form and occurs when pregnant women without a
previous history of diabetes develop a high blood glucose level.

Type 2 diabetes is by far the most common form of diabetes on a global scale. During the past
few decades, type 2 diabetes has reached epidemic proportions in many part of the world. The
World Health Organization (WHO) has predicted that the global prevalence of type 2 diabetes
will be more than double from 135 million in 1995 to 300 million in 2025. Some of the countries
expecting greatest increases, such as India (up 19 million to 57 million), China (up 16 million to
37 million), Pakistan, Indonesia and some of the poorest. According to IDF Atlas 2015 now
China ranks first with 109.6 million followed by India 69.3 millions of diabetes cases, though
interestingly 46.5% of adults with diabetes are still undiagnosed. According to the Global report
on diabetes by World Health Organization, Geneva, 2016, the number of people with diabetes
has risen from 108 million in 1980 to 422 million in 2014.

All forms of diabetes are associated with the insidious development of specific damage to the
small vessels of certain organs. The small vessels (microvascular) complications of diabetes
primarily affect the retina, the renal glomerulus and the peripheral nervous system. All three
tissues are freely permeable to glucose and are closely linked to glycemic control. The ultimate
clinical consequences of diabetic microvascular diseases are failure of related major systems and
visual impairments, chronic renal failure and neuropathic foot ulceration.
Diabetic Neuropathy (DN) - Neuropathy is the most common complication of diabetes. Globally diabetic neuropathy affects approximately 131 million people as of 2010 (1.9% of the population). These conditions are thought to result from diabetic microvascular injury involving small blood vessels that supply nerves. Diabetic Neuropathy is diagnosed by thorough clinical examination and electrophysiological tests.

Diabetic Retinopathy (DR) - Diabetes is the principal cause of partial sight and blind registration in adult diabetic patients. It’s remain asymptomatic until well advanced. It has been said that hypertension has a major influence on the progression of retinopathy in type 2 diabetes. Direct fundoscopy is done to diagnose it. Digital fluoroseic angiography and fundus photography are important tools in Diabetic Retinopathy screening, monitoring and management.

1.2. STUDY OF LIFESTYLE FACTORS

Diabetes affects an estimated 25.8 million Americans of all ages — over 8% of the population. The most common form is type 2 diabetes, which accounts for 90% to 95% of all diagnosed cases in adults. Genes do play a role in type 2 diabetes, but lifestyle choices are also important. We can, for example, have a genetic mutation that may make us susceptible to type 2, but if we take good care of our body, we may not develop diabetes. Recent studies show that lifestyle factors are nonetheless strongly associated with new-onset diabetes among the elderly. In some recent studies, lab values were included to determine whether lifestyle factors were independent of known predictors of diabetes. Lifestyle factors may have provided little explanatory power, but that would be because measures such as fasting glucose and dyslipidemia may represent a history of poor lifestyle choices. Among the elderly, those currently practicing low-risk lifestyles likely have been doing so for much of their lives. Duration of diabetes, family history all have important impact on the occurrence of the disease. Some studies suggest that there
are enormous benefits to low-risk lifestyles that extend well into advanced age. Previous researches have tied several lifestyle factors to a reduced risk of type 2 diabetes, but the studies have tended to focus on the impact of one risk factor at a time.

1.3. STUDY OF BIOCHEMICAL FACTORS

Glycemic control in diabetes mellitus is a cornerstone in reducing morbidity and mortality of the disease. Achieving glycemic control or reducing hyperglycemia significantly decreases the microvascular and macrovascular complications of diabetes.

Control of plasma glucose in patients with diabetes can be assessed by measurement of glycated hemoglobin (HbA1c), fasting blood sugar (FBS), and postprandial blood sugar (PPBS). That HbA1c reflects contributions from both basal (fasting) and postprandial hyperglycemia. Estimation of glycated hemoglobin (HbA1c) remains the gold standard for assessment of glycemic control. In 2003, Monnier et al. (Monnier L et al., 2003) published a landmark study describing the relative contributions of BHG and PPHG to overall hyperglycemic exposure at different levels of A1C. But fasting or postprandial plasma glucose (PPG) is a better predictor of glycemic control in resource-poor settings when HbA1c is not available.

In addition to elevated blood sugar level in type 2 diabetes mellitus, plasma creatinine and urea are also important parameters in diabetic patients. Aldler et al., 2003, in their study observed that raised plasma creatinine and urea levels in diabetic patient may indicate a pre-renal problem. Researcher also studied that high urea levels in diabetes mellitus patients could be attributed to a fall in the filtering capacity of the kidney thus leading to accumulation of waste products within the system. Judykay T (Judykay T, 2007) in his submission suggested that high creatinine levels observed in diabetic patients may be due to impaired function of the nephrons.
Type 2 diabetes is associated with a clustering of interrelated plasma lipid which includes cholesterol, and elevated triglyceride levels. There is evidence that each of the dyslipidemic features is associated with increased risk of cardiovascular disease, the leading cause of death in patients with type 2 diabetes. Altered metabolism of triglyceride-rich lipoproteins is crucial in the pathophysiology of the atherogenic dyslipidemia of diabetes. Although behavioral interventions can improve diabetic dyslipidemia to some extent, pharmacological therapy will be needed to reach treatment goals in many patients.

1.4. MOLECULAR STUDY

In 1962, Neel proposed that certain populations have high prevalence of genetic traits which once conferred survival advantages during protracted periods of meager nutrient supply but which may now be detrimental due to reduced habitual levels of physical activity. While studies of different form of diabetes have yielded important evidence for single gene defects. Many plausible (candidate) genes have been found to have their function in altering the genes encoding signaling intermediates within the intracellular pathways of insulin action.

The receptor for advanced glycation end-products (RAGE) has been implicated in the pathogenesis of diabetic microvascular complications. RAGE is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules, and RAGE gene is located on chromosome 6p21.3 at the major histocompatibility complex locus in the class III region and composed of 11 exons as well as a 39 UTR. RAGE has been shown by in vivo and in vitro studies to play a role in the pathogenesis of diabetic microvascular complications. (Schmidt A.M. et al., 2000).

2245G/A RAGE polymorphism (rs55640627), situated at intron 8 region, is of interest due to it’s relatively high prevalence, and the nucleotide change can be rapidly screened using the
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polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. This polymorphism creates a PstI restriction site (CTGCA↑G). The nucleotide change can be rapidly screened by polymerase chain reaction (PCR)-RFLP method. In this study, we aimed to investigate the association of 2245G/A RAGE gene polymorphisms with neuropathy and retinopathy in the Eastern-Indian type 2 diabetic population.

Another polymorphism Gly82Ser (rs2070600) is particularly interesting because of relatively high prevalence and the polymorphism results in the creation of an AluI restriction site (AG↑CT). The nucleotide change can be rapidly screened by polymerase chain reaction (PCR)-RFLP method. Gly82Ser polymorphism is said to have significance because it occurs at a predicted N-linked glycosylation site and in the same immunoglobulin variable domain as the AGE binding site (Hudson et al., 1998).

1.5 STUDY OF ENVIRONMENTAL FACTOR

The problems of protection against exposure to arsenic through drinking water has assumed considerable importance, due to widespread effect of arsenic poisoning of large human populations numbering several millions in West Bengal & Bangladesh. The effects of environmental toxicants like arsenic were related to lifestyle and also to different kinds of addiction. The prevalence of diabetes mellitus is 2-fold higher in some arsenic affected places. West Bengal and Bangladesh form a geologic continuity, and the occurrence of arsenic in drinking water seems to depend on arsenic-rich sediments. An estimated 36 million people in the Bengal Delta, India are at risk from drinking arsenic contaminated water. In a community-based survey of diabetes mellitus in Bangladesh (Rahman M et. al, 1998) observed a dose-response trend between the prevalence of diabetes mellitus and the arsenic level in drinking water. Particularly high arsenic levels have been reported in West Bengal, adjacent to Bangladesh
(Datta DV. et al., 1974; Garai R. et al., 1984; Guha Mazumder DN. et al., 1992; Guha Mazumder DN. et al., 1988 and Mandal BK. et al., 1996). At the time of an Arsenic survey at 1988 by School of Environmental Studies (SOES), West Bengal, 22 villages in 12 blocks/Police stations (PSs) of 5 districts were known to be affected by arsenic. The present results indicated that 3417 affected villages in 111 blocks of 9 highly affected districts are arsenic-contaminated. So the objective of this study is to find out the effect of arsenic in etiology of diabetes mellitus.

1.6. STUDY OF ENZYMES

Aldose reductase (ALR2; EC: 1.1.1.21), is the first and rate-limiting enzyme in the polyol pathway. It reduces glucose to sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase. Increased activity of the ubiquitous polyol pathway leads to intracellular accumulation of osmotically active sorbitol and fructose through the action of this enzyme. Depletion of myo-inositol and impairment of Na⁺-K⁺-ATPase activity are associated with disturbances that have been implicated, particularly in the pathogenesis of diabetic neuropathy.

Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, ageing process, inflammation, diabetes, immunodepression, neurodegenerative condition and other disease conditions (Maxwell S.J. et al, 1995). These free radicals, which are atoms or molecules with an unpaired electron, are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Hemnani T et al, 1998).

Superoxide dismutase (SOD) (EC 1.15.1.1) is a family of metalloenzymes which is known to accelerate spontaneous dismutation of the superoxide radical to hydrogen peroxide and
molecular oxygen (McCord J.M. et al, 1969). SOD is widely distributed among aerobically living organisms and has been inferred to play an important role in controlling superoxide levels in cellular compartments (McCord J.M. et al, 1971 & Fridovich I. Et al, 1978). At the onset of diabetes, there seems to be an increase in the activity of superoxide dismutase to neutralize the excess of superoxide anions produced by the mitochondria, due to high concentration of circulating glucose, which prevents oxidative damage to tissues. (Likidlilid A. et al, 2000).

Several studies have suggested that reactive oxygen species (ROS) are implicated in the etiology of type 2 diabetes as well as in the development of severe microangiopathic complications such as diabetic retinopathy and diabetic neuropathy. Chronic extracellular hyperglycemia in diabetes stimulates ROS production and increases oxidative stress. Glutathione peroxidase (GPx) (EC 1.11.1.9) detoxify cytotoxic secondary metabolites of ROS. Many studies have looked at GPx activity in specific disease states independently and cardiovascular disease and diabetes have both been shown to be associated with alterations in GPx activity. With chronic oxidative stress, as in diabetes, one may expect increased GPx activity as a result of upregulation of the enzyme.

1.7. STUDY GROUP

Clinically diagnosed type 2 diabetic patients had been screened for this study from Endocrine department of Ramakrishna Mission Seva Pratishthan, Kolkata, India, from November 2012 to June 2015. Out of all clinically diagnosed type 2 diabetic cases, 112 Diabetes Neuropathy (DN) patients and 28 Diabetes Retinopathy (DR) patients were chosen according to inclusion criteria of this study. In addition 30 age sex match controls were included in this study.
1.8. EXPERIMENTAL DESIGN

In the present study, attempt has been made to study the predisposing factors of type 2 diabetes mellitus in our population.

i) Detailed history was taken from all cases regarding general information, socio-economic status, medical history, other complications related to diabetes mellitus.

ii) Blood was collected in EDTA (Ethylene diamine tetra acetic acid) vial for the study of genetic polymorphism and for the enzyme estimation.

iii) Genetic polymorphism was studied by isolated DNA followed by PCR and Restriction digestion

iv) Hair sample was collected for arsenic estimation.

v) Atomic absorption spectrometry was carried out to estimate the arsenic content in hair sample.

vi) Blood levels of enzyme Aldose reductase was estimated from the whole blood.

vii) Blood levels of enzyme Super Oxide Dismutase (SOD) was estimated.

viii) Blood levels of enzyme Glutathione Peroxidase (GPx) was estimated.

1.9. EXPERIMENTAL PROCEDURE

A detailed structured questionnaire was designed which covers general information on socio-demographic, age, family history of patients, general medical history, types of drug used, different bio-chemical parameters like blood sugar level, glycated haemoglobin (HbA1c), Urea, Creatinine, lipid profile etc. and occupational history.

1. Collection of Blood Sample: 5ml peripheral blood was collected in EDTA vial.

2. Molecular Study of RAGE gene: Genomic DNA was extracted from each sample of blood using a commercial kit (QIAGEN kit). PCR was performed from the isolated DNA.
a) 2245 G/A polymorphism Study: This study was done by the method based on Kankova K et al 2001. In this study, a two-step nested PCR was used since this polymorphism lies in a highly homologous region. Restriction analysis was performed with PCR products digested overnight with restriction nuclease PstI (Thermo Scientific, Lithuania) at 37°C.

b) Gly 82 Ser polymorphism Study: A PCR-RFLP assay was used to determine the RAGE Gly82Ser polymorphism by the method based on Kanakova et al., 1999. The PCR products were digested by the restriction enzyme AluI (Thermo Scientific, Lithuania) at 37°C.

3. Study of Environmental Pollution:

a) Estimation of Arsenic: Hair samples were digested with 5ml of concentrated Nitric Acid & 3ml of concentrated sulfuric acid following the method of Agahian et al 1990. Flow-injection-hybride generation-atomic absorption spectrometry (FI-HG-AAS) was used for estimation of arsenic in the collected bio-samples.

4. Study of Enzyme Estimation:


b) Estimation of Superoxide Dismutase activity: Blood level of SOD was estimated by the method of Woolliams et al, 1983.

c) Estimation of Glutathione peroxidase: Blood level of glutathione peroxidase was estimated by the method of Paglia & Valentine, 1967.

The data obtained from healthy controls and patients had been compared and statistical analysis had been carried out following Student’s t test.