DISCUSSION
5.1. DISCUSSION – GENERAL

The term 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 and 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 also common. 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. The main goals of diabetes care are good metabolic control, and minimization of complications. These are affected by patient compliance, which is the extent to which a person’s behavior coincides with medical or other health care regimen.

This work is a case-control study on the clinically diagnosed Diabetic Neuropathy and Diabetic Retinopathy patients. In our study, a total of 112 Diabetes Neuropathy (DN) patients and 28 Diabetes Retinopathy (DR) patients were screened from the Endocrine department of Ramakrishna Mission Seva Pratishthan, Kolkata, India, from November 2012 to June 2015.

Our present study deals with:
i. Lifestyle Factors of Diabetes Cases:

Lifestyle factors include gender, age, family history of Diabetes, duration of diabetes. Presence of hypertension, dyslipidemia, hypothyroidism are the ill effects of these factors. Lifestyle of urban and rural habitants are totally different. Lifestyle factors have strong influence on Diabetes Mellitus.

ii. Biochemical Factors of Diabetes cases:

Fasting sugar, post pandial sugar levels, HbA1c, Urea, Creatinine and Lipid profiles are critical biochemical factors for DM.

iii. RAGE gene polymorphism study of Diabetes:

Gene polymorphism has a major role on complication of Diabetes. Here we studied two polymorphism, 2245 G/A and G82S polymorphisms of diabetic cases.

iv. Effect of Environmental Pollution in Diabetes:

Arsenic has toxic effect on human health. As West Bengal is an arsenic prone area, we studied the arsenic level in Diabetic cases.

v. Study of Enzymes on Diabetes cases:

Levels of enzymes changes in diseased condition. During diabetes the fate of aldose reductase, a rate limiting enzyme of polyol pathway and super oxide dismutase and glutathione peroxidase, enzymes related to oxidative stressed condition, changes according to the severity of the disease.

5.2. DETAILED HISTORY OF STUDIED CASES

Health care delivery in India is provided either by doctors in the health centers, clinics, district, municipal and tertiary teaching hospitals run by the central and state governments; or through
private general practitioners, specialists in their clinics, nursing homes or large corporate hospitals. The quality and cost of care varies considerably from place to place, depending on the available resources, training and interest in diabetes of the treating doctor and the patients’ ability to pay for it. Generally, care provided in government institutions is free or at low subsidized cost. These institutions are crowded, ill equipped, and have scant resources. The quality of care suffers in this setting. Due to the scant and limited resources, the system is geared towards care of acute pressing illness with virtually no infrastructure for chronic diseases like diabetes. The absence of dramatic symptoms and the general paucity of symptoms in type 2 diabetes is perhaps the biggest barrier to early diagnosis. While delay in diagnosis is a phenomenon noted even in developed countries. The findings of the current study pointed out to the low level of adequate compliance among diabetic patients, with many physical and financial barriers. Their compliance is related to their income, mode of discovery of the disease, history of hypo or hyperglycemia, diet-related barriers, and level of knowledge about the disease. This low compliance was reflected on the lack of control of diabetes among them as implied by the high percentages of complications, obesity, and high random blood sugar levels.

The prevailing poverty, ignorance, illiteracy and poor health consciousness further adds to the problem. Patients can access any level of care (primary, secondary or tertiary) based on close location, knowledge of its existence and resources. Thus many sociological factors determine long term outcome of illness. A study of these factors and their influence on the prognosis and outcome are necessary to tackle diabetes in the community.

5.2.1. GENDER

Studies have shown varying results when predicting gender as a risk factor for developing DM. In the Joslin clinic patients, there appeared to be excess females over males in the older-onset
group, however among those with PDR, males were equal to females (Aiello LM. et al, 1981). In the clinic cohort in Chennai DR appeared to be prevalent more in the males compared to females (sex ratio 2 : 1) (Rema M. et al, 1996). A similar preponderance has been reported from the CURES (Chennai Urban Rural Epidemiology Study) Eye study (Rema M. et al, 2005), UKPDS (UK Prospective Diabetes Study) study (Harris MI. et al,1992) and the Hyderabad study (Dandona L. et al, 1999). In our study the data obtained from table 4.1.1. has shown that majority of the cases were male in both type of complications. Male and female ratio was 1.5:1 on average in our study.

5.2.2. AREAS

The place of residence are important determinants of how quickly a diagnosis will be made. Almost three year delay between city and semi-urban area was seen in a study. According to the table 4.1.3. more than 50 % of cases were from Kolkata. As our study centre was located in Kolkata (urban area) so most of the patients were from there.

5.2.3. EDUCATIONAL STATUS

In accordance with the study, Rena, et al. (Rena R. et al, 2001) and Sweileh, et al. (Sweileh W.M. et al, 2005) confirmed that illiteracy does negatively affect diabetic patients’ knowledge and compliance. Similarly, Abdel Hamid (Abd El. 2005) reported that the educated patients usually pay more attention to follow the best lifestyle by selecting healthy food and maintaining physical activity. In this study, education appears to have a major affect on diabetes prognosis. From this study it is not possible to say whether this is related to greater understanding of the illness and therefore greater commitment of self care or is a reflection of a better socioeconomic status and therefore better access to medical care, or perhaps both. In this study, inspite of a longer mean duration of diabetes, (perhaps reflecting earlier diagnosis) those with higher
education has very low in number in our study (8.93% in case of neuropathy and 3.57% in case of retinopathy).

5.2.4. MONTHLY INCOME

These barriers of such studies are commensurate with the generally low socio-economic characteristics of the patients in the study sample, where most of them were illiterate, unemployed, and from rural areas. They may perceive more difficulties in following dietary and medication regimens for a long-term disease as DM. On the same line, O'Rahilly (O'rahilly S. et al, 2005) highlighted that patient's compliance and subsequent glycemic control are markedly influenced by barriers related to dietary habits, lifestyle, and treatment regimens. According to the present study findings, patients with insufficient income tended to be less compliant to diabetic management, compared to those with sufficient income. Studies demonstrated a positive relation between obesity and socio-economic level (Moore, et al., 2010 and Rehkopf D.H. et al, 2011). Moreover, Teresa, et al. (Teresa T. et al, 2007) mentioned that people with higher socioeconomic levels are more likely to develop type-2 diabetes. Aslam and Habib (Aslam M. et al, 2003) emphasized that DM is a disease that requires continuing medical care and education to prevent or reduce the risk of long-term complications.

Higher family income increases the likelihood of proper care being provided to persons with diabetes. More so if the affected family member is actively working (gainfully employed or a housewife). This greater care should translate into fewer diabetes related complications. In neuropathy cases, most of them (43.75%) came from an average income of Rs.11000-15000.
5.2.5. DURATION OF DISEASE

The duration of diabetes is the most important independent determinant of long term diabetes complication and is a function of current age and the age at diagnosis. It is probably the strongest predictor for development and progression of diabetic complications. In persons with type 1 diabetes with less than 5 yr of duration, the prevalence of retinopathy was about 10 per cent, whereas it ranged from 25 to 40 per cent in individuals with type 2 diabetes. In India, virtually all studies have shown an increased prevalence of Neuropathy and Retinopathy as the duration of diabetes increased (Rema M. et al, 2005; Rema M. et al, 2000 and Dandona L. et al, 1999). In the study conducted by Dandona et al (Dandona L. et al, 1999) in type 2 diabetes, it is reported that 87.5 per cent of those with >15 yr duration of diabetes had DR compared with 18.9 per cent of those who had <15 yr duration. In addition, it has been demonstrated that for every five year increase in duration of diabetes, the risk for DR increased by 1.89 times (Rema M. et al, 2005).

In our study, from table 4.1.7. we had found that, 33.33% of male cases and 44.19 % of female cases with 6-10 years duration of diabetes had Diabetic Neuropathy. On the other hand, 50% of male cases and 41.67 % of female cases with >15 years duration of diabetes had Diabetic Retinopathy.

5.2.6. RELATED COMPLICATIONS LIKE HYPERTENSION

Increased blood pressure has been hypothesized, through the effects of increased sheer stress of blood flow, to damage the retinal capillary endothelial cells in eyes of people with diabetes (Kohner EM 1989). The possible mechanisms by which hypertension may affect DM are haemodynamic (impaired auto regulation and hyperperfusion) and through VEGF (vascular endothelial growth factor). This hypothesis has been supported by observations from clinical studies which showed an association between hypertension and the presence and severity of
neuropathy and retinopathy in people with diabetes (Rema M. et al, 2005 and Fujisawa T. et al, 1999). The UKPDS showed that the incidence of retinopathy was associated with systolic blood pressure (Kostraba JN. et al, 1991). In the Indian context, hypertension was not a significant confounding factor however uncontrolled hypertension did influence the progression of DM (Rema M. et al, 2005) where as hypertension was the most common complication found in diabetic cases both in male and female ones.

5.3. ANTHROPOMETRIC MEASUREMENT

5.3.1. BMI

The barriers identified in the current study were mostly physical and financial ones. The physical ones were related to diet, exercise, and medications. The great majority of patients were either overweight or obese, which could be an outcome of lack of exercise, or a factor discouraging exercise. In agreement with this, Jin J., et al. (Jin J.et al, 2008) found that patients with high BMI scored lower in their perceived compliance to self-care and self-care abilities, and were more likely to perceive physical disabilities and barriers to exercise. But in our study we have found that patients were having lower BMI than control group. May be this was an indication of poor health status.

5.4. BIOCHEMICAL FACTORS

5.4.1. BLOOD SUGAR PROFILE

There was strong evidence to suggest that the development and progression of Diabetes is influenced by the level of hyperglycaemia (Rema M. et al, 2005 ; Rema M. et al, 2000 and Klein R. et al, 1998). The protective effect of glycaemic control on the development and progression of DM had been investigated in both type 1 (WESDR) and Diabetes Control and Complications
Trial- DCCT) and type 2 diabetic patients (UKPDS) (Klein R. et al, 1998; The DCCT Research Group, 1993 and UK Prospective Diabetes Study (UKPDS) Group, 1998). The DCCT Research Group, 1993 demonstrated that intensive therapy reduced the mean risk of diabetic complications by 76 per cent as compared with conventional therapy in the primary-prevention cohort. Rema et al (Rema M. et al, 2005) had shown that the visual outcome of laser photocoagulation for eyes with PDR was also dependent on the degree of glycaemic control. For every 2 per cent elevation of HbA1c, the risk for DM increased by a factor of 1.7 (Rema M. et al, 2005). In the UKPDS (UK Prospective Diabetes Study (UKPDS) Group, 1998), the risk reduction in eye complications for every 1 per cent decrease in HbA1c was 19 per cent. The mean value of fasting, post-prandial and HbA1C were all elevated in our study. The mean values according to the table 4.3.1.1. were 166.92±9.41, 275.20±15.94 and 8.78±0.22 respectively, which were much higher than the standard control values. Thus it was observed that high glycemic values play an important role in the onset of progression of diabetes.

5.4.2. KIDNEY PROFILE

A link between renal and retinal angiopathy in diabetes had been long recognized, an effect that may be mediated through an increase in blood pressure, fibrinogen levels and lipoproteins (Root HF. et al, 1954). Cross-sectional (Cruickshanks KJ. et al, 1993) and longitudinal studies (Klein R. et al, 1993 and Mathiesen ER. et al, 1995) report a relationship between microalbuminuria, proteinuria and retinopathy. Mohan et al (Mohan V. et al, 2000) reported that the prevalence of neuropathy and retinopathy was significantly higher in type 2 south Indian diabetic patients. Level of urea and creatinine were increased in those patients. A recent study done by Klein et al (Klein R. et al, 2005) demonstrated a significant association between DM and preclinical morphologic changes of diabetic nephropathy in type 1 diabetic patients. In our study urea level
was significantly higher in diabetic cases though creatinine values were not significantly increased in the studied group.

5.4.3. LIPID PROFILE

Individuals with elevated cholesterol or triglyceride levels are more likely to have or develop retinal hard exudates, which can be associated with risk of vision loss, independent of the extent of macular oedema (Chew EY. et al, 1996). Several investigators had reported on the association of lipids with Diabetes, but the results had not been consistent. The ETDRS (Ferris FL 3rd. et al, 1996) and the WESDR group (Klein BEK. Et al, 1991) found a statistically significant association between elevated serum cholesterol and the severity of retinal hard exudation in patients with DR. Another studies had demonstrated that decreasing dietary polyunsaturated fats may had an association with shrinkage of exudates and a treatment apt to lower plasma lipid levels reduced the risk size of perimacular hard exudates. It had also been shown that in type 2 diabetic subjects there was an increase in the lipid peroxidation in plasma and this is accentuated in patients with diabetic complications (Sundaram RK . et al, 1996). A recent paper of Rema M. et al,2005 showed an association of DM with cholesterol and serum triglycerides. This association was maintained even after adjusting for age, as age by itself is a significant risk factor for hyperlipidaemia. We have observed in our study that triglyceride values are significantly higher in Retinopathy cases only. But cholesterol were significantly higher in both neuropathy and retinopathy cases.
5.6. MOLECULAR STUDY

5.6.1. RAGE GENE

Nowadays, genome-wide association study is encouraging and widely conducted to help in developing more accurate diagnostic and therapeutic strategies of various kinds of human diseases (Wallace C. et al, 2007). However, as only a subset of human genetic variation has been identified, it is unknown what proportion of the total genetic variation can be captured in this way, and it is likely that additional technological approaches will be required to capture all common variation (Wallace C. et al, 2007).

RAGE gene polymorphisms are attractive candidates to influence DM because of pathophysiological data correlating diabetic complications like neuropathy and retinopathy and advanced glycation end products (AGEs). RAGE, which maps to chromosome 6p21.3, is a member of the immunoglobulin super family. AGEs result from the non-enzymatic glycation of proteins and lipids. They were found at increased levels in diabetes and can lead to increased oxidative stress and receptor mediated activation and secretion of various cytokines (Brownlee M. et al, 1988 and Schmidt AM. Et al, 1999).

We investigated associations of RAGE polymorphism with or without the risk of Diabetic neuropathy and retinopathy in a case control based study in Eastern-Indian population. In this study, we observed an association between RAGE gene polymorphisms with diabetic complications.

Most of the RAGE polymorphisms that have been identified comprise either rare coding changes or are located in non-coding regions (Hudson BI. Et al, 1998 and Kankova K. et al,2001). 2245G/A RAGE (rs55640627) polymorphism, situated at intron 8 region, is of interest due to its
relatively high prevalence, and the nucleotide change can be rapidly screened using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Gly82Ser (rs2070600) polymorphism in RAGE is potentially interesting since it occurs at a predicted N-linked glycosylation motif in the AGE binding site, thereby influencing AGE-RAGE interactions (Hudson BI et al, 1998). Kumaramanickavel et al. (Kumaramanickavel G. et al, 2002) previously reported that this polymorphism was associated with decreased risk for the development of DR in a Southern Indian population essentially similar to the one studied here. However, additional studies investigating the same locus did not show significant association for this polymorphism in Chinese and Japanese populations (Liu L. et al, 1999 and Yoshioka K. et al. 2005), and a recent study showed association of the A allele (serine) with DR in a Han Chinese population (Zhang HM et al, 2009). As also suggested by Zhang et al. (Zhang HM et al, 2009) in their report, this might represent either different molecular pathogenesis of the disease in the two populations or simply a different population history resulting in different haplotype structure of the associated region.

5.6.1.1. POLYMORPHISM STUDY OF 2245 G/A OF RAGE GENE:

The role of RAGE in the pathogenesis of diabetes related complications was supported by some previous studies. AGE-RAGE interaction leads to the formation of products which are responsible for those complications. Soluble RAGE (sRAGE) is a naturally occurring inhibitor of signalling pathways induced by RAGE as it can remove AGE by acting as a decoy and blocking the AGE-RAGE interaction (Al-Mesallamy HO et al, 2010). In 2003, Moore et al showed that sRAGE was able to reduce AGE-induced leucocyte adhesion to endothelial cell monolayers (Moore TC et al, 2003). Although the functional effect of the intronic polymorphism
is unknown, the possibility of its quantitative impact on RAGE expression should not be ruled out. sRAGE is produced by alternative splicing of RAGE mRNA, which involves regions between intron 7 and 9 (Schlueter C. et al, 2003). The intron 8 region in which the 2245G/A polymorphism is present could hypothetically be involved in this regulatory process. The level of sRAGE has been shown to be significantly reduced in diabetic retinopathy patients compared with healthy controls (Schlueter C. et al, 2003 and Grossin N. et al, 2008). Kankova et al performed a pilot study in 2002 by investigating the relationship of three RAGE intron polymorphisms with diabetic retinopathy in Caucasians (Kankova K. et al, 2002). They reported that intron polymorphism 2245G/A was probably not involved in the genetic modification of susceptibility to the development of proliferative diabetic retinopathy.

In this study, we found that 2245A allele was significantly present (p<0.001) in the Diabetic Neuropathy (87.5 %) and Diabetic Retinopathy group (72.73 %) compared with the healthy control group (19.23 %) (table 4.4.1.). We hypothesise that individuals with increased 2245A allele frequency could have greater AGE-RAGE interaction due to low sRAGE level and a higher risk of developing neuropathy and retinopathy. However, the actual mechanism of how it affects the disease development still needs to be shown in functional studies. It is possible that the effect of 2245A allele may be related to linkage disequilibrium with a nearby causative factor that was not investigated in this study. The negative finding by Kankova et al (Kankova K. et al, 2002) could be due to differences in genetic background and population history (Ethnic variation).

In conclusion, in this study it is showing that 2245A allele of RAGE gene is associated with the development of neuropathy and retinopathy in East-Indian population. However, larger studies are necessary to support and substantiate this study.
5.6.1.2. POLYMORPHISM STUDY OF Gly82Ser OF RAGE GENE:

The RAGE Gly82Ser polymorphism occurs at a predicted N-linked glycosylation site and in the same immunoglobulin variable domain as the AGE-binding site (Hudson BI . et al,1998). Ser82 has been reported to enhance ligand affinity and up-regulate receptor signaling through mitogen-activated protein kinases and nuclear factor-κB (Hofmann MA. et al,2002). Several epidemiologic studies have investigated the association between the polymorphism and various diseases (Yoshioka K. et al, 2005).

In a case-control study, Hofmann et al. (Hofmann MA. et al,2002) showed that the Ser82 allele exhibited an association with rheumatoid arthritis with relative risk of 2.6 (P < 0.001). There were reports on significant association between the polymorphism and type 2 diabetic complications (Kankova K. et al,1999 and Kumaramanickavel G. et al,2002). Prevost et al. (Prevost G . et al,2005) suggested that the polymorphism was associated with progression to diabetic advanced nephropathy in Caucasian type 1 diabetic patients (adjusted OR, 3.17; 95% CI, 1.32-7.85). In a large population study in Korea, an association between the variant genotypes and a significantly decreased risk of coronary artery disease was shown (OR, 0.749; 95% CI, 0.579-0.969; Yoon SJ.et al,2007). Therefore, it has been indicated that the polymorphism within the ligand-binding domain of RAGE is a functional genetic variant association with activation of signal transduction pathways.

Our data also strongly suggest that patients with the serine allele of the Gly82Ser (rs2070600) polymorphism in the RAGE gene showed a increased risk for diabetic neuropathy and retinopathy in the East-Indian population.

In conclusion, we were able to confirm association of the 2245G/A and G82S polymorphisms of RAGE with diabetic neuropathy and retinopathy in the population of Eastern-India.
5.7. ENVIRONMENTAL FACTOR

5.7.1. ARSENIC LEVEL:

Arsenic, a metalloid, occurs naturally, being the twentieth most abundant element in earth’s crust. The inorganic forms consisting mostly of arsenic and arsenate compounds are toxic to human health. Humans are exposed to arsenic primarily from air, food and water. Drinking water may be contaminated with arsenic from arsenical pesticide, natural mineral deposits or improperly disposed arsenical chemicals (Guha Mazumder, 2008).

Arsenic contamination of ground water in West Bengal basin in India is unfolding as one of the worst natural geoenvironmental disaster to date. Chronic exposure of humans to high concentration of arsenic in drinking water is associated with skin lesion, peripheral vascular disease, hypertension, blackfoot disease and high risk of cancer (Guha Majumder, 2003). It is estimated that over 8 lakh people in West Bengal are chronically exposed to arsenic through drinking water (Das et al, 1996).

Human uptake of arsenic mainly occurs via the food chain (dietary sources and drinking water) and occupartional exposure (Soros et al, 2003). The Food and Agriculture Organisation World Health Organisation (FAO/WHO) recommended a provisional tolerable weekly intake of not more than 15 g inorganic AS Kg⁻¹ body weight (Kohlmeyer et al, 2003). The population of West Bengal and Bangladesh do not only drink the arsenic contaminated ground water, it is also used to irrigate the agricultural land, specially during the dry season. Rice is the prevailing crop in these countries. High arsenic concentration in irrigated soil and water may lead to elevated concentration of arsenic in rice grains or in rice straw, the latter is used as cattle feed (Abedin et al, 2002). As rice is the base of diet in this area, the majority of population can be considered to uptake arsenic above the recommended value. The levels of arsenic obtained in straw samples
are significantly higher than the background levels, being the major species as(V). Since straw is widely used as cattle feed in Bangladesh and India, their high concentration of inorganic arsenic may result in adverse health effects on cattle and increase human arsenic exposure via the animal-human pathway (Sanz et al, 2005).

When arsenic is absorbed in the human body, the major portion is excreted in the urine, a small portion via the farces and through the skin, hair, and nails and possibly a trace through the lung. Even if only a small amount of arsenic is absorbed, a amount of absorbed portion is deposited in skin, hair and nails where it is firmly bound to keratin. Arsenic in the hair and nails had thus been used as an index for monitoring the exposure of victims to arsenic (Borgofio and Greiber, 1971, Pirl et al 1983). Where long term exposure has been measured using hair as biomarker of choice, an increase in hair arsenic concentration has been observed associated with increasing arsenic concentration in drinking water (Armienta et al., 1997; Chiou et al., 1997; Lin et al., 1998; Olguin et al, 1983 and Valentine et al, 1979) reported an increase in hair arsenic concentration associated with an increase in soil, dust and air arsenic concentration. The potential presence exogenous arsenic, hair and toe nails have been considered useful markers for environmental contamination.

The concentration of arsenic in hair were clearly increased in people consuming drinking water with high arsenic concentration. In studies carried out in California and Neveda, a concentration of 400 pg/1 in drinking water corresponded to about 1.2 mg arsenic/kg of hair and 100pg/1 in water corresponded to about 0.5 mg arsenic/kg in hair (Valentine et al 1979). In Alaska an average od 400 pg/1 in drinking water corresponded to 3.3 mg/kg in hair (Harringtonet al, 1978). In Hungary, people with drinking water concentration ranging from 50 to 100 pg/l, had an average hair arsenic concentration of 3mg/kg (Borzsonyi et, al, 1992).
The results of this study support the association between a long-term arsenic exposure and diabetes mellitus, as found in our study. This was a prospective follow-up study that assessed the incidence of diabetes mellitus in the arsenic-hyperglycemic patients by comparing the data obtained from nonendemic control areas (control cases) and by comparing a higher arsenic exposure group (diabetic cases). We observed NIDDM because all of the study subjects were >30 years of age. There are only a few reports on the incidence of NIDDM. According to the study of Bender et al. (Bender AP et al., 1986) in Minnesota, the incidence rate of NIDDM in Caucasians is 1.2/1,000 person-years. In Pima Indians, the incidence rate is 18.5/1,000 person-years for all ages combined and 46/1,000 person-years in subjects > 25 of age (Knowler WC et al., 1978). After standardization to the white population in the United States in 1970, the incidence rates of NIDDM are 1.34/1,000 and 26.5/1,000 person-years for Caucasians and Pima Indians, respectively (Knowler WC et al., 1978).

The administration of arsenic has been demonstrated to cause hyperglycemia in experimental animals and to affect the functions of insulin receptor and glucose transportation (Boquist L et al., 1988; Douen AG et al., 1988 and Douen AG et al., 1988). Arsenic has been found to cause mitochondrial damage, degeneration, and necrosis of β cells in the islets of mice after intraperitoneal injection of arsenite plus hydroxylamine, with a consequence of transient hyperglycemia (Douen AG et al., 1988). Sulphydryl groups play important structural and functional roles in both insulin receptors (Pike LJ et al., 1986) and glucose transporters (May JMet et al., 1985). Jhun et al. (Jhun BH et al., 1991) demonstrated the existence of a phenylarsine oxide sensitive GLUT4 degradation in rat adipocytes, which might have pathophysiologic significance, giving rise to clinical problems of insulin resistance.
Arsenic may also compete with zinc in metal-binding proteins that display vicinal dithiols contained in zinc fingers of DNA binding and repair proteins. This competitive binding causes conformational change and altered biologic function in proteins (Engel RR \textit{et al.}, 1994). These effects of arsenic may explain some of the possible mechanisms of its diabetogenic effect.

The use of average drinking water and the lack of individual measures of arsenic make it possible to underestimate exposure due to subject variability in water consumption and to other sources of arsenic exposure in those areas, such as contaminated food and cooking water. On the other hand, because arsenic exposure was assessed at the village level and diabetes diagnosis was often not performed according to standard procedures. This ecologic association could reflect the uncertain comparability of exposure groups in terms of socioeconomic development, study selection factors and other diabetes risk factors.

In this study 140 hair samples from patients (112 neuropathy cases and 28 retinopathy cases) of As-affected areas of West Bengal and 30 healthy control cases were analyzed. Table 4.5.1. shows the statistical representation of As in biological samples from West Bengal. The normal level of As in hair ranges from 80 to 250 μg/kg, and 1000 μg/kg is an indication of toxicity (Arnold et al. 1990). In our study, the level of As in hair ranged from 940 μg/kg - 4090 μg/kg in diabetic neuropathy cases with an average of 1960 μg/kg and 340 μg/kg - 3200 μg/kg in diabetic retinopathy cases with an average of 2680 μg/kg. In healthy cases the arsenic level was ranged between 68 μg/kg – 230 μg/kg with an average of 180 μg/kg.

In our study, Early polyneuropathy was diagnosed in 20% of neuropathy patients, Sensory-motor polyneuropathy was diagnosed in 39% of neuropathy patients and finally Sensory polyneuropathy was diagnosed in 41% of patients. Nerve conduction studies revealed dysfunction of the sensory and motor nerves. Basu et al. (1996) reported a sensory predominant
distal polyneuropathy in eight arsenicosis patients exposed to As-contaminated water (range: 200–2000 μg/L) from West Bengal. Guha Mazumder et al. (1997) investigated neuronal involvement among 29 subjects from West Bengal of India. In our study, we have found in early polyneuropathy cases there was an average 2870 μg/kg of arsenic, in Sensory-motor polyneuropathy cases there was an average 1920 μg/kg of arsenic and in Sensory polyneuropathy cases there was an average 580 μg/kg of arsenic.

Our findings support the hypothesis that arsenic is diabetogenic in humans. Although the pathophysiologic mechanisms of arsenic require further investigation, the health hazards of arsenic exposure should be attended and remedial measures should be taken.

5.8. STUDY OF ENZYMES

5.8.1. ALDOSE REDUCTASE ACTIVITY IN THE STUDIED CASES:

5.8.1.1. INTRODUCTION

It has been hypothesized that uncontrolled hyperglycemia is the major factor to various secondary complications. When intracellular glucose levels are high polyol pathway for glucose metabolism is activated (Gabbay KH, 1973) and this activation is immediately linked to hyperglycemia and as a result it develops complications (Gabbay KH, 1973 and Williamson JR et al., 1993). Increased oxidative stress also induce diabetic complications like retinopathy (Brownlee M, 2001). As aldose reductase was present in cells of retina, it became evident for the occurrence of diabetic retinopathy (Dagher Z et al., 2004 and Asnaghi V et al., 2003). So aldose reductase becomes an intriguing target for the treatment of secondary complications.
5.8.1.2. ALDOSE REDUCTASE ACTIVITY AND DIABETES MELLITUS

The present study investigated the activity of erythrocyte aldose reductase in DR patients as well as patients with DN. The results showed that the activity of aldose reductase is significantly higher in diabetic patients with complications like neuropathy (3.89 units/g Hb) and retinopathy (3.79 units/g Hb) as compared to nondiabetic patients (1.80 units/g Hb) as discussed in the table 4.6.1. Though the difference between diabetic neuropathy and diabetic retinopathy is significantly low.

Certain increase in oxidative stress had been associated with diabetic complications (Brownlee M, 2001). In some study it has been found that this increased oxidative stress help in oxidation of certain molecule like cystein which in turn modulate aldose reductase activity (Kaiserova K et al., 2006). So it can be concluded that specific activity of certain protein may also contribute to the development of diabetic complications. In this study we have also found that aldose reductase activity is higher in neuropathy and retinopathy cases than normal, though there is no significant differences between diseased state. So from this result we may also conclude that high value of aldose reductase may initiate the disease but not involve in its progression.

Diabetic complications generate with times. Diabetic neuropathy and retinopathy are such complications which are linked to diabetes duration of patients (Oishi N et al., 2002). In this study we observed that diabetic retinopathy happens with patients who have diabetes for more than 10-15 years on average. In contrast neuropathy happens with patients with a history of diabetes for more than 5 years on average. So this study also supports that diabetes complications have an association with duration of this disease. Other parameters like HbA1C, blood sugar level have no significant difference.
Based on our study, we can hypothesize that aldose reductase activity may act as an predisposing factor in diabetes. Diabetes patient with certain high aldose reductase level is prone to these secondary complications like diabetic neuropathy and diabetic retinopathy.

**5.8.2. SUPER OXIDE DISMUTASE ACTIVITY IN THE STUDIED CASE:**

**5.8.2.1. INTRODUCTION**

SOD previously known as erythrocuprein (human) or hemocuprein (bovine) exists as a family of metalloproteins and is widely distributed in mammalian tissues. Erythrocytes only contain the copper/zinc SOD isozyme, which is coded by a gene located on chromosome 21. By virtue of their physiological role, erythrocytes are exposed to continuous oxidative stress, because oxygen radicals are continuously generated by auto-oxidation of haemoglobin (Misra & Fridovich, 1972).

The nature of the superoxide radical is such that it may act either as a reductant or as an oxidant. (In the dismutation reaction, half is oxidized to $0\%$ by the other half, which is in turn reduced to $\text{H}_2\text{O}_2$). Several early lines of indirect chemical evidence (Fridovich, I , 1958 and Fridovich, I , 1961) had implicated the involvement of the superoxide radical in certain reactions catalyzed by xanthine oxidase, finally leading to the proposal in 1962 (Fridovich, I , 1962) that enzyme-bound superoxide radicals were involved. Yamaeaki and Piette (Yamazaki, I , 1963), in a study of the peroxidase-oxidase reaction, proposed the superoxide radical as the active intermediate involved in this reaction but lacked conclusive evidence for this proposal. They noted that, because of the rapid spontaneous second order dismutation reaction, $\text{O}_2$ - could never accumulate to a concentration sufficiently large to be detected by electron spin resonance. More recently, however, a rapid-freezing technique has allowed electron spin resonance to confirm the production of the superoxide radical by xanthine oxidase (Knowles, R. F., 1969).
The abundance of superoxide dismutase activity in the variety of animal tissues assayed suggests that the enzyme might play a significant, even vital, role in protecting the organism against the damaging effects of the superoxide radical.

5.8.2.2. SUPER OXIDE DISMUTASE ACTIVITY AND DIABETES MELLITUS

Diabetes is a social disease which is an important medical problem due to its prevalence, treatment, and accompanying complications. Studies to-date have allowed the explanation of the pathomechanisms of many important unfavourable changes in the organism during the course of diabetes; however, changes in the activity of enzymes of the antioxidant system still remain the subject for discussion and evoke much controversy (Clapés S, 2013 and Leal CAM, 2011). The antioxidant barrier, which consists of antioxidant enzymes and fine-particle size antioxidants, counteracts the hazardous effect of free radicals. With the high production of free radicals, the antioxidant system increases its activity which, in time, may lead to its insufficiency or exhaustion. An increased production of free radicals is considered as one of the key factors leading to damage in the course of uncontrolled diabetes (Clapés S, 2013). Changes in the antioxidant system vary in the degrees of intensity, but they always cause an intensification of the primary disease, thus leading to the development of complications (Walsh SW, 2007).

Diabetes is associated with the state of increased oxidative stress. In conditions of hyperglycaemia there occurs an intensification of glucose metabolism in the cells of the endothelium, granulocytes, monocytes and blood platelets, which is accompanied by an increased production of free oxygen species. The studies by Nishikawa showed that irrespective of the route of changes dominant in a given type of cells, an increase in the glycolytic activity leads to an increased synthesis of pyruvate (Nishikava T, 2000). Pyruvate, in turn, is transported to the mitochondria and subject to further transformations in the tricarboxylic acid cycle. This
results in an increased formation of dinucleotides NADH and FADH2, which participate in oxidative phosphorylation. During the intensive processes in the course of the respiratory chain, a part of the electrons may leave the main reaction chain, originating the formation of the superoxide anion radical (O2●-). In patients ill with diabetes, ROS, together with glycation end-products, lead to changes in vascular function and disturbance of the cell homeostasis as a result of an increase in the level of lipid peroxidation, changes in intercellular matrix, as well as an inhibition of nitric oxide synthesis and activity (Książek K, 2001).

A relationship is observed between an elevated level of glucose and oxidative stress. Our studies showed a significant increase in the synthesis Super oxide dismutase activity in patients with diabetic complications, compared to healthy cases (Table no. 4.7.1.). In our study, the group of patients with neuropathy, the activity of these enzymes was 190.83±11.96 U/g Hb whereas in patients with retinopathy had an activity of 304.45±75.61 U/g Hb. Both have statistically higher values than the control groups 81±6.25 U/g Hb. It is suggested that the intensity of oxidative stress depends on both metabolic disorders in diabetes, and the duration of the disease (Zozulińska D, 1996). Our study also supports this as because retinopathy is a more late stage manifestation of diabetic complications.

Hence, in order to avoid such transformations, possibly the earliest metabolic control of diabetes is important as the main causative agent of these disorders.

5.8.3. GLUTATHIONE PEROXIDASE ACTIVITY IN THE STUDIED CASE:

5.8.3.1. INTRODUCTION

Glutathione peroxidase (GPx) is an endogenous antioxidant enzyme that detoxifies hydrogen peroxide (H2O2) and fatty acid hydroperoxides (fatty-OOH). GPx is a homotetramer with a
molecular mass of 80 kDa, constructed from four identical subunits, each of which contains one atom of selenium (Se). Glutathione peroxidase demonstrates a special affinity for hydrogen peroxide and glutathione. It uses reduced glutathione to detoxify peroxidases, releasing oxidized glutathione in the process. Oxidized glutathione is recycled by glutathione reductase back to reduced glutathione using riboflavin (Vitamin B12) as a cofactor and NADPH as a reducing agent (an anti-oxidizing substance).

The central role of selenium in glutathione peroxidase activity provides a possible focus for intervention. Selenium supplementation may be able to up-regulate glutathione peroxidase activity to restore some degree of balance with the overexpressed SOD.

5.8.3.2. GLUTATHIONE PEROXIDASE ACTIVITY AND DIABETES MELLITTUS

ROS have been implicated as important mediators of β-cell dysfunction in islet. Superoxide radicals and NO, produced in the extracellular compartment by activated recipient can in turn generate \( \text{H}_2\text{O}_2 \), hydroxyl radical, and peroxynitrite. Each of these molecules can damage the β-cell membrane and (with the exception of superoxide) readily cross into the cytosol and mitochondria to cause further damage to key enzymes, lipids, and DNA. This situation is worsened by intracellular generation of superoxide radicals by xanthine oxidase and NO by inducible NO synthase.

The possible sources of increased oxidative stress might include increased generation of free radicals or impaired host antioxidant defense system. Reduced GPx activity can be found in numerous pathologic conditions and diseases, such as in chronic etilism, chronic renal insufficiency, hypertension, endemic nephropathy (Djordjević et al 1998). Altered GSH-Px activity was also be detected in DM (Kaji et al.,1985; Cser et al., 1993; Matkovics et al., 1982). Previous studies have shown that levels of free radicals are increased in diabetes and
obesity (Hu FB et al., 2001). Many clinical studies have recognized a fair association between obesity leading to oxidative stress in T2DM (Must A et al., 1999). The correlation between GPx activity and glycemia (measured by FPG or HbA1C) has been validated by some studies (Ruiz C et al., 1999). A large number of studies demonstrated that GPx activity in the blood, erythrocytes, and leukocytes was similar in DM patients (types 1 and 2) and in healthy controls (Walter RM et al., 1991; Leonard M.B. et al., 1995; Akkus I et al., 1996). On the other hand, there are studies clearly confirming altered activity of glutathione-dependent enzymes in DM (Godin et al., 1988; Dohi et al., 1988). Murakami et al., 1991 studied the erythrocytes in DM and concluded that reduced erythrocyte GSH was caused by reduced activity of gamma glutamylcystein synthetase in connection with its glycation. Yoshida (1995) confirmed that in the erythrocytes of diabetics with poorly controlled disease the synthesis of GSH and thiol transport were damaged, rendering the cells more sensitive to oxidative damage.

Results of experimental studies, conducted both in vivo and in vitro, suggest that the peripheral nervous system is sensitive to oxidative damage (Schmeichel et al., 2003; Russel et al., 1999; Russel et al., 2002). Neurons take over glucose from the blood by the concentration-dependent transport, so that hyperglycemia is always associated with increased glucose values in the neurons, which results in oxidative stress (Tomlinson and Gardiner, 2008). On the other hand, antioxidative defense in peripheral nerves is thought to be limited due to primary lower values of glutathione and glutathione-dependent enzymes (GSH-Px and GSH-r) (Romero et al., 1991; Schmeichel et al., 2003), which further increases the sensitivity of nerves to oxidative damage.

In this study, we investigated the effect of oxidative stress status in T2DM and found that there was increase in GPx activity of diabetic patients as compared to healthy controls. According to Table. 4.8.1. the mean value of GPx activity in neuropathy and retinopathy cases were
155.97±13.67 U/g Hb and 132.88±26.34 U/g Hb respectively. In comparison to healthy control where the value was 88.19±12.37 U/g Hb, the GPx values in patients were not significantly higher.

In the erythrocytes of diabetic patients with poorly controlled disease the synthesis of GPx were damaged, rendering the cells more sensitive to oxidative damage.