“Biochemical studies and genetic polymorphism involved in Diabetes in North Indian Population”

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SUMMARY

Diabetes mellitus is a persistent metabolic disorder that is plaguing populations worldwide. Although there is now superior understanding about the various facets of T2DM, its multi-factorial pathogenesis still leaves us with more questions than answers. There is little confusion about the fact that our lifestyles have had a profound impact on the evolution of T2DM. Metabolic processes happening in our bodies are not able to keep up with our misbalanced lifestyles and therefore we must seriously consider how to defy this deadly epidemic. It’s time to reassess the status of diabetes as a whole.

The main aim of the present study was to understand the following-

1. Screening of Type 2 diabetic patients and controls based upon inclusion and exclusion criteria.
2. Analysis of various biochemical, oxidative and antioxidant parameters including fasting plasma glucose level, glycated haemoglobin, MDA, Catalase, SOD, GPx, GR, G6PD and Aldose reductase in normal as well as diabetic subjects so as to determine their status as well as correlation in the given population.
3. Estimating genetic polymorphism of TGFß, MTHFR and PTPN22 genes related to diabetes
4. To get further insights into disease pathogenesis, proteomic studies are proposed which would help us in better and efficient diagnosis and prognosis of diabetes.

T2DM Patients (n=120) as well as healthy controls (n=120) were screened on the basis of inclusion and exclusion criteria and various socio-economic, demographic and anthropometric features were recorded.Gender distribution in controls was 57.5% males (n=69) and 42.5% females (n=51). The age of healthy controls ranged from 30 to 73 years with a mean age of 50.76±1.0 years. Gender distribution in diabetic patients was 69.16% males (n=83) and 30.83% females (n=37). The age of diabetic patients ranged

from 30 to 75 years with a mean age of 53.13±0.91 years.Majority of the patients (62.5%) belonged to the age group of 46-60 followed by >60 age group (20.83%). Majority of the diabetic subjects were married (80.83%). The education profile pointed out that about 40.83% patients were graduates. Servicemen appeared to be the frontrunners in the occupation section (50.83% diabetics). 18.33% (n=22) of the patients replied yes when asked about smoking and mild physical activity was the most prevalent form of exercise among the patients (84.16%). Refined oil was the chosen cooking medium among 60.83% patients and 60% of them admitted to consuming junk food sometimes. The differences in values between these features were statistically significant as compared to controls ($p<0.05$).Our findings revealed that once diagnosed, type 2 diabetic patients made healthier lifestyle choices, however their physical activity profile needed improvement.67.5% diabetics had a family history indicating the strong genetic component of the disease. 69.87% of the diabetic males showed positive family history, while 62.16% of the diabetic females exhibited positive family history.

HbA1c which is an indicator of glycemic control was significantly elevated in patients (7.89±0.083) as compared to controls (5.57±0.089) ($p<0.001$). MDA which is a marker of lipid peroxidation showed significantly higher values in patients (1.57±0.49) as compared to controls (0.98±0.02) ($p<0.001$). Significantly higher values of fasting Triglycerides were observed in patients (257.36±7.27) as compared to controls (139.14±2.74)($p<0.001$). Total Cholesterol was significantly elevated in patients (234.07±4.1) as compared to controls (183.52±3.29) ($p<0.001$). LDL-c was also observed to be significantly elevated in patients (148.61±4.23) as compared to controls (115.63±3.43) ($p<0.001$), whereas HDL-c values were significantly lower in patients (33.98±0.52) as compared to controls (40.06±0.61) ($p<0.001$). VLDL-c values were also significantly enhanced in patients (51.47±1.45) as compared to controls

(27.83±0.55) ($p<0.001$) (Fig.). This high prevalence of hypertriglyceridemia, high LDL, low HDL and hypercholesterolemia in the diabetic patients are known risk factors for cardiovascular disease.

Significantly higher Uric acid values were observed in patients (5.86±0.16) as compared to controls (3.94±0.08) ($p<0.001$) and Hyperuricemia is linked to development of T2DM and its complications especially cardiovascular and renal complications.

Creatinine values were also significantly increased in patients (1.06±0.02) as compared to controls (0.99±0.013) ($p<0.05$). It is believed that one can plot the inverse of creatinine (I/ Cr) over time and get a straight line which can thus be used for monitoring the development of nephropathy. Nephropathy leads to end stage renal disease which is a major cause of mortality in diabetes hence these values must be carefully monitored over time.Frequency analysis of the various biochemical parameters showed that the most commonly encountered abnormality among the diabetic patients was high TG values or hypertriglyceridemia (95.83%, n=115), followed by low HDL-c values in 83.33% of the patients (n=100). Glycaemic control as evidenced by HbA1c values was found to be suboptimal in 62.5% of the patients. Majority of the patients were obese (76.66%, n=92). It was observed that 73.5% of the male patients (n=61) had obesity while 83.8% of the female patients (n=31) suffered from it.Correlation analysis revealed that significant correlation existed between many biochemical parameters. Fasting plasma glucose (FPG) showed significant positive correlation with HbA1c ($r=0.513$, $p<0.0001$), with MDA ($r=0.258$, $p=0.004$), with HDL ($r=0.210$, $p=0.021$) and with LDL ($r=0.235$. $p=0.0096$). HbA1c showed significant positive correlation with MDA ($r=0.232$, $p=0.01$). Total cholesterol (TC) showed significant negative correlation with HDL ($r=-0.272$, $p=0.0026$) and highly significant positive correlation with LDL ($r=0.947$, $p<0.0001$). Triglycerides (TG) showed highly significant negative correlation with HDL (r=-0.364, p<0.0001). HDL showed highly significant negative correlation with LDL (r=-0.262, p=0.0038). Presence of dyslipedaemia and obesity among the patient led us to explore the prevalence of metabolic syndrome in our diabetic patients. Metabolic Syndrome was highly prevalent in diabetic subjects with 58.33 % patients (n=70) displaying three out of five components.

Activity profile of antioxidant enzymes showed that the activity of SOD was significantly increased in patients (1929.38±47.11) as compared to controls (1115.66±50.82) (p<0.001). Activity of GPx enzymes in patients (12.15±0.15) was found to be significantly decreased as compared to controls (29.64±0.38) (p<0.001). Similarly, activity of catalase enzyme was also significantly diminished in patients (1.45±0.02) as compared to controls (2.76±0.05) (p<0.001). Activity of GR enzyme was significantly enhanced in patients (31.71±0.76) as compared to controls (16.14±0.27) (p<0.001). Activity of G6PD witnessed significant decrease in patients (7.0±0.12) as compared to controls (9.75±0.19) (p<0.001). On the contrary, activity of AR was observed to be significantly elevated in patients (4.8±0.07) as compared to controls (3.47±0.05) (p<0.001). Such a situation represented a tug of war between the pro-oxidant effect of free radicals and the antioxidant effect of the enzymes. High values of SOD and GR were holding the fort but diminished Catalase and GPx activities were on the verge of tipping the balance in favour of oxidative stress. Moreover, significant positive correlation was revealed between the activities of enzymes GR and GPx (r=0.1828, p=0.045) whereas significant negative correlation between SOD and catalase activities (r=-0.261, p=0.003) was found.

The level of copper is significantly increased in diabetic patients (161.34±1.81) as compared to controls (120.32±2.04) (p<0.0001). On the contrary, the level of zinc is significantly reduced in diabetic patients (90.06±1.36) as compared to controls

(94.73±1.44) (p=0.0195). Copper ions in excess cause redox imbalance hence promote pro-oxidant action whereas zinc ions Zn possesses antioxidant properties and can prevent macromolecules from free radical induced oxidation. Their antagonistic relationship further lowered the defense status in the body of the patients.

Our study estimated the parameters associated with iron metabolism in relation to glycated haemoglobin (HbA1c) values in the subjects. 50 healthy controls, 50 type 2 diabetic patients with HbA1c <7 (good glycaemic control) and 50 type 2 diabetic patients with HbA1c >7 (poor glycaemic control) were selected from the original set of subjects. Mean serum free iron concentration in Group I (controls), Group II (T2DM patients with HbA1c <7), and Group III (T2DM patients with HbA1c>7) was 105.34 ± 3.5, 107.33 ± 3.45, and 125.58 ±3.74 µg/dL, respectively. Mean serum TIBC (Total Iron Binding Capacity) concentration in Group I, Group II, and Group III was 311.39 ±5.47, 309.63 ±6.1, and 284.2 ± 3.18 µg/dL, respectively. Further, mean serum transferrin saturation (%) in Group I, Group II, and Group III was 34.17 ± 1.21, 35.02 ± 1.2, and 44.39 ±1.07 µg/dL, respectively. An increase in the levels of serum free iron concentration and serum transferrin saturation levels with poor glycaemic control in our study indicate an important role of free iron in the development of diabetic complications. Thus, monitoring the prevalence of iron overload is beneficial in the long run.

Allele specific PCR was used to detect A1298C SNP in diabetic and control DNA samples and No significant association for risk of developing T2DM was observed for SNP allele C (OR, 1.17, 95% CI, 0.81-1.61, p = 0.403).

We used allele specific PCR to detect T869C SNP in diabetic and control DNA samples. TT homozygous genotype was prevalent in controls while in diabetics TC heterozygous genotype was prevalent. Significant association for risk of developing

T2DM was observed for SNP allele C (OR, 2.69, 95% CI, 1.75-4.14, $p < 0.05$). Upon logistic regression analysis, SNP T869C displayed significant association in dominant model (OR, 3.62 95% CI, 2.12-6.19, $p <0.05$), co-dominant model-I (OR, 4.91, 95% CI, 1.48-16.3, $p = 0.005$) and co-dominant model-II (OR, 3.46, 95% CI, 1.98-6.04, $p <0.001$). Thus T869C mutation could be used as a marker for T2DM development as well as progression of nephropathy (shown by the presence of CC genotype) which is a major co-morbidity attached with T2DM.

PTPN22 1858 C/T SNP was found to be non-polymorphic in our study population.

^{1}H-NMR based serum metabolic profiling of the subjects was done. The subjects included diabetic patients without complications (DB, n=20) and diabetic patients who had developed complications (DC, n=25), both from the original set of patients and normal asymptomatic controls (NC, n=18). The discriminatory metabolites analysed in serum were mainly related to lipid, amino acid, glucose, and energy metabolism. When compared to the DB, the DC patients had increased levels of succinate, citrate, glucose, threonine, PUFA and urea by the decreased levels of LDL/VLDL, lipids, N-acetyl glycoproteins(NAG), pyruvate, creatine, and amino acids like valine, arginine, glutamate, methionine, and proline.

These results corroborate with the biochemical as well as genetic data of the patients. TGF ß T869C polymorphism that causes mutation of leucine to proline is implicated in diabetic nephropathy. Hence, the down regulation of proline in patients who had ultimately developed nephropathy (the risk of which was indicated by our T868C polymorphism) seemed to be the effect of ongoing medication followed by the patients.

The awareness and knowledge profile of the patients showed a glimpse about the reason behind this dismal state of affairs. Only 52.5% knew that they suffered from type 2 diabetes and 54.2% were aware of the fact that it is preventable. Only 50.8% were acquainted with the connection of T2DM with family history. Knowledge regarding lifestyle risk factors like obesity and sedentary behaviour was evident in 68.33% and 61.66% patients respectively. 67.5% patients gave credit to their attending physician for providing information about the disease. The most commonly recognized diabetic co-morbidity was heart problem as reported by 59.2% patients. Frequent urination was found to be the most commonly recognized symptom among 59.4% patients. Economic burden of the disease was found to be the foremost cause of non-adherence among the patients. Perhaps, that is the reason 30% of them sought alternative forms of treatment that were cheaper.

Thus, studying diabetes with the aid of one approach may not be sufficient. Hence, a combined attack involving various biochemical, genetic, metabolomic as well as social factors involved in type 2 diabetes and its complications will go a long way in understanding the mechanism behind its aetiology, complications, possible treatment and cure and most importantly its prevention. Such a multifaceted approach may provide the answers we are looking for.