CONCLUSIONS

1. The present study revealed 47.87 percent prevalence of depression, which was observed to be slightly higher in women in the population of Punjab.

2. Besides many possible risk variables, sedentary lifestyle was observed to be the strongest predictor that approximately added 4 fold higher risk of depression. Other independent predictors were being a woman, duration of diabetes >5 years, BMI ≥ 30kg.m⁻², LDL > 100mg/dl and TG > 150mg/dl.

3. Linear regression analysis revealed significant direct relationship between advancing glucose levels and depression.

4. It was affirmed that T allele of rs2020936 and rs3794808 within SLC6A4 gene influenced significantly to the risk of depression as well as diabetes in the population of Punjab. Major alleles of rs3758653 and rs916455 and minor allele of rs747302 within DRD4 gene conferred significant risk of depression, whereas major alleles of rs3758653 and rs1800955 contributed to the risk of diabetes.

5. Haplotypes TAT within SLC6A4 gene was observed to be susceptibility marker, carriers of which were at 2.19 times higher risk of depression than those subjects who did not possess it. TCTC haplotype within DRD4 gene was analysed to be a risky marker increasing 4.15 times higher risk of depression whereas, TCTT haplotype within same gene increased the risk of diabetes by 3.10 times in comparison to those subjects who did not have it.

6. Several Gene-Gene and Gene-Environment interactions were explored, which showed that SNPs within SLC6A4 and DRD4 gene communicate with each other and with the risk variables, hence, contributing substantially to the risk of both depression and diabetes.
7. Furthermore, two way epistatic effects were observed between SNPs rs2020936-rs747302, rs3794808-rs1800955 and rs2020942-rs747302 for the risk of depression and rs3794808-rs3758653, rs3758653-rs916455 for the development of diabetes in the population of Punjab.
SUMMARY

Diabetes Mellitus (DM) is a group of complex metabolic disorders with basic anomaly of insulin secretion and insulin action in target tissues such as muscles and liver. India is having the highest prevalence of type 2 diabetes mellitus (T2DM) which is escalating continuously. Moreover, depression is also highly prevalent in Indians, especially in T2DM patients. T2DM has been observed to be associated with an increased risk of depression and the depression related symptoms are two fold higher in patients with type 2 diabetes than healthy non-diabetic subjects. Both of these disorders may coexist and have bidirectional relationship. In diabetes, poor glycemic control influences Hypothalamic-Pituitary-Adrenal Axis (HPA axis) which activates the neurobiology of various mood disorders including depression and in depressed subjects, higher levels of cortisol makes the cells resistant for insulin action, hence influences the risk of hyperglycemia. Both diabetic and depressed subjects have poorer self-management and non-adherence to anti-diabetic, lipid lowering and antihypertensive treatment. It is well known in the scientific arena that depression due to diabetes is strongly associated with poor health, lower quality of life, lack of self-care, impaired glycemic control, increased risk of developing diabetes related complications and mortality.

Genetic epidemiology and twin studies have shown that depression is influenced by genetic factors with considerable heritability component. The influence of genes and genetic variants that predispose a diabetic subject to the risk of depression has been examined finitely in India. Some studies have suggested that dopamine receptor type 4 (DRD4) and serotonin transporter (SERT) genes play a significant role in the development of depression. At the cellular level, DRD4 mRNA acts as a peripheral marker of central dopaminergic
function in major depression and **serotonin transporter** encoded by solute carrier family 6, member 4 (SLC6A4) gene is the target of an important class of antidepressant drugs named as the **serotonin** selective reuptake inhibitors (SSLI). DRD4 is also expressed in human and rodent amygdaloid nuclei, with the highest levels observed in the basal and central nuclei. In the basal nucleus, the levels of the DRD4 mRNA are significantly higher in patients with major depression compared with control patients. In the pursuit of understanding diabetes and its implications, innumerable studies have been conducted on different parameters that may influence its development or its imperative sequels, however, role and relevance of DRD4 and SLC6A4 genes as the genetic determinants of depression in T2DM remained to be investigated in India. Consequently, the present study set forth aim to identify those genetic variants within these genes that participate and contribute significantly to the risk of depression in T2DM subjects of Punjab.

To achieve the set-forth objectives, initially 689 subjects were screened to have data on confirmed T2DM subjects from outpatient doors (OPDs) of various tertiary health care hospitals of Punjab. Following strict inclusion and exclusion criteria. 426 T2DM subjects diagnosed with American Diabetes Association (ADA) guidelines were included to participate in the study. 27 subjects were further excluded because of having genotyping errors or unclear results. Finally, a cohort of 399 confirmed T2DM subjects were included in the present study. All T2DM subjects were tested for the presence of depression by employing Patients Health Questionnaire-9 (PHQ-9) which is self-administered version of Primary Care Evaluation of Mental Disorders (Prime-MD). Its reliability to assess depression has been validated in India. PHQ-9 ≥10 score has been confirmed with 88 percent sensitivity and specificity for diagnosing depression in diabetes. Other methods such as Hamilton depression rating scale (HAMD), Montgomery-Asberg depression rating scale (MADRS), Centre for Epidemiological Studies depression scale (CES-D) can detect depression but PHQ-9 is the only questionnaire which has been
validated to diagnose depression in diabetes, hence, was used to test depression in T2DM patients of Punjab.

After testing with PHQ-9, T2DM subjects were categorized into two groups. Group1 (subjects with depression; n=191) and group 2 (subjects without depression; n=208). Data on control group (subjects without diabetes and depression) was collected in order to examine the influence of various variables on diabetes. For this, initially 497 subjects were screened. After following inclusion-exclusion criteria, 181 subjects were included to participate as control subjects (Group 3). In this way present cross sectional study involved three groups i.e. diabetic subjects with depression (n=191), diabetic subjects without depression (n=208) and non-diabetic, non-depressed subjects or controls (n=181). The differences between first two groups (diabetic subjects with and without depression) revealed the effect of diabetes on depression, whereas differences between the last two groups (diabetic subjects without depression versus controls) revealed the effects for the development or risk of diabetes.

Subjects were allowed to participate only after submitting their informed consent before participation. Information regarding all the variables that might influence diabetes and depression was collected by conducting their interview or from their health records. Required sample size and its power was calculated by the software G Power, which confirmed that 580 T2DM subjects involved in this study were appropriate to prevent spuriousness and ambiguity in the results with more than 82 percent power.

The demographic, biochemical and clinical characteristics of the diabetic subjects (with depression and without depression) and controls (non-diabetic, non-depressed subjects) were compared. Women were found to be more depressed in comparison to men. Glucose levels were observed to be more pronounced in subjects with depression than non-depressed subjects. Both
systolic and diastolic blood pressure values were observed to be higher in depressed subjects in comparison to non-depressed subjects however, these variables were significantly higher comparing non-depressed diabetics with controls. Mean Body Mass Index (BMI) was observed to be significantly higher in depressed than non-depressed subjects, which was higher when compared with controls. The number of sedentary individuals was found to be more in depressed subjects than non-depressed subjects however, this number further decreased in normal individuals. Low density lipoprotein (LDL) levels were found to be significantly dissimilar among all the three groups, whereas, total cholesterol (TC) and high density lipoprotein levels (HDL) were different between depressed and non-depressed subjects.

Gender wise analysis revealed that men and women differed significantly for age, glucose levels, systolic and diastolic blood pressures, smoking, alcohol drinking, TC, triglycerides (TG) and HDL. In the present study women were having higher Patient Health Questionnaire-9 score (PHQ-9) in comparison to men (12.77 vs. 10.58) hence, were observed to be more depressed.

In order to understand the effect of different variables for the risk of depression in diabetes as well as the individual risk of diabetes, odds ratios were calculated which exposed that age neither influenced depression nor diabetes however, being a woman was a risky proposition for depression. Other factors that influenced the risk of depression significantly were duration of diabetes (DOD) >5 years, systolic blood pressure (SBP) >120mmHg and diastolic blood pressure (DBP) >80mmHg, BMI within the range of 23-29.99 kg.m⁻² as well as BMI ≥30 kg.m⁻², sedentary life style, TC >200 mg/dl, LDL >100 mg/dl, TG >150 mg/dl and HDL <40 mg/dl. The factors that played significant role for the development of
diabetes were observed to be DBP >80mmHg, BMI ≥23 kg.m⁻², sedentary life style, TC >200mg/dl, LDL >100mg/dl, TG >150mg/dl and HDL < 40mg/dl.

Nonetheless, this investigation was done by univariate regression analysis whereby the effect of other variable may confound the outcome; therefore multivariable regression model (backward step wise) was performed to identify those variables which independently predict the risk of depression and diabetes.

It was revealed that sedentary life style was the strongest predictor which approximately conferred 4 fold higher risk of depression. Women subjects were at 1.3 times higher risk of being depressed than their counterparts. Other independent predictors were duration of diabetes >5 years, LDL >100mg/dl and TG >150mg/dl. Similarly independent predictors for diabetes were sedentary life style, LDL >100mg/dl and TG > 150mg/dl which almost doubled the risk of diabetes. The present study revealed 47.87 percent prevalence of depression in T2DM subjects of Punjab.

The relationship of advancing glucose levels with depression was examined by regressing depression scores of diabetic subjects upon glucose levels, which revealed that increasing glucose levels were significantly correlated with increasing depression scores. The statistically significant regression coefficient suggested that more the glucose levels more were the chances of getting depression.

For the accomplishment of first objective, the genetic contribution of SLC6A4 and DRD4 genes in depression was examined by polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP), which involved 3 SNPs of SLC6A4 gene and 4 SNPs of DRD4 gene. Allele frequencies were calculated from genotype numbers by gene counting method. Their departure
from Hardy Weinberg equilibrium was examined by chi-square analysis. It was observed that all the alleles of seven SNPs were in Hardy-Weinberg Equilibrium.

To recognize the genetic influence of diabetes on depression by the SNPs of SLC6A4 gene, genotypes and alleles were compared between diabetic subjects with depression and diabetic subjects without depression. Odds ratios revealed that T allele of rs2020936 added 1.52 times the risk of depression whereas, C allele was observed to be protective. Another SNP rs2020942 of SLC6A4 gene did not influence the risk of depression and diabetes whereas, T allele of rs3794808 conferred 1.74 folds higher risk of depression. T allele of rs2020936 and T allele of rs3794808 added risk of diabetes by 1.34 and 1.36 times respectively whereas, rs2020942 failed to show any effect on the risk of diabetes.

Genetic contribution of DRD4 gene for the risk of depression was analysed which revealed that the major alleles of rs3758653 and rs916455 and minor allele of rs747302 significantly influenced the risk of depression. However, SNP rs1800955 did not contribute to this risk. It was revealed that major alleles of rs3758653 and rs1800955 contributed considerably to the risk of diabetes whereas, rs747302 and rs916455 did not add any risk of diabetes in normal population.

In the genetic model analysis for the SNPs within SLC6A4 gene, the authority of T allele of rs2020936 for the risk of depression and participation of C allele for its protective effect from the chances of getting depression was evident in both dominant and recessive models. SNP rs2020942 did not show any correlation with risk of depression in this analysis. The exposition of C allele of rs3794808 for its protective effect was evident in both dominant and recessive model. When analysed for the risk of diabetes, it was revealed that T allele of rs2020936 and rs3794808 contributed to the risk of diabetes whereas, none of the alleles of rs2020942 influenced diabetes in normal population.
Minor alleles of rs3758653 and rs916455 of DRD4 gene showed their effect in recessive model for the risk of depression. Major allele of rs747302 influenced depression through recessive model whereas; no association of rs1800955 with depression was observed. However, none of the SNPs showed contribution for the risk of diabetes in any model, although major alleles of rs3758653 and rs1800955 submitted their participation in the risk of diabetes.

Haplotypes were generated from the genotype numbers of SNPs within SLC6A4 and DRD4 genes and their association for the risk of depression was analysed by calculating odds ratios by taking the most frequent haplotype as referent. Eight expected haplotypes were observed within SLC6A4 gene. Two haplotypes i.e CGC and CAC were having frequency less than five percent hence, excluded from the analysis. It was observed that TAT haplotype was a risky haplotype that increased the risk of depression by 2.19 times. Of expected sixteen haplotypes within 4 SNPs of DRD4, eleven were observed. Haplotypes TCCC and CGCC had lesser frequency than five percent therefore, not included in further analysis. It was revealed that TCTC haplotype emerged to be a susceptibility haplotype which imparted 4.15 times increased risk of depression in diabetic subjects.

Haplotype analysis for the risk of diabetes revealed that those subjects who carried TCTT haplotype of DRD4 gene were at 3.10 times increased risk of diabetes than other subjects. However, none of the haplotypes within SLC6A4 gene showed any association with diabetes.

For achieving second objective of the research proposal, Gene-Gene interaction analysis was done from the genotype data with the help of software epiSNP. Out of several SNP-SNP interactive effects only significant SNP-SNP
interactions for the risk of depression and diabetes were deduced. It was observed that interaction between SNP rs2020936 of SLC6A4 gene and SNP rs3758653 of DRD4 gene showed significant interactive effect for depression. Similar was the interaction between SNPs rs3794808 and rs747302. However, interactions between SNPs rs3794808 and rs3758653 exhibited dominance x dominance (DD) mode for their contribution in the risk of depression, whereas, rs3794808, while interacting with rs1800955 and rs916455 expressed significant risk of depression with additive x additive (AA) and DD effects respectively. SNP rs2020936 interacted with rs747302 and rs916455 expressing dominance x additive (DA) and interactive (I) form of effects for the risk of depression. Similarly, rs2020942 in concert with rs747302 and rs916455 confirmed their role for the risk of depression through I and DA interactive effects.

The interactions of SLC6A4 and DRD4 gene through SNPs rs3794808 and rs3758653 showed significant association for diabetes. However, rs3794808 in the presence of rs747302 and rs916455 of DRD4 gene showed risk of diabetes through I and DA mode of interaction. SNPs rs2020936 and rs916455 showed confirmation of their participation for the risk of diabetes; however, former exhibited AD effect in the presence of rs3758653 of DRD4 gene.

Gene × Environmental (G x E) interaction analysis involved significant quantitative variables and SNPs within SLC6A4 and DRD4 genes. Their interactive effect corresponds to M: overall marker effect, A: additive effect and D dominance effect. It was revealed that rs2020936 of SLC6A4 gene in the presence of duration of diabetes (DOD) >5years, BMI ≥30 kg/m² and TG >150mg/dl influenced risk of depression through marker, dominance and marker effect respectively. SNP rs2020942 interacted with TC >200mg/dl to cause additive effect. Another SNP rs3794808 of SLC6A4 gene interacted with BMI ≥30 kg/m², DOD >5 years, LDL
>100mg/dl, HDL <40mg/dl to exhibit risk of depression through marker, dominance, dominance and additive effect respectively. SNP rs3758653 of DRD4 gene expressed marker, additive, additive and dominance effect in the presence of DOD >5 years, BMI ≥ 30kg/m², TC >200mg/dl, LDL >100mg/dl respectively. Another SNP rs747302 collaborated with DOD >5 years, BMI ≥ 30 kg/m² and HDL<40mg/dl through dominance, marker and additive effect respectively. SNP rs1800955 was associated with higher levels of TC >200mg/dl through dominance effect. SNP rs916455 expressed dominance and additive effect when present along with LDL >100mg/dl and DOD >5 years respectively.

For the risk of diabetes rs2020936 showed dominance and additive effect with DOD >5 years and LDL >100mg/dl respectively, whereas SNP rs2020942 was observed to be associated with TC >200mg/dl and BMI ≥30 kg/m² through marker effect. Another SNP rs3794808 of SLC6A4 gene collaborated with TG >150mg/dl, LDL >100mg/dl and TC >200mg/dl which exhibited the risk of diabetes through dominance, additive and additive mode respectively. G × E interaction analysis of the SNPs within DRD4 gene demonstrated the interaction between SNP rs3758653 with LDL >100mg/dl, TG >150mg/dl and HDL <40mg/dl, rs747302 with BMI ≥30kg/m², TC >200mg/dl and LDL >100mg/dl whereas, rs916455 correlated significantly with DOD >5 years by additive effect for the risk of diabetes.

In order to complete third objective, SNPplot software was used to investigate epistatic effects of 3 SNPs of SLC6A4 gene and 4 SNPs of DRD4 gene. Bonferroni correction was applied to the significance level because of multiple comparions. It was revealed that two way epistatic effects existed between rs2020936 of SLC6A4 gene and rs747302 of DRD4 gene which worsened the risk of depression among diabetic subjects. The other epistatic effects observed were between SNPs rs3794808 and rs1800955 and between SNPs rs2020942 and rs747302.
For the investigation of those SNPs which impinged upon other SNPs for increasing the risk of diabetes, SNP-SNP interaction analysis revealed that SNPs rs3794808 and rs3758653 had epistatic effects which supplemented considerable risk of developing diabetes. Both SNPs rs3758653 and rs916455 exhibited two way epistatic effects for the risk of diabetes in the population of Punjab.