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ORIGINAL ARTICLE

Association of C(-1019)G with Risk of Development of Major Depression in Diabetics with Respect to Various Pakistani Ethnicities

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ABSTRACT

Objective: To determine whether the ethnicity is associated with the risk of development of Major Depressive Disorder (MDD) in adult diabetic population with respect to the genetic association with single nucleotide polymorphism (SNP) C(-1019)G.

Material and Methods: A total of 400 subjects were included in the study, out of which 200 were cases and 200 were age, gender and ethnicity matched healthy controls. Out of 200 cases , 100 cases had diabetes mellitus (DM) only and 100 cases had DM with MDD. Both males and females were included in the study having ages 25 years and above. Cases of both type I and type II DM were included in the study. Blood samples were collected and DNA was extracted by Chelax method. Real-time PCR was carried out to determine respective allelic frequencies of C(-1019)G genotype using TaqMan SNP genotyping assays and master mix.

Results: According to our results no significant association was found between C(-1019)G genotype and risk of development of MDD in Pakistani diabetic population with respect to Ethnicity but diseased genotype was found more in Pathan population.

Conclusion: There is no significant association of SNP C(-1019)G of 5-Hydroxytryptamine 1A receptor gene with MDD in Pakistani diabetics with respect to ethnicity.

Keywords: Diabetes mellitus, major depressive disorder, 5-hydroxytryptamine 1A receptor gene, single nucleotide polymorphism, polymerase chain reaction.

INTRODUCTION

Diabetes Mellitus (DM) has a major burden on world's morbidity and mortality rates (1–3). According to International Diabetic Federation (IDF), in the year 2019 diabetes caused 4.2 million deaths worldwide (4,5). The proportion of people with diabetes is increasing in most countries (1,4,5). Approximately 463 million adults (20 to 79 years) were living with diabetes in the year 2019 and this number is expected to increase to 700 million till the year 2045 according to IDF (4,5).

There is some evidence that prevalence of complications of diabetes such as nephropathy, neuropathy, retinopathy etc. is found to be more in diabetics having depression as compared to those individuals without these conditions (6). In addition, depression has been associated with poor compliance and adherence to treatment like diet and exercise regimes and poor glycaemic control resulting in constant hyperglycaemia and elevated HbA1c levels (7–9). Such patients also have poor adherence to selfcare attitude (10).

According to Diagnostic and Statistical Manual of Mental Disorders edition 5 (DSM-5) published by the American Psychiatric Association, there are now 8 specific disorders described under the heading of depressive disorders with major depressive disorder (MDD) or major depression being one of them (11). Clinical depression, which is the most severe form of MDD is 2 times more prevalent in diabetic patients as compared to non-diabetics (12–15).

Serotonin also called 5-Hydroxytryptamine (5-HT), is a neurotransmitter mainly involved in the pathophysiology of MDD (16–18). This neurotransmitter is produced by raphe nuclei (found in brainstem) and enterochromaffin cells (19–21).

The serotonin receptors or 5-Hydorxytrimptamine Receptors (5HTRs) have 14 subtypes out of which 5 subtypes are of significance (22,23). These include 5HT1A , 5HT1B , 5HT4 , 5HT6 and 5HT7 (22).

Gene polymorphism refers to multiple forms of a single gene (24,25). Single nucleotide polymorphism (SNP) is a single nucleotide substitution in a genome that helps to determine association between gene variants and different diseases (26).

A single nucleotide polymorphism C(-1019)G (rs6295) exist in the promotor region of the gene encoding 5-HT1A Receptors on chromosome number 5 (24,27). This SNP is a known causative agent of MDD.

MATERIAL AND METHODS

The study was done at the Department of Biochemistry, Islamic International Medical College Rawalpindi in collaboration with Railway General Hospital Rawalpindi, Armed Forces Institute of Pathology (AFIP), Rawalpindi and Institute of Biomedical and Genetic Engineering (IB&GE), Islamabad. It was a case control study. The research protocol was approved from the Ethics Review Committee of Islamic International Medical College, Rawalpindi. Duration of study was 1 year from September 2020 to September 2021.

In our current we have taken 200 cases (100 males and 100 females) and 200 controls (100 males and 100 females). The cases were further divided into two groups. Group 1 consisted of cases of both type 1 and type 2 DM and group 2 consisted of cases of both type 1 and type 2 DM with MDD. All the subjects were adults having ages 25 years and above. Cases of all other chronic illness and other metabolic disorders were excluded. Blood samples were collected after taking informed consent from all the subjects, following the standard techniques and then stored in EDTA bottles at 6 to 8 °C. The sampling technique was nonprobability convenient sampling. DNA was then extracted from whole blood using Chelax method and was stored at -80°C until PCR amplification. PCR was performed using original TaqMan assay (Catalogue # 4351379) and master mix (Catalogue # 4371353) using real time PCR following the instructions of the manufacturer.

Statistical analysis was carried out using commercial statistical software package, SPSS 26 software for Microsoft Windows. Possible association of SNP rs6295 with MDD in Pakistani diabetics was determined by computing odds ratio (OR) and 95% confidence intervals (CIs). Frequencies and percentages were determined for descriptive statistics. p-value less than 0.05 was considered to indicate a statistically significant difference.

RESULT

There were 200 cases and 200 healthy age, sex and ethnicity matched controls. Out of 200 cases, 100 cases had DM and 100

cases had DM with MDD. In Punjabis, among 100 cases of DM 20 (5.00%) subjects had CC genotype, 39 (9.75%) subjects had CG genotype and 10 (2.50%) subjects of only DM had GG genotype. Among 100 cases of DM with MDD 29 (7.25%) had CC genotype, 33 (8.25%) had CG genotype and 7 (1.75%) had GG genotype. In Controls, out of 200 subjects among Punjabis 43 (10.75%) subjects had CC genotype, 61 (15.25%) subjects had CG genotype and 20 (5.00%) subjects had GG Genotype.

In Pathans, among 100 cases of DM 6 (1.50%) subjects had CC genotype, 11 (2.75%) subjects had CG genotype and 7 (1.75%) subjects of only DM had GG genotype. Among 100 cases of DM with MDD 8 (2.00%) had CC genotype, 4 (1.00%) had CG genotype and 5 (1.25%) had GG genotype. In Controls, out of 200 subjects among Pathans 13 (3.25%) subjects had CC genotype, 28 (7.00%) subjects had CG genotype and 9 (2.25%) subjects had GG Genotype.

In Kashmiris, among 100 cases of DM 2 (0.50%) subjects had CC genotype, 5 (1.25%) subjects had CG genotype and none had GG genotype. Among 100 cases of DM with MDD none had CC genotype, 12 (3.00%) had CG genotype and 2 (0.50%) had GG genotype. In Controls, out of 200 subjects among Kashmiris 7 (1.75%) subjects had CC genotype, 14 (3.50%) subjects had CG genotype and 2 (0.50%) subjects had GG Genotype.

No significant association was found between any ethnic group included in our study i.e. Punjabi, Pathan and Kashmiri and C(-1019)G rs6295 genotype with the risk of development of MDD in diabetics. However diseased genotype I.e. GG was found to be more in Pathans.

Table 1: Association of 5-HT1A receptor gene SNP C(-1019)G rs6295 Genotype with ethnicity of cases and control

Parameter	N=400			OR (95% CI) P
	Cases n=200		Controls n=200	
Genotype in Ethnicity	DM 100	DM with MDD 100		
Punjabi CC CG GG	20(5.00%)* 39(9.75%) 10(2.50%)	29(7.25%) 33(8.25%) 7(1.75%)	43(10.75%) 61(15.25%) 20(5.00%)	Ref I 1.71 (0.82-3.57) 0.15[a] 1.21 (0.41-3.53) 0.73[a] Ref I 1.37 (0.71-2.67) 0.35[b] 0.78 (0.33-1.85) 0.57[b] Ref I 0.80 (0.43-1.51) 0.49[c] 0.65 (0.25-1.69) 0.37[c]
Pathan CC CG GG	6(1.50%) 11(2.75%) 7(1.75%)	8(2.00%) 4(1.00%) 5(1.25%)	13(3.25%) 28(7.00%) 9(2.25%)	Ref I 3.67 (0.77-17.43) 0.10[a] 0.51 (0.10-2.57) -[a] Ref I 0.85 (0.26-2.81) 0.79[b] 1.98 (0.59-6.63) 0.26[b] Ref I 0.23 (0.06-0.91) -[c] 3.89 (0.86-17.68) -[c]
Kashmiri CC CG GG	2(0.50%) 5(1.25%) 0(0.00%)	0(0.00%) 12(3.00%) 2(0.50%)	7(1.75%) 14(3.50%) 5(1.25%)	Ref I Ref I 1.25 (0.19-8.14) -[b] Ref I 0.47 (0.08-2.86) -[c]

[a] Association of genotype of group DM and group DM with MDD
[b] Association of genotype of group DM and Controls
[c] Association of genotype of group DM with MDD and Controls
Given percentages are out of total sample i.e. N=400

Table 2: Percentages of GG genotype with ethnicity in the study population

Parameter	N=400			Sub-Total	%GG
	Cases n=200		Controls n=200		
Genotype in Ethnicity	DM 100	DM with MDD 100			
Punjabi CC CG GG	20 39 10	29 33 7	43 61 20	92 133 37	$\frac{37}{262}\times 100$
Sub-Total	69	69	124	Total = 262	14.12%
Pathan CC CG GG	6 11 7	8 4 5	13 28 9	27 43 21	$\frac{21}{91}\times 100$
Sub-Total	24	17	50	Total = 91	23.08%
Kashmiri CC CG GG	2 5 0	0 12 2	7 14 5	9 31 7	$\frac{7}{47}\times 100$
Sub-Total	7	14	26	Total = 47	14.89%

DISCUSSION

Diabetes mellitus and major depressive disorder are two chronic conditions which share common pathological pathways. Certain inflammatory components and hyperactivation of the hypothalamic pituitary adrenal excess are known to cause both diabetes and depression. The presence of diabetes mellitus can ultimately result in major depressive disorder if diabetes remains uncontrol for a long period of time. In this way MDD is a chronic complication of uncontrolled diabetes mellitus. Likewise, MDD through the same pathological pathways which are neuroinflammatory in origin, causes diabetes mellitus if MDD remains undiagnosed and untreated

Genetic studies in this context provide a strong evidence for such conditions. Single nucleotide polymorphism of 5-Hydroxytryptamine 1A receptor gene C(-1019)G rs6295 exist in the promoter region of this gene on chromosome number 5 and is a known cause of MDD.

Different ethnicities have different genetic makeup. If genetically significant association is found with a specific ethnicity this means that the specific ethnicity is at a higher risk of developing that specific disease.

To the best of our knowledge this is the first study on any diabetic population in context of this SNP. Therefore, no data for any population is available for association of C(-1019)G genotype and risk of development of MDD in diabetics. However, studies have been conducted on association of this SNP with other diseases and in case of genetic studies such associations also provide clues. S. Vincentiis et al. conducted research on association of C(-1019)G with depression in patients of temporal lobe epilepsy caused by hippocampal sclerosis in Brazilian population found that there was no difference between the group with temporal lobe epilepsy with MDD and the control group regarding gender and ethnicity (28).

No significant association was found between any of the ethnic groups included in our study (I.e. Punjabi, Pathan and Kashmiri) and C(-1019)G genotype with the risk of development of MDD. However diseased genotype was found to be more in Pathans which means that they are more likely to develop the disease. In case of genetic studies not finding a significant association is also important.

REFERENCES

1. Hernández ER. Biomarkers and Depression Associated to Type 2 Diabetes A Pilot Study. Biomed J Sci Tech Res [Internet]. 2019 Jan 25;13(4). Available from: https://biomedres.us/fulltexts/BJSTR.MS.ID.002437.php
2. Gong Q, Zhang P, Wang J, Ma J, An Y, Chen Y, et al. Morbidity and mortality after lifestyle intervention for people with impaired glucose tolerance: 30-year results of the Da Qing Diabetes Prevention Outcome Study. lancet Diabetes Endocrinol. 2019;7(6):452–61.
3. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of diabetes 2017. Hindawi; 2018.
4. Duke, L., Fereira de Moura, A., Silvia Gorban de Lapertosa, S., Lucy Hammond L., Jacobs E., Abha Kaundal, A., ... Rivas Gonzalez M. IDF Diabetes Atlas 9th edition 2019. International Diabetes Federation Diabetes Atlas, Ninth Edition. 2019.
5. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2019;157:107843.
6. Lin EHB, Heckbert SR, Rutter CM, Katon WJ, Ciechanowski P, Ludman EJ, et al. Depression and Increased Mortality in Diabetes: Unexpected Causes of Death. Ann Fam Med [Internet]. 2009 Sep 1;7(5):414–21. Available from: http://www.annfammed.org/cgi/doi/10.1370/afm.998
7. Roy MS, Roy A, Affouf M. Depression is a Risk Factor for Poor Glycemic Control and Retinopathy in African-Americans With Type 1 Diabetes. Psychosom Med [Internet]. 2007 Jul;69(6):537–42. Available from: http://journals.lww.com/00006842-200707000-00009
8. Van Tilburg MAL, McCaskill CC, Lane JD, Edwards CL, Bethel A, Feinglos MN, et al. Depressed Mood Is a Factor in Glycemic Control in Type 1 Diabetes. Psychosom Med [Internet]. 2001 Jul;63(4):551–5. Available from: http://journals.lww.com/00006842-200107000-00005
9. Lustman PJ, Griffith LS, Freedland KE, Clouse RE. The course of major depression in diabetes. Gen Hosp Psychiatry [Internet]. 1997 Mar;19(2):138–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0163834396001703
10. Gonzalez JS, Peyrot M, McCarl LA, Collins EM, Serpa L, Mimiaga MJ, et al. Depression and Diabetes Treatment Nonadherence: A Meta-Analysis. Diabetes Care [Internet]. 2008 Dec 1;31(12):2398–403. Available from: http://care.diabetesjournals.org/cgi/doi/10.2337/dc08-1341
11. American Psychiatric Association. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders Fifth Edition. Arlington. 2013.
12. Ali S, Stone MA, Peters JL, Davies MJ, Khunti K. The prevalence of co-morbid depression in adults with Type 2 diabetes: A systematic review and meta-analysis. Diabet Med [Internet]. 2006;23(11):1165–73. Available from: https://pubmed.ncbi.nlm.nih.gov/17054590/
13. Darwish L, Beroncal E, Sison MV, Swardfager W. Depression in people with type 2 diabetes: current perspectives. Diabetes, Metab Syndr Obes targets Ther. 2018;11:333.
14. Sharif S, Raza MT, Mushtaq S, Afreen B, Hashmi BA, Ali MH. Frequency of depression in patients with type 2 diabetes mellitus and its relationship with glycemic control and diabetic microvascular complications. Cureus. 2019;11(7).
15. Sartorius N. Depression and diabetes. Dialogues Clin Neurosci. 2018;20(1):47.
16. Yohn CN, Gergues MM, Samuels BA. The role of 5-HT receptors in depression. Mol Brain [Internet]. 2017 Dec 24;10(1):28. Available from: https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-017-0306-y
17. Kraus C, Castrén E, Kasper S, Lanzenberger R. Serotonin and neuroplasticity–links between molecular, functional and structural pathophysiology in depression. Neurosci Biobehav Rev. 2017;77:317–26.
18. Żmudzka E, Sałaciak K, Sapa J, Pytka K. Serotonin receptors in depression and anxiety: Insights from animal studies. Life Sci. 2018;210:106–24.
19. Nichols CD. Serotonin. In: Encyclopedia of the Neurological Sciences. 2014.
20. Jones LA, Sun EW, Martin AM, Keating DJ. The ever-changing roles of serotonin. Int J Biochem Cell Biol. 2020;125:105776.
21. Beecher K, Belmer A, Bartlett SE. Anatomy of the serotonin transporter. In: Serotonin. Elsevier; 2019. p. 121–33.
22. Pitsillou E, Bresnehan SM, Kagarakis EA, Wijoyo SJ, Liang J, Hung A, et al. The cellular and molecular basis of major depressive disorder: towards a unified model for understanding clinical depression. Mol Biol Rep. 2020 Jan;47(1):753–70.
23. Pourhamzeh M, Moravej FG, Arabi M, Shahriari E, Mehrabi S, Ward R, et al. The Roles of Serotonin in Neuropsychiatric Disorders. Cell Mol Neurobiol. 2021;1–22.
24. Han D, Qiao Z, Qi D, Yang J, Yang X, Ma J, et al. Epistatic Interaction Between 5-HT1A and Vascular Endothelial Growth Factor Gene Polymorphisms in the Northern Chinese Han Population With Major Depressive Disorder [Internet]. Vol. 10, Frontiers in Psychiatry . 2019. p. 218. Available from: https://www.frontiersin.org/article/10.3389/fpsyt.2019.00218
25. Parris MS, Grunebaum MF, Galfalvy HC, Andronikashvili A, Burke AK, Yin H, et al. Attempted suicide and oxytocin-related gene polymorphisms. J Affect Disord. 2018;238:62–8.
26. Scutt G, Overall A, Scott R, Patel B, Hachoumi L, Yeoman M, et al. Does the 5-HT1A rs6295 polymorphism influence the safety and efficacy of citalopram therapy in the oldest old? Ther Adv Drug Saf [Internet]. 2018 Apr 23;9(7):355–66. Available from: https://doi.org/10.1177/2042098618770620
27. Li H, Gao Y, Zou Y, Qiao S, Zhi W, Xu X, et al. Associations Between a rat 5-HT1A Receptor Gene Promoter Region Polymorphism (rs198585630) and Cognitive Alterations Induced by Microwave Exposure. 2021;
28. Vincentiis S, Alcantara JA, Rzezak P, Kerr DS, Gattaz WF, van der Linden Jr H, et al. Genetic polymorphisms of the serotonin transporter are not related with depression in temporal lobe epilepsy caused by hippocampal sclerosis. Epilepsy Behav. 2021;117:107854.