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Original Article

Psychiatry/Mental Health Section

Tryptophan Hydroxylase 2 Gene Polymorphism in Anxiety and Depressive Disorder in Kashmiri Population

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ABSTRACT

Background: The gene of tryptophan hydroxylase is widely recognized as a major candidate gene in many psychiatric disorders. However, no study has been done which investigates tryptophan hydroxylase 2 gene polymorphism in anxiety and depressive disorders in Kashmiri population (India).

Objectives: To study tryptophan hydroxylase 2 (TPH2) C 11993 A gene polymorphism in anxiety and depressive disorders.

Method: Sixty patients of depression disorder, 60 patients of anxiety disorder and 40 unrelated healthy volunteers (control) were studied in a case control design. Polymorphism was determined using polymerase chain reaction (PCR) and agarose gel electrophoresis after digestion with HAP II enzyme. Genotypes and allele frequencies were compared using Chi-square tests, Fischer's exact test, odds ratio, 95% confidence interval (C.I) and p-value of <0.05 was considered to be statistical significant.

Results: The mean age ± SD of anxiety, depression and control group was 32.73±10.99, 32.20±10 and 29.75±10.12 respectively and the difference was found to be statistically non significant (p=0.349).The mean HAM-A (Hamilton rating scale for anxiety) score and HAM-D (Hamilton rating scale for depression) score was high in both groups (anxiety and depression) and found to be statistically significant (p=0.001).Depression group had AA genotype (55.2%) than control (37.5%) and was found to be statistically non significant (p=0.890).Comparison of allelic frequency revealed no association of A allele in anxiety group (76.67%) compared with control (75.5%) and was found to be statistically non significant (p= 0.866), OR 1.09 (0.56-2.11).

Conclusion: TPH2C 11993 A gene was not found to be associated with major depressive disorder (MDD) and anxiety disorder in Kashmiri population.

Keywords: HAM-A, HAM-D, Psychiatric disorders

INTRODUCTION

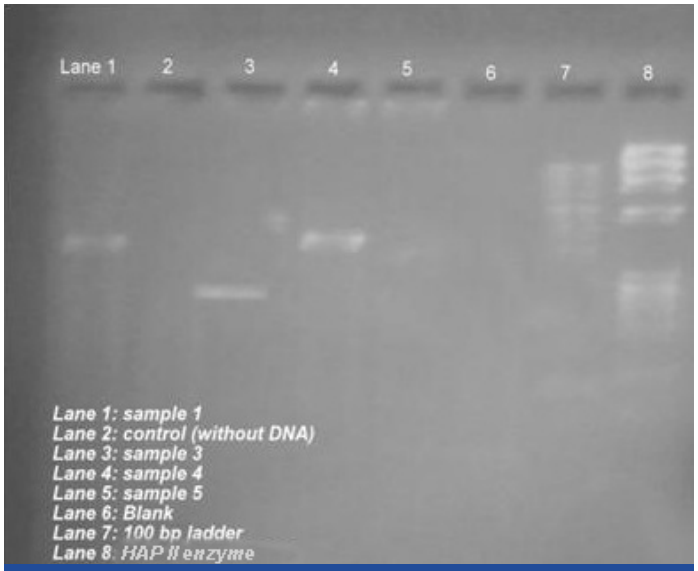
The current concept of mental disorders centres around the view that they arise out of interaction between an individual's genetic endowment and environmental factors. A variety of genetic, biological, psychological and social factors are postulated to be causal factors, leading to the emergence of anxiety and depressive disorders [1]. Depression is characterized by persistence and pervasive low mood with loss of energy and interest, and various other signs and symptoms (change in apatite, change in sleep and thoughts of death and suicide) for a period of at least two weeks [1]. Depression is one of the most widely prevalent disorder with average lifetime prevalence of 12% [1]. It has been observed that 12.6% of people above 18 suffer from anxiety disorders in any one year [2]. Anxiety disorders are characterized by sustained feeling of impending threat along with disturbed autonomic functions and associated cognitive disturbances. Panic disorder, Obsessive Compulsive Disorder, Phobic anxiety disorder, Generalized Anxiety Disorders and Post Traumatic Stress Disorder are generally considered as anxiety disorders [2]. Serotonergic drugs like lysergic acid diethylamide (LSD) and methylene dioxy-N-methy amphetamine (MDMA) were found to be associated with the development of acute and chronic anxiety disorders. It was found that patients with panic disorders had lower levels of circulating serotonin compared with controls in one study [3]. The gene of tryptophan hydroxylase, the rate limiting enzyme in the synthesis of serotonin, is widely recognized as a major candidate gene in many psychiatric disorders [4-6].Recently a new isoform of the enzyme (TPH2) has been identified.TPH2 is predominantly expressed in the brain stem, where the serotonergic raphe nuclei are located [4-7]. Therefore we planned to study the role of TPH2 gene in anxiety and depressive disorders in our Kashmiri population (India).

MATERIALS AND METHODS

The study was conducted in Postgraduate Department of Psychiatry, Government Medical college, Srinagar, Kashmir (India), which is the lone tertiary psychiatric hospital in Kashmir and caters to the majority of psychiatric patients of Kashmir. A total of 160 unrelated individuals (60 with unipolar major depression, 60 with anxiety disorder, and 40 healthy volunteers) were enrolled in the study from March 2012 to April 2013 in Postgraduate Department of Psychiatry, Government Medical college, Srinagar, Kashmir (India). 40 unrelated healthy volunteers belonging to the same state were selected (from Kashmiri university) after excluding mental disorders by comprehensive clinical interview by two experienced psychiatrists. The diagnoses of major depression and anxiety disorder were made based on Diagnostic and Statistical Manual of Mental Disorder (DSM IV TR) criteria [8]. Bipolarity was excluded on the basis of compete history taking and detailed mental status examination. Diagnoses were confirmed by two consultant psychiatrists independently. The inclusion criteria included persons suffering from depressive disorders and anxiety disorders, patients above 18 years of age and patients willing to participate in the study, by means of informed consent. The exclusion criteria included persons below 18 years of age, all depressive disorders and anxiety disorders due to general medical conditions and due to psycho-active substances use and exclusion was done before selection of patients. Data obtained were meticulously recorded on a specially designed proforma. Observer rating scales like Hamilton anxiety rating scale (HAM-A) and Hamilton rating scale for depression (HAM-D) were administered to anxiety and depression patients. HAM-A is a 14 item scale, used to assess severity of anxiety and HAM-D is a 17 item scale, used to assess severity of depression. These scales have good validity and have been used previously [9, 10].

DNA Extraction: For genotyping gene, DNA was extracted from a portion of whole blood, using GENEI Genomic Extraction Kit, supplied by the Messers Bangalore Genei, India.

Determination of TPH2 C 1193 A gene polymorphism: Polymerase chain reaction was carried out with primer. (5 - ATGTGTGAAAGCCTTTGACCCAAAG ACA) and Reverse (5 - TGCGTTATATGACATTGACTGAACT GC) and with specific protocols [11]. PCR amplified fragments were digested with Hap II restrictive endonuclease and were analysed by electrophoresis on 1.5% Polyacramide gel. Sequencing was done subsequently, using an automatic sequencer – Three genotypes CC-AC-AA were observed [Table/Fig-1]. PCR amplification of TPH2 gene.



[Table/Fig-1]: PCR amplification of TPH2 gene

STATISTICAL ANALYSIS

Genotype and allele frequencies were compared using Chi-square test, Fischer exact test, odds ratio, 95% confidence interval (C.I) and p-value of < 0.05 was considered to be significant. The quantitative data was analysed by one way analysis of variance (ANOVA) and two sample independent t-tests.

RESULTS

The mean age ± SD of anxiety, depression and control group was 32.73±10.99, 32.20±10 and 29.75±10.12 respectively and was found to be statistically non-significant (p= 0.349). The mean HAM-A in anxiety group was 28.2 and was found to be statistically higher (p =0.001) than depressive group having mean HAM-A of 16.62. However mean HAM-D of anxiety group was 15 and found to be significantly lower (p=0.001) than depressive group, with mean value of 25.1 [Table/Fig-2].

	Anxiety n=60	Depression n=60	Control n=40	p-value
Age (Mean ± SD)	32.73± 10.99	32.20 ± 10	29.75 ± 10.12	0.349
Male	35(58.33)	36 (60)	25(62.5)	
Female	30(41.66)	24(40)	15(37.5)	
HAM-A	28.20±5.14	16.62±5.67	-	≤0.0001*
HAM-D	15.13±6.11	25.17±5.66	-	≤0.0001*

* Significant at p < 0.05. Values within parenthesis are percentages

[Table/Fig-2]: Mean age and HAM-A and HAM-D in studied groups

Depression group (n=60)	
Depression severe	38 (63.33)
Depression severe	38 (63.33)
Anxiety group (n=60)	
Generalised anxiety disorder	11 (18.33)
Panic disorder	35(58.33)
Posttraumatic stress disorder	7(11.67)
Obsessive compulsive disorder	7(11.67)
Values within parenthesis are percentages	

[Table/Fig-4]: Showing subgroups in depressive and anxiety groups

The three groups of patients were compared with respect to genotypic and allelic frequency [Table/Fig-3]. It was found that patients suffering from anxiety group had AA genotype (63.3%) than control (57.5 %) and was found to be statistically non-significant (p=0.652).Similarly depression group had AA genotype (55.2 %) than control (57.5%) and was found to be statistically non-significant (p=0.890), which implies that there is no association between AA genotype and type of disorder. (Anxiety and Depression group). There was also no significant difference between depression and anxiety group with regard to both genotype distribution. p=0.630) and allele frequency (p=0.460) OR =1.25 (0.69 to 2.25). Comparison of allelic frequency revealed no association of A allele in anxiety group (76.67%), compared with control (75.5%) and was found to be statistically non-significant (p= 0.866, OR 1.09 (0.56-2.11). There was also no association of A allele frequency in depression group (72.41%) compared to control (75.55%) (p=0.460, OR=1.25 (0.69 to 2.25) [Table/Fig-3].

Severe depression (63.33%) and mild-moderate depression (36.67%) were two subgroups in depression group. In anxiety group, panic disorder (58.33%) and generalized anxiety disorder (18.33%) constituted major diagnosis [Table/Fig-4].

DISCUSSION

5HTTLPR (5-hydroxytryptamine-transporter linked promoter region) polymorphism is the most widely studied genetic variant in psychiatry and the discovery of a central nervous system (CNS) specific tryptophan hydroxylase added further impetus to the study of the serotonin system in various psychiatric disorders [11]. The study was conducted in Kashmir, which is a distressed society, due to continuous violence witnessed by the local population and susceptibility towards anxiety and depressive disorders is increasing day by day [12]. Our findings suggested that TPH 2 gene was not found associated with major depressive disorder. Nazree et al., recently reported lack of association between TPH2 gene polymorphism with major depressive disorder in three ethnic groups of Indian, Chinese and Malaysian population [13]. Similar findings have been reported by Garriock et al., who also observed lack of association of TPH2 polymorphisms with major depression and treatment resistance [14]. However our findings are in contrary to Tsai SJ et al., who recently observed that TPH2 genetic variants may play a role in MDD susceptibility [15]. Shen X et al., also reported that

Genotypic Frequency						Allelic frequency			
Group	AA	AC	CC	Chi-square test	p- value	A	C	p- value	OR (95% CI)
Anxiety	38 (63.3)	16(26.7)	6(10)	0.856	0.652[a]	92(76.67)	28(23.33)	0.866[a]	1.09(0.56-2.11)[a*]
Depressive	32(55.2)	20(34.5)	6(10.3)	0.925	0.630[b]	84(72.41)	32(27.59)	0.460[b]	1.25(0.69-2.25)[b]
Control	23(57.5)	14(35)	3 (7.5)	0.233	0.890[c]	60(75)	20(25)	0.744[c]	0.87(0.45-1.67)[c*]

* Significant at p < 0.05.Values within parenthesis are percentages
a : Anxiety group vs Control group, b: Depression group vs Anxiety group, c: Depression group vs Control group

[Table/Fig-3]: Genotypic and allelic frequency of TPH2 gene in the studied group

TPH2 gene may have a gender dependent effect on susceptibility to major depressive disorder in Chinese Han population and observed that TPH2 polymorphisms was associated with major depressive disorder [16]. Similar findings have been reported by Zill et al., [17]. Some authors suggest that this inconsistency might be due to the fact that ethnic variation might play a role in this regard [18]. It appears that no investigator has studied C 1193A TPH2 polymorphism in anxiety disorder so far. The TPH2 polymorphism genotype and allele frequency was found to be statically non-significant in case of anxiety disorder. Lin YM et al., and Youn JS reported association of anxiety with TPH2 polymorphism [6, 19]. Similar findings have been reported earlier also [20, 21]. But all these have studied different polymorphism of TPH2, so basically there are no replicative studies to derive any valid conclusion.

Though there was no deliberate effort to match the control and patient groups for age, both of them were found to be matched for age. Barring social anxiety disorder, anxiety disorders are known to be more common in women. Depression is also known to be more common in women [1]. However in the present study gender distribution in anxiety and depressive groups of patients was found to be same as that of controls.

The mean HAM-A scores for anxiety and depressive group of patients was 28.2 and 17 respectively. Difference in the mean scores was found to be statistically significant (p = 0.001). But 24 out of 60 (40%) depressed patients had HAM-A scores in mild to moderate range (18 to 30) of anxiety. A score of 18 and above is considered as mild anxiety (Ham-A) [10]. Though the difference is highly significant, the fact that a substantial number (40%) of depressive patients had at least mild levels of anxiety, lends credence to the contention that depression and anxiety are two facets of same disorder. This finding of our supports the view of Sun et al., [22] and K Himmelhoch et al., [23], who stated that depression and anxiety are two faces of the same disorder. This argument can be further advanced by the fact that 53% of anxiety patients in this study had moderate to severe levels of depression and an additional 40% had mild levels of depression. It appears the phenotype anxiety cannot be readily distinguished from phenotype depression.

CONCLUSION

Our preliminary study suggests that TPH 2 gene was not found associated with MDD and anxiety disorder in Kashmiri population. There were high HAM-A and HAM-D scores in anxiety and depression patients.

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