Single Nucleotide Polymorphism rs10501087 in Brain Derived Neurotrophic Factor gene role in the pathophysiology of Major Depressive Disorder in Iraqis

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Abstract

Objective: To determine the possible genetic polymorphism rs10501087 genetic variants of BDNF gene and its relationship with major depressive disorder in Baghdad city.Method: Case control study, that the blood samples had taken from 120 participants were collected from 60 patients diagnosed as MDD according to DSM-V criteria by psychiatrist and 60 normal controls not suffer from any mood disorders and all participants had did not undergo any chronic disease. The collected blood divided into two samples, one for ELISA to estimate BDNF concentration and the other one to PCR for genotyping study.Results: Using statistical analysis for the evaluation the serum levels of BDNF and genetic variation, the levels of BDNF concentrations in depressed patients were estimated and there were significantly lower than healthy control group which was 729.12 pg.ml-1 in patients with depression compared with healthy control group which was 1169.5 pg.ml-1 p value ($p<0.005$).The results of HRM for rs10501087 in patients and healthy control groups showed that 14 (23%) of patients had wild type genotype (T/T), 13 (22%) of patients had the mutant variant (C/C), and 33 (55%) of patients had the heterozygous genotype (T/C). On the other hand, the screened controls, 26 (43%) were genotyped as T/T, 13 (22%) were genotyped as C/C and 21 (35%) were genotyped as T/C. Moreover, odd ratio for TT and CC genotypes in the studied groups, were 1.0 and 0.1857 respectively while for the heterozygous genotype it was 2.918, which motioned that T>C polymorphism was a risk factor for incidence of MDDConclusion: Brain Derived Neurotrophic Factor protein that estimated by ELISA method was decreasing significantly in depressive patients when compared with healthy control group. In other hand, there were significant differences ($P\leq0.05$) between heterozygous T/C in BDNF gene SNP 10501087 variation in patients with MDD and healthy control groups P value (0.0123)

1. Introduction

Major Depressive disease (MDD) is a mental illness also called clinical depression which disease affects sensations and thoughts, it caused from genetic and environmental factors. Depression is a disease causing the second leading cause of disability in the world, ranging in severity from mild to severe [1]. It is characterized not only by a depressed mood or sadness, also loss of interest by a combination of physical, cognitive, motivational factors, increasing or decreasing in eating and sleep habits and may suicidal behavior and suicidal thoughts or thinking in ending life, lack the ability to enjoyment, low energy and mood, feelings of guilty and worthlessness [2].
There is powerful relation between BDNF SNPs and affective disorders may include MDD. Some studies are focused on the findings obtained from rs10501087 polymorphisms and mental illnesses. Other studies approve impact relation of the BDNF SNP rs10501087 on antidepressant response and resistant MDD for treatment [3]. Suicidal behavior refers to the occurrence of suicide attempts that range from suicide death, to highly lethal but failed in suicide attempts, to suicide attempts of low lethality. It is strongly linked with psychiatric well as rs10501087 showed significant genotypic and haplotypic association with suicide risk. Considering the sample size, the present findings need to be replicated in larger samples to confirm or refute a role of BDNF in the investigated suicidal behavior phenotypes, Disorder in particular mood and substance abuse disorder. The rs10501087 SNP as well as rs6265 SNP, in analyzing the response the medicinal treatment demonstrated a significant genotyping and haplo-typing correlation to psychiatric major problems especially suicidal behavior or suicidal risk [4].

Methodology

Study design

Blood samples from 120 participants, 60 from depressive patients diagnosed according to DSM-V criteria and 60 normal healthy control person not undergo from any psychiatric illnesses

Study settings

The study was carried out in Baghdad provinces in Iraq (for Iraqis only), from various referral hospitals (Al-Imamain Al-Kadhmain Medical City and Ibn-Rushud Psychiatric Training Hospital), the study started from May 2020 t0 February 2022 (two years duration).

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Inclusion criteria

1- Patients both males and females with Major Depressive Disorder aged between 18 to 35 years old.
2- Normal persons both males and females aged from 18 to 35 years.
3- Both age and sex matched for cases and controls.

Exclusion criteria

1- Any physical Diseases.
2- Any Mental Disorder including Alcoholism's and other substances Abuser.
3- Pregnancy.
4- Patients taking psychotropic medications.

Two tubes to divide the blood samples of participants into whole blood samples in EDTA tubes produced a Buffy coat, and gel tubes for serums to estimates the levels of BDNF concentrations by ELISA approach.

The first divided sample was then used to extract the participants nucleic acid to identify their genetic rs6265 SNP variations for molecular genotyping analysis, 2 to 2.5 milliliters of venous whole blood were drawn from each subject and placed in EDTA anticoagulant tubes and stores at 2-8°C for PCR later. The second divided sample also 2 to 2.5 milliliters to separate the plasma for biochemical analysis was spun at 10,000 rpm for five minutes to extract serum that stored under -20 to -80 °C in Eppendorf tubes. By using the ELISA method, biochemical studies were conducted for measuring levels of BDNF in all subjects. By using High Resolution Melting (HRM) PCR, the genotypes of BDNF gene were identified. The kits used in this study are listed in table 1. The primers that are used in this study (MacrogenTM made in Korea) are listed in table 2.

Table 1: Kits used in this study

KITS	Company/ Origin
RDEEH0043Human BDNF (Brain Derived Neurotrophic Factor) ELISA Kit	China
Presto™ Mini gDNA Kit	Geneaid made in Taiwan
GoTag qPCR Master Mix, Nuclease Free Water, Quantifluor dsDNA System.	Promega made in USA
Primers	Macrogen made in Korea

Table 2: Primers used in the study

Primer Name	Seq.	Annealing Temp. (°C)	Product size (bp)
rs10501087-F	5'-CAAGGGATTTGGCTTCCTTCA-3'	60	130
rs10501087-R	5'-GGAAAGGCCTCCATGCAATG-3'	660	

Statistical Analyses

The statistical analyses were performed using Graph Pad Prism version 9.0 (Graph Pad software Inc., La Jolla, CA). Student T-test and one way ANOVA (Tukey Test) were used to determine whether group variance was significant or not. Chi-square was employed to test count variances. Alleles and genotypes of gene SNPs were presented as number and percentage frequencies. Hardy-Weinberg Equilibrium analysis of genotype frequency was performed using Fisher Test. Statistical differences were defined significantly as * in P=<0.05 or ** in P=<0.01.

2. Results

The study included 120 participants divided into two groups 60 patients with depression as case group, and 60 normal healthy persons as control one.

Table 3: The levels of BDNF in patients with depression and healthy controls.

Total Subject (120)	Patients with BDNF (60) Pg.ml-1	Healthy Control (60) Pg.ml-1	P value
level of BDNF	729.12(_+628.4)	1169.5(_+585.9)	0.0026**
BDNF brain-derived neurotrophic **factor, *** (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

In this study, the level of BDNF concentration in depressed patients was estimated and there was significantly lower than healthy control group which was 729.12 $pg.ml^{-1}$ in patients with BDNF compared with healthy control group which was 1169.5 $pg.ml^{-1}$ p value (P < 0.005).

Table 4: Relationship between rs10501087 genotypes in Patient group and control group.

Genotype rs10501087)(	Healthy No. (%)	Patients No. (%)	Chi-Square (χ2)	P-value	O.R. (C.I.)
TT-wild	26 (43%)	14 (23%)	----	-----	1.00
TC	21 (35%)	33 (55%)	6.267	0.0123*	2.918 (1.287 to 6.462)
CC	13 (22%)	13 (22%)	1.467	0.2259 NS	0.1857 (0.6475 to 4.771)
Total	60 (100%)	60 (100%)			
Allele	Frequency				
T	73 (0.6)	61 (0.508)	2.433	0.1188 NS	1.502 (0.9038 to 2.527)
C	47 (0.4)	59 (0.492)			
* (P≤0.05), ** (P value <0.0001), NS: Non-Significant.					

The results of HRM for rs10501087 in patients and healthy control groups showed that 14 (23%) of patients had wild type genotype (T/T), 13 (22%) of patients had the mutant variant (C/C), and 33 (55%) of patients had the heterozygous genotype (T/C). On the other hand, the screened controls, 26 (43%) were genotyped as T/T, 13 (22%) were genotyped as C/C and 21 (35%) were genotyped as T/C.

The studied mutant genotypes CC was non significantly different at ($p<0.05$) between each BDNF patients group and healthy control group which was (0.2259), while there was significant differences ($P\leq0.05$) between heterozygous T/C in BDNF patients and healthy control groups P value (0.0123). Moreover, odd ratio for TT and CC genotypes in the studied groups, were 1.0 and 0.1857 respectively while for the heterozygous genotype it was 2.918, which motioned that T>C polymorphism was a risk factor for incidence of MDD.

3. Discussion

Brain-Derived Neurotrophic Factor (BDNF) concentration in serum that estimated by ELISA:

BDNF, also its receptor neurotrophic tyrosine kinase receptor type 2 indicated an vital function essentially in MDD, suicidal attempts, thinking or behavior, and medicines that treated emergent suicidal ideation [5][6].

From the results it was shown that the BDNF levels in patients as a general have low level of concentration (729.12 pg ml^{-1}) in serum that was highly significantly differences **($P\leq0.005$)** when compared with healthy control group (1169.5 pg ml^{-1}) as shown in the results previously, Neurotrophic growth factors like BDNF were correlated to MDD. A diversity of clinical studies had concerned reduced BDNF indicating via its receptor TrkB (neurotrophic receptor tyrosine kinase 2) in the pathophysiology of mental or mood diseases, and that in line with this study which was there was detected decreasing in BDNF serum level in all patients [7]. Signaling BDNF had linked in mood disorders of the pathophysiology in humans, the protein BDNF level was decreasing in the serums of depressed patients [8].

BDNF levels alterations in serum was described in psychiatric and other neurodegenerative disorders. Therefore, BDNF in serum has represented an important peripheral biomarker for such diseases, and it is easily detected by non-invasive methods with the benefit of existence measurable in vivo. In this study used serum to detect the BDNF levels [9]. BDNF deficiencies in patients with depression were a well-established of clinical evidence associating the participation of BDNF in the pathobiology of depression [10]. Also, peripheral decreases in mature BDNF in serum and plasma was found in patients with depression [11]. Rs10501087 in BDNF gene The results of HRM for rs10501087 in patients and healthy control group showed that 14 (23%) of patients had wild type genotype (T/T), 13 (22%) of patients had the mutant variant (C/C), and 33 (55%) of patients had the heterozygous genotype (T/C). Otherwise, the examined controls, 26 (43%) were genotyped as T/T, 13 (22%) were genotyped as C/C and 21 (35%) were genotyped as T/C.

However, the results of rs10501087 genotypes (wild TT, heterozygous TC and mutant CC) indicated that there was significant differences between all depressive patients (as a general) heterozygotes genotype (TC) and control group, also between mutant genotype CC in moderate BDNF group and healthy control, as well heterozygotes genotype (TC) in sever depressive patients and healthy control as shown in table (3-13) and (3-14). While there was non-significant differences between all rest groups and healthy control. BDNF gene located on chromosome 11:27648561, The rs10501087 was intronic SNP (NCBI), so this snip location on intron so it may be affected gene regulation.

The difference in results may be because of a number of factors like outcome assessment, kind of medication, sample size, population organization, and quite notably, the density and rich variety in the regulation of BDNF numerous transcripts, if the SNP located in the coding and noncoding sequences, and in the proBDNF and mature BDNF translation product sequences [12][13].

The most universal genetic correlation study to date to have shown the association between BDNF sequence variation with both MDD and response to treatment. Given that a number of alternative BDNF transcripts have been found to display complex splicing and expression styles and that the findings in various studies stay inconsistent [14].

The common occurrence of disease-associated loci in intronic and intergenic regions was usually documented to possible regulatory DNA function. SNP or differences at a single nucleotide might result big conformational alterations in the structure of DNA by effect the state of the chromatin and interactions amongst remote loci [15][16].

Moreover, SNP or genetic differences at individual nucleotides can interrupt protein–DNA or RNA–DNA interactions[17][18], changing the binding of promoters and enhancers by regulatory proteins or RNA molecules, or regulating deposition of epigenetic signs [19].

The transcripts were found in gene rewards, introns and enriched in exons, however giving a non-uniform distribution through the targeted haploblocks. But non-conserved, they were developed for epigenetic markers of active transcription, showing tissue specificity and enrichment for weak active enhancers [20].

As mental disorders, especially the depression was existing in more of 90% of suicides [21]. Numerous studies have reported associations between the brain-derived neurotrophic factor (BDNF) gene variation and psychiatric disorders, as well as suicidal behavior, though with conflicting results. In European population, Genotyping was performed for the functional Val66Met polymorphism rs6265

and 7 additional tagging single nucleotide polymorphisms within the BDNF gene including rs10501087 the results showed that the BDNF single markers nor haplotypes were originate to be related with suicide risk and generation history of suicide attempts. Analyzing treatment response phenotypes, the efficient Val66Met polymorphism as well as rs10501087 presented significant genotypic and haplotypic association with suicide risk in remitters [4].

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