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INFLUENCE OF SEROTONIN TRANSPORTER-LINKED POLYMORPHIC REGION (5-HTTLPR) VARAINTS ON CLINICAL OUTCOMES IN THAI PATIENTS WITH DEPRESSIVE DISORDER

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ABSTRACT

The influence of the serotonin transporter polymorphisms on fluoxetine clinical outcomes was determined in 69 Thai patients with major depressive disorder. The results indicated that patients with *l/l* genotype had a significantly better response to fluoxetine treatment when compared with *s* allele carriers either evaluated based on the Thai HRS-D scores or psychiatrist efficacy evaluation ($p = 0.001$). At the same time, carriers with *s* allele had significantly higher rate of various side effects than the *l/l* genotype group ($p = 0.002$). These preliminary data might be used to reduce or prevent adverse effects and improve prescribing efficacy for depressive patients with different genotypes.

Keywords: Fluoxetine, 5-HTTLPR, Clinical Outcomes

INTRODUCTION

Major depressive disorder (MDD) is widely distributed in the population. MDD is associated with substantial symptom severity and role impairment. Selective serotonin reuptake inhibitors (SSRIs) have become first line drug in the treatment of depression partly because of their better tolerability and safety profiles. Fluoxetine is the most frequently prescribed drug among the new generation of antidepressants. It is also approved for the management of a variety of mental disorders. The pharmacological activity of fluoxetine is due to specific serotonin reuptake inhibition at the presynaptic serotonergic nerve terminal.[1]

Allelic variation in 5-HTT function may lead to both increased susceptibility to anxious or depressive features and less favorable antidepressant responses in patient affected by mood disorders.[2-3] The most widely investigated gene is that of the brain serotonin (5-HT) transporter (5-HTT). For interindividual difference in drug response, two polymorphisms have been observed. The deletion polymorphism in the promoter region of 5-HTT was known as short (*s*) variant, whereas the long (*l*) variant was the insertion polymorphism. Several studies have reported the association between variation in these genotypes and clinical response (efficacy and side effect) to SSRIs in Caucasian and Asian population.[4-7] The results were inconsistent to conclude whether the insertion allele could influence response to any antidepressant. This study was performed to evaluate the association of the serotonin transporter polymorphisms and clinical outcomes (efficacy and adverse effects) of fluoxetine for depression treatment in Thai patients. If association is found, the finding of 5-HTTLPR variants might be a predictor for treatment response of fluoxetine in clinical setting.

METHODS

Study design and Subjects

Study design: The cross-sectional and prospective study has been performed. All collecting data were obtained from outpatient department at Srithanya Hospital, Mental Health Department, Nonthaburi, Thailand.

Recruitment of participants and data collecting: After the approval from both Ethical Committee of Srithanya hospital and Department of Mental Health, the target participants were recruited into study and informed consent were obtained from all participants. Eligible subjects were adult outpatients (age more than 18 years old) who was diagnosed as depressive patients by DSM-IV criteria and had been prescribed with fluoxetine for more than 8 weeks. The good history of drug compliance was confirmed by interviewing the patients. Exclusion criteria were patients with 1) abnormal liver function; 2) diagnosed depression with psychosis features; and 3) loss of follow up. Demographic data, clinical data, dosage regimen of fluoxetine and concomitant drugs were recorded.

Blood sample collection: After the same dosage regimen was taken for more than eight weeks, the steady state condition was assumed to achieve. Blood sample (15 ml) of each participant was collected before taking fluoxetine in the morning. The whole blood samples for genotyping were stored with EDTA at -40ºC. Blood sample was also determined for fluoxetine and norfluoxetine (known active metabolite) plasma concentration for pharmacokinetic parameters calculation.

Drug efficacy and side effects monitoring: Clinical outcomes of each patient were monitored at hospital visits. Drug efficacy was assessed by the 17-item Hamilton depression rating scale (Thai HRS-D 17) and side effects were recorded in the data collecting form by interviewing. For responder without side effects, the same prescribed dose of fluoxetine for depression treatment was continually taken and the monitoring process was ongoing. For non-responder or responder with side effects, psychiatrist might consider adjusting the fluoxetine dosage regimen or switch therapy with other antidepressants. Switching to other antidepressants would be the terminal of project monitoring. If the new fluoxetine dosage regimen was prescribed, the monitoring process was continually ongoing. Each time, after the same dosage regimen had been taken for more than eight weeks and steady-state condition was assumed to achieve. Blood sample (10 ml) was again collected to determine the fluoxetine and norfluoxetine plasma concentration.

DNA extraction and genotyping

DNA extraction: DNA was prepared from leukocytes (buffy coat) of the whole blood sample using an Illustra Blood GenomicPrep Mini Spin Kit, UK according to the manufacturer's instruction.

Genotyping for 5-HTTLPR: Short and long variants of serotonin transporter were the investigated polymorphisms. For GC-rich genotyping, fragments of serotonin transporter gene (serotonin gene-linked polymorphic region: 5-HTTLPR) were amplified by PCR using primers with 5'-GGCGTTGCCGCTCTGAATTGC-3' plus 5'-GAGGGACTGAGCTGGACAACCAC-3'.[8] Briefly, PCR amplification was performed in a final volume of 12.5 μL containing 100 ng of genomic DNA, 0.2 μmol/L of each dNTP (Biolabs), forward primer (Proligo) and reverse primer (Proligo);, 1.25 μL of buffer (Qiagen) with 1.5 mmol/L $MgCl_2$, 1X PCRx enhancer solution (Qiagen) and 0.625 U of Taq DNA polymerase (Qiagen). A GC-rich region composed of 20-23 bp repeating units was amplified for 39 cycles of 95ºC for 30 sec, 61ºC for 30 sec, and 72ºC for 1 min, followed by a final extension of 72ºC for 10 min. Polymorphisms of 5-HTTLPR were determined based on their fragment size in 2% agarose gel using electrophoresis technique. The sizes of the *s* and *l* 5-HTTLPR alleles were 469-470 base pairs and 511-513 base pairs, respectively.

Statistical analysis: Data analysis for descriptive statistics and inferential statistics were generated by the software SPSS for windows version 17.0 (SPSS Co., Ltd, Bangkok, Thailand). Clinical data, demographic data and fluoxetine pharmacokinetic parameters were presented by descriptive statistics. Association of serotonin transporter polymorphism and clinical outcomes (Thai HRS-D score, psychiatrist evaluation and side effects) were investigated by Chi-square test. All statistical significant level (α) was set at 0.05.

RESULTS

Influence of serotonin transporter polymorphisms on clinical outcomes: Sixty nine participants were recruited into the study. The prevalence of serotonin transporter genotypes in this study was 60.9%, 24.6% and 14.5% for *s/s*, *s/l* and *l/l* genotype, respectively. Allele frequencies of short allele and long allele were 73.2% and 26.8%, respectively. Demographic data and pharmacokinetic parameters among different serotonin transporter genotypes showed no significant different (table I). Therefore, the factors of demographic data and dosage regimen should have minimum confound on the analysis of influence of serotonin transporter polymorphism on clinical outcomes.

Analysis of the results by chi-square test revealed that patients with *l/l* genotype had a significantly better response to fluoxetine treatment when

compared with *s* allele carriers whether determined from the Thai HRS-D scores or psychiatrist efficacy evaluation (table II). On the contrary, carriers with *s* allele had significantly higher rate of various side effects than the *l/l* genotype group as shown in table III. Five participants had to switch from fluoxetine to another drug regimen for depression treatment. Among these, four cases were with *s/s* genotype, whereas, one case was with *l/l* genotype.

DISCUSSION

The prevalence of serotonin transporter genotypes in this study was 60.9%, 24.6% and 14.5% for *s/s*, *s/l* and *l/l* genotype, respectively. Allele frequencies of short allele and long allele were 73.2% and 26.8%, respectively. Whereas, the prevalence study by Tencomnao *et al* in Thai patients with depressive disorder reported that there was 54.0%, 36.9% and 9.1% for *s/s*, *s/l* and *l/l* genotype, respectively.[9] Allele frequencies were fairly similar to this study. There were 72.5% and 27.5% for short and long allele, respectively.

From this study, the association of the serotonin transporter polymorphisms and clinical outcome of fluoxetine was determined. The result revealed that patients with *l/l* genotype had a significantly better response to fluoxetine treatment when compared with *s* allele carriers. Carriers with *s* allele had significantly higher rate of various side effects than the *l/l* genotype group. The result obtained from this study was strongly consistent with the study of Susuki *et al*.[10] They have investigated and reported that polymorphisms of serotonin receptor and polymorphisms of CYP2D6 synergistically predicted fluvoxamine-induced side effects in 100 Japanese depressed patients. Study by Perlis *et al* reported that the short variant might identify patients at risk for developing insomnia or agitation with fluoxetine treatment in major depressive patients (n = 36).[11]

The result was also consistent with a study in 121 Chinese patients with depressive disorder by Yu *et al*.[12] Analysis of the results revealed that patients with *l/l* genotype had a significantly better response to SSRI (fluoxetine) when compared with *s* allele carriers, as evaluated by Hamilton Depression Rating Scale-score ($p = 0.013$). The corresponding study in 51 Caucasian elderly depressed patients by Rausch *et al* confirmed that the *l* allele variants were associated with better SSRI response ($p < 0.02$).[13]

The conflicting results of prediction for response and side effects of various SSRIs have also been reported for the serotonin transporter polymorphism. Takahashi *et al* investigated the association between serotonergic polymorphisms and incidence of nausea in 66 Japanese patients.[14] The research result suggested that three polymorphisms in serotonergic system did not affect the development of fluvoxamine-induced nausea, and that incidence of nausea was not a phenomenon that predicts the treatment response to fluvoxamine. Studies by Zanardi *et al* [15] and Pollock *et al* [16] revealed that *l* allele variant was associated with a worse paroxetine response. Kim *et al* [17] reported that the *s/s* subjects showed the better response for fluoxetine or paroxetine in Korean Patients with depressive disorder. Yoshida *et al* [18] also reported that the *s/s* Japanese subjects had the better response for fluvoxamine when compared with the *l/l* variant group.

There are several possible explanations for this discrepancy. Asian study was developed with small sample size. Allele frequency of the long allele variants was different among Caucasian and Asian population. Variations of the study results could have also been caused by the difference in the diagnosis features, duration of treatment, clinical improvement evaluation and type of side effects or adverse effects evaluation. Evaluation by depression rating scales was different in those studies. Several studies used a global rating scale instead of the specific rating scales. Moreover, there was no structure for adverse drug reaction form in the study. Some serious adverse effects such as agitation or akathesia were difficult to detect. Therefore, several confounding factors might not be detected and/or control.

CONCLUSION

The influence of the serotonin transporter polymorphisms was determined for fluoxetine pharmacodynamic or clinical outcomes. It was found that patients with *l/l* genotype had a significantly better response to fluoxetine treatment when compared with s allele carriers either evaluated by the Thai HRS-D scores or psychiatrist. Among patients with different serotonin transporter polymorphisms, carriers with *s* allele had significantly higher rate of various side effects than the *l/l* genotype group. This study provides the preliminary data which might be used to reduce or prevent adverse effects and improve prescribing efficacy for depression patients with different genotypes. The limitation of this study and consideration for further study include the following. Firstly, serious side effects of fluoxetine were not regarded in this study. Secondly, power of the studying can be increased by recruiting more participants.

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Table I: Demographic data and pharmacokinetic parameters among serotonin transporter genotypes

Data	Average (mean±SD)	Serotonin transporter genotypes s/s (N=42)	s/l (N=17)	l/l (N=10)	P-value
Gender					
Male (N=26)		17(40.5%)	5 (29.4%)	4 (40.0%)	
Female (N=43)		25 (59.5%)	12 (70.6%)	6 (60.0%)	**0.732**
Age (years)	38.6 ± 10.3	39.9(24-62)	36.0(20-61)	37.5(22-54)	**0.414**
Weight (kg)	63.8 ± 14.6	65.5 ± 16.4	58.6 ± 10.4	65.8 ± 11.8	**0.132**
Dose (mg/day)	28.28 (10-80)	28.42 (10-80)	26.47 (10-60)	31.11 (20-80)	**0.759**
Fluoxetine concentration (μmol/L)	1.39 ± 1.94	1.20 ± 1.93	1.78 ± 1.89	1.45 ± 2.19	**0.605**
Norfluoxetine concentration (μmol/L)	2.62 ± 3.13	2.20 ± 2.64	3.50 ± 4.38	2.76 ± 1.98	**0.365**
SUM (μmol/L)	4.01 ± 4.89	3.40 ± 4.47	5.27 ± 6.16	4.21 ± 3.83	**0.426**
Concentration Dose ratio μg/ml/mg/kg dose	0.023 ± 0.033	0.020 ± 0.032	0.032 ± 0.037	0.022 ± 0.033	**0.503**

Table II: Comparison of drug efficacy among different genotypes

Drug efficacy	Serotonin transporter genotypes *s/s* (N=42)	*s/l* (N=17)	*l/l* (N=10)
Method I : Thai HRS-D 17			
≤ 7	29 (69.0%)	14 (82.4%)	8 (80.0%)
> 7			
$\chi 2 = 13.76$ P = 0.001	9 (21.4%)	3 (17.6%)	1 (10.0%)
Method II : Psychiatrist Efficacy evaluation			
Improve without side effect	22 (52.4%)	11 (64.7%)	7 (70.0%)
Currently use with side effect	16 (38.1%)	6 (35.3%)	2 (20.0%)
$\chi 2 = 9.05$ P = 0.011			

Table III: Comparison of side effects among different genotypes

Side effects	Serotonin transporter genotypes *s/s* (N=42)	*s/l* (N=17)	*l/l* (N=10)
Headache (N=7)	4 (9.5%)	2 (11.7%)	1 (10.0%)
Drowsiness and fatigue (N=6)	4 (9.5%)	1 (5.9%)	1 (10.0%)
Other symptoms (N=11)	8 (19.1%)	3 (17.6%)	0 (0.0%)
All adverse events (N=24) **$\chi 2 = 13.00$ P = 0.002**	16 (38.1%)	6 (35.3%)	2 (20.0%)

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