Family-Based Association Study of Serotonin Transporter Promoter in Suicidal Adolescents: No Association With Suicidality but Possible Role in Violence Traits

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The serotonin transporter–linked promoter region polymorphism (5-HTTLPR) is thought to be associated with some serotonin dysfunction–related psychopathologies such as depression and anxiety disorders. Suicide and suicide-related behaviors such as violence, aggression, and impulsivity have been reproducibly associated with serotonin dysfunction and are partially genetic. This study examined the association of 5-HTTLPR with suicidal behavior and related traits in Israeli suicidal adolescent inpatients using the haplotype relative risk (HRR) method that controls for artifacts caused by population stratification. Forty-eight inpatient adolescents who recently attempted suicide were assessed by structured interviews for detailed clinical history, diagnoses, suicide intent, suicide risk, impulsivity, violence, and depression. Blood samples were collected and DNA extracted from patients and their biological parents. The 5-HTTLPR allele frequencies were tested for association with suicidality by the HRR method. In addition, the relationship between genotypes and phenotypic severity of several clinical parameters was analyzed. No significant allelic association of the 5-HTTLPR polymorphism with suicidal behavior was found (chi square = 0.023; P = 0.88). Analysis of variance of the suicide-related trait measures for the three genotypes demonstrated a significant difference in violence measures between patients carrying the LL and LS genotypes (9.50 ± 4.04 vs. 5.36 ± 4.03; P = 0.029). This study suggests that the 5-HTTLPR polymorphism is unlikely to have major relevance to the pathogenesis of suicidal behavior in adolescence but may contribute to violent behavior in this population.

KEY WORDS: suicide; adolescence; haplotype relative risk; 5-HTTLPR; violence; aggression

INTRODUCTION

An association between serotonin (5-HT) dysfunction and both suicide and suicide-related behaviors, such as violence, aggression, and impulsivity, has been demonstrated in a number of studies [Coccaro, 1989; Coccaro et al., 1989; Arango et al., 1995; Mann and Malone, 1997; Mann, 1998]. In adolescents, aggression and violence may be as important in some kinds of suicidal behaviors as is depression [Apter et al., 1995]. Brent et al. [1996] screened 58 adolescent suicide probands and concluded that liability to suicidal
behavior might be familial, transmitted as a trait independent of axis I or II diagnoses. Adoption studies suggest that there may be a genetic vulnerability to suicide, which may be partially independent of the presence of a psychiatric disorder [Roy, 1983, 1986, 1997; Roy et al., 1991]. Roy et al. [1991] showed that the concordance rate for suicide in monozygotic twins is 11.3% vs. 1.8% for dizygotic twins. Meta-analysis of several studies have demonstrated even higher differences: 13.2% in monozygotic pairs vs. 0.7% in dizygotic pairs, indicating that suicidal behavior is at least partially genetically determined [Roy et al., 1991; Roy et al., 1997].

One candidate gene potentially influencing suicidal behavior is the serotonin transporter (5-HTT) gene. This gene codes for the serotonin transporter, which is localized in the outer membrane of the presynaptic serotonergic neurons [Ramamoorthy et al., 1993]. The serotonin transporter is a sodium-dependent transporter with high affinity for serotonin. It has an important role in controlling serotonin availability in the synapse by regulating the reuptake of serotonin. 5-HTT is the major target of serotonin reuptake inhibitors [Sanders-Bush and Mayer, 1996; Heils et al., 1997]. The gene encoding the 5-HTT protein (SLC6A4) was mapped to the long arm of chromosome 17 [Lesch et al., 1993; Ramamoorthy et al., 1993; Gelernter et al., 1995]. Lesch et al. [1994] have shown the existence of a region of variable number tandem repeats (VNTR) of 17 base pairs (bp) in the second intron with three major alleles. Another polymorphism found in the promoter region is the 5-HTT–linked polymorphic region (5-HTTLPR) that consists of two common alleles, a short (S) and long (L) variants, differing by 44 bp [Heils et al., 1996].

Lesch et al. [1995] have shown that there are no differences in amino acid sequence between mood disorder probands and healthy population. No major mutation was found in the SLC6A4 coding region in both affected probands and control subjects [Di Bella et al., 1996]. Since no sequence changes were found in the gene encoding the 5-HTT protein, it was hypothesized that an alteration in the expression of the gene may be found in serotonin-related psychopathologies.

Studies on SLC6A4 Gene Polymorphism and 5-HTTLPR in Psychiatry

Numerous case control association studies were performed to trace possible association of the SLC6A4 polymorphism in intron 2 with psychopathologies [Collier et al., 1996a, 1996b; Stober et al., 1996; Evans et al., 1997]. Since findings regarding SLC6A4 association with affective disorders were inconsistent [Furlong et al., 1998], a search for association with functional alleles in the promoter region, namely the 5-HTTLPR, was initiated. Collier et al. [1996b] demonstrated that the S-allele is more frequent in patients with affective disorder than in a control group. In a large meta-analysis, Furlong et al. [1998] showed a significant association of S-allele with affective disorders. Our group showed no association in unipolar affective disorder patients from Ashkenazi and non-Ashkenazi Jewish origin in a case control study [Frisch et al., 1999]. The same lack of association to affective disorders and suicide attempts was demonstrated by Ohara et al. [1998] in a case control study in 80 patients with mood disorder and 92 healthy controls.

Association of 5-HTTLPR polymorphism was demonstrated in anxiety-related traits [Lesch et al., 1996] but was not found in Israeli population [Eebstein et al., 1997]. Studies on patients who suffer from obsessive-compulsive disorder have been inconsistent [Billett et al., 1997; McDougle et al., 1998; Frisch et al., 2000]. Alcohol dependence and ethanol tolerance were found to be associated to the S-allele in few studies [Lesch et al., 1998; Turker et al., 1998] or showed no association in other studies [Gelernter et al., 1997]. Hallikainen et al. [1999] found association between S-allele and type 2 alcoholism with habitual impulsive violent behavior.

Role of 5-HTTLPR in Suicidal Behavior

The role of 5-HTTLPR polymorphism in suicidal behavior was studied by Russ et al. [2000] and Geijer et al. [2000]. In both case control studies, there was no significant difference in the frequency of the S-allele on promoter alleles between suicidal patients and control group. However, Du et al. [1999] reported on a modest association between the L-allele of the 5-HTTLPR and suicidality in depressed patients, while Bondy et al. [2000] found a possible association of the S-allele with violent suicide victims.

Family-Based Studies

All studies that have been reported up till now were of case control design. The allelic frequencies of many polymorphisms in candidate genes vary between populations [Gelernter et al., 1997, 1998, 1999], and even between ethnic groups in the same country, e.g., Ashkenazi and non-Ashkenazi Jews in Israel [Frisch et al., 1999]. This makes case control association studies problematic since one cannot be sure that the control sample was drawn from exactly the same genetic population as the suicidal patients. The present study attempts to overcome this methodological difficulty by using the haplotype relative risk (HRR) method [Knapp et al., 1993]. The advantage of family-based association methods compared to case control association methods is widely accepted [Ott, 1989; Knapp et al., 1993; Schaid and Sommer, 1994; Spielman and Ewens, 1996]. This method is less prone to produce false positive and false negative associations in the case of hidden population stratification. The use of the HRR method requires a triplet of the patient and both his parents. The HRR method allows the using of the HRR method [Knapp et al., 1993]. The advantage of family-based association methods compared to case control association methods is widely accepted [Ott, 1989; Knapp et al., 1993; Schaid and Sommer, 1994; Spielman and Ewens, 1996]. This method is less prone to produce false positive and false negative associations in the case of hidden population stratification. The use of the HRR method requires a triplet of the patient and both his parents. The HRR method allows the using of the haplotype relative risk (HRR) method [Knapp et al., 1993]. The advantage of family-based association methods compared to case control association methods is widely accepted [Ott, 1989; Knapp et al., 1993; Schaid and Sommer, 1994; Spielman and Ewens, 1996]. This method is less prone to produce false positive and false negative associations in the case of hidden population stratification. The use of the HRR method requires a triplet of the patient and both his parents. The HRR method allows the using of the haplotype relative risk (HRR) method [Knapp et al., 1993]. The advantage of family-based association methods compared to case control association methods is widely accepted [Ott, 1989; Knapp et al., 1993; Schaid and Sommer, 1994; Spielman and Ewens, 1996]. This method is less prone to produce false positive and false negative associations in the case of hidden population stratification. The use of the HRR method requires a triplet of the patient and both his parents. The HRR method allows the using of the haplotype relative risk (HRR) method [Knapp et al., 1993]. The advantage of family-based association methods compared to case control association methods is widely accepted [Ott, 1989; Knapp et al., 1993; Schaid and Sommer, 1994; Spielman and Ewens, 1996].
than the case control method [Knapp et al., 1993; Schaid and Sommer, 1994].

To the best of our knowledge, no HRR study has been published yet on the 5-HTT promoter polymorphism and suicidal behavior in an adolescent population. The suicidal behavior phenotype in this study was assessed by a number of independent valid and reliable state and trait questionnaires for this age group.

MATERIALS AND METHODS

Patient Sample

The study was conducted in the adolescent psychiatric unit of a university-affiliated psychiatric hospital in Israel. Because of a recent suicide attempt, the sample consisted of 48 consecutive patients admitted to the unit over a period of 3.5 years. This sample is part of a larger sample that was analyzed for the tryptophan hydroxylase gene polymorphism in a previous study (data not shown). There were 32 (67%) girls and 16 (33%) boys, aged 15 to 24 (mean, 18.9 ± 2.5) years. Forty-seven patients were Jewish: 24 (51%) Ashkenazi, 23 (49%) non-Ashkenazi (North African, Iraqi, and Yemenite origin), and 1 Muslim. Individuals were considered to be of Ashkenazi origin if all four grandparents originated from Eastern Europe. Diagnoses were established according to the DSM IV criteria [American Psychiatric Association, 1994]. Most of the patients had more than one comorbid diagnosis. Diagnoses were major depression (n = 17), dysthymia (n = 10), bipolar I disorder (n = 3), schizophrenia (n = 14), eating disorder (n = 8), obsessive-compulsive disorder (n = 8), conduct disorder (n = 4), substance abuse (n = 8), depression due to substance abuse (n = 3), adjustment disorder (n = 3), brief psychotic disorder (n = 1), posttraumatic stress disorder (n = 1), panic disorder (n = 3), and attention-deficit hyperactivity disorder (n = 1). In 17 patients, borderline personality disorder (axis II) was the primary diagnosis. The mean score on the Global Assessment of Functioning [First et al., 1994] was 56.73 (SD = 17.14; range, 20–90).

Methods of suicide attempt included ingestion of pills (15 patients), cutting veins (5 patients), jumping (13 patients), hanging (4 patients), ingestion of poison (2 patients), suffocation by drowning or other means (9 patients). For the patients with more than one suicide attempt, we referred to the severest method. The mean number of previous suicide attempts in the study population was 2.3 (range, 0–4).

Study Design

According to the HRR method, parents of the study patients served as the control group. There were 39 probands for whom both parents were available (trios) and 9 probands for whom one parent was available (duos).

The study was approved by the hospital review board and written informed consent was obtained from all subjects and their parents after the nature of the study was fully explained.

Blood Collection

Ten milliliters of venous blood were drawn from each subject and his or her biological parents (if available) into EDTA-containing tubes. DNA isolation was performed as described by Miller et al. [1998].

Clinical Assessment

The clinical phenotype of the suicidal subjects was determined by five questionnaires and a diagnostic semistructured interview as follows.

Past Feelings and Acts of Violence Scale (PFAVS), a 12-item questionnaire checking risk of violence, anger, and legal problems linked to violence. The scale is reliable in detecting history of violence and predicts violence as the reason for hospitalization. It is mainly a trait questionnaire. Answers are rated from never to always on a four-point Likert scale, and the score ranges from 0 to 36. Internal validity is 0.77 [Plutchik and Van Praag, 1990].

Impulsivity Scale (IS) is a 15-item questionnaire for impulsivity. Answers are rated on a four-point Likert scale, and scores range from 15 to 60. The internal validity is 0.77 [Plutchik and Van Praag, 1986; Apter et al., 1990].

Suicide Risk Scale (SRS), a 26-item pencil-and-paper questionnaire, checks for the actual risk for suicide. Answers are given as yes or no. Score ranges from 26 to 52 (lower scores means higher risk). The internal validity is 0.84 [Plutchik et al., 1989; Apter et al., 1990].

Beck Depression Inventory (BDI) is a well-known tool for measuring depression. The scores range from 0 to 63; scores above 9 are considered positive for depression. The internal validity is 0.73–0.92 [Beck and Steer, 1987; Beck et al., 1988].

Beck Suicide Intent Scale (BSIS) is a 15-item questionnaire on the seriousness of the suicide attempt. The scores range from 0 to 30. The questionnaire has been found to differentiate between suicide attempters and completers [Beck et al., 1974a, 1974b].

Schedule for Affective Disorders and Schizophrenia for Children-Patient Version (K-SADS-P) is a structured interview for axis I diagnosis in children and adolescents. The K-SADS-P has been translated into Hebrew and showed good interrater reliability in our earlier studies [Apter et al., 1989; Shanee et al., 1997]. In patients older than 18 years, we used the Hebrew version of the Structured Clinical Interview for DSM IV (SCID), version 2.0 [First et al., 1994], translated by Shalev et al.

Procedure

Patients were interviewed on admission, during the first week after the most recent suicide attempt. Two patients were unable or refused to answer the questionnaires. Blood was drawn during the same week and was delivered to the laboratory immediately. When DNA extraction was not possible immediately, the blood was stored for up to 24 hr at 4°C.
Genotyping

The insertion/deletion polymorphism in the regulatory region of the serotonin transporter gene was studied according to Lesch et al. [1996]. The GC-rich region was amplified with Pfu exo minus DNA polymerase (Stratagene, La Jolla, CA) in the presence of 7-Deaza-dGTP. The two amplification products were separated on 2% agarose gel and classified as long (L = 528 bp) and short (S = 484 bp) alleles.

Statistical Analysis

Transmitted and nontransmitted allele frequencies were calculated from genotypes of probands and parents according to the HRR method [Knapp et al., 1993; Schaid and Sommer, 1994] and chi-square test was performed.

Analysis of variance was performed within the patient group to test whether there are significant differences among the three categories of genotype, LL, SS, LS, for the following scales: Past Feelings and Acts of Violence, Impulsivity, Suicide Risk, Beck Depression Inventory, and Beck Suicide Intent Scale. Posthoc comparison tests were carried out for the significant findings using Bonferroni correction.

RESULTS

Patient Sample

Table I lists the means and standard deviations of the questionnaire scores. The patients in the sample showed low to moderate violence rate (by the PFAVS), moderate to high impulsivity (by the IS), moderate to high suicide risk and intent (by the SRS and the BSIS, respectively), and a wide range of depression (by the BDI).

HRR Analysis

When trios only were considered (n = 39), the chi-square value was 0.026 with \( P = 0.87 \). When both trios and duos (n = 48) were considered, the chi-square was 0.023 with \( P = 0.88 \) (Table II).

Analysis of Variance Among Three Genotypes (LL/LS/SS)

The results of the analyses are presented in Table III. No significant differences were found among the three genotypes for scores on the IS, SRS, BDI, and the BSIS. Differences were significant for the PFAVS (\( f = 3.716; df = 2.43; P = 0.032 \)). To pinpoint the source of the difference, a posthoc comparison analysis using the Bonferroni test was carried out. A significant difference was found between LL and LS (\( p = 0.029 \)). The LL genotypes had mean score of 9.50 (SD = 4.04) and the LS genotypes 5.36 (SD = 4.03). SS mean score was 6.19 (SD = 2.95; Table III).

DISCUSSION

This study used the family–based HRR method to compare the frequencies of 5-HTT promoter alleles in suicidal adolescents to the nontransmitted alleles from their parents. The results showed no association between suicidal behavior and the alleles of this polymorphic region. Recent case control studies found association of suicidality to the S-allele [Bondy et al., 2000] and L-allele [Du et al., 1999], while no association was reported by Geijer et al. [2000] and Russ et al. [2000]. As family-based studies are considered to be more robust than case control studies and avoid errors introduced via population stratification, our results may help resolve the debate in the literature on the role of the 5-HTTLPR polymorphism in suicidal behavior, with the caveat that our sample size is rather small.

Analysis of variance failed to reveal an association of this polymorphism to certain behaviors connected to suicide, such as impulsivity, depression, suicide intent, or to the number or severity of the suicide attempts. However, such association was found for violence traits as measured by the PFAVS questionnaire. The ANOVA followed by posthoc Bonferroni test showed significant difference between LL and LS genotypes. This questionnaire has been found to be highly sensitive and reliable in detecting aggression and violence [Plutchik and Van Praag, 1990], two important factors in the evaluation of suicidal adolescents [Coccaro, 1989; Coccaro et al., 1989; Apter et al., 1990, 1993, 1995; Gould et al., 1992]. The PAFVS significantly discriminated between psychiatric inpatients with and without violent behavior [Plutchik and Van Praag, 1986]. In a previous study done in our unit, a subgroup of violent patients showed higher suicide risk and impulsivity compared with the nonviolent subgroup [Apter et al., 1995]. It should be noted that two studies [Coccaro, 1989; Coccaro et al., 1989] emphasized that impulsive aggressive behavior with suicidality tends to correlate

**TABLE I. Clinical Assessment of Inpatient Suicidal Adolescents**

<table>
<thead>
<tr>
<th>Scale</th>
<th>n</th>
<th>Range (scale range)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFAVS</td>
<td>46</td>
<td>1–20 (0–36)</td>
<td>6.37 (3.91)</td>
</tr>
<tr>
<td>IS</td>
<td>48</td>
<td>21–48 (15–60)</td>
<td>34.42 (5.75)</td>
</tr>
<tr>
<td>SRS</td>
<td>46</td>
<td>28–52 (26–52)</td>
<td>36.35 (5.96)</td>
</tr>
<tr>
<td>BDI</td>
<td>46</td>
<td>1–49 (0–63)</td>
<td>23.28 (14.57)</td>
</tr>
<tr>
<td>BSIS</td>
<td>46</td>
<td>2–26 (0–30)</td>
<td>13.43 (6.51)</td>
</tr>
</tbody>
</table>

*PFAVS = Past Feeling and Acts of Violence Scale; IS = Impulsivity Scale; SRS = Suicide Risk Scale; BDI = Beck Depression Inventory; BSIS = Beck Suicide Intent Scale.

**TABLE II. HRR Analysis of 5-HTTLPR Alleles in Suicidal adolescents (n = 48)**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Transmitted</th>
<th>Nontransmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>528 bp (L)</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>484bp (S)</td>
<td>36</td>
<td>37</td>
</tr>
</tbody>
</table>

*5-HTTLPR = 5–HTT–linked polymorphic region. L = long allele; S = short allele. Chi-Square = 0.023; \( p = 0.88 \).
with dysregulation of central serotonin activity. In this study, we observed an association between the serotonin genotype with aggressive behavior but not with impulsivity.

**Study Limitations**

The sample size needed to demonstrate a genetic association is difficult to predict, since it depends on degree of association, linkage disequilibrium, accuracy of phenotypic and allelic frequencies data, as well as the expected size of the effect.

Another limitation of this study is its restriction to adolescent inpatients, which may represent an extreme end of the suicidal spectrum. The relevance of our findings to outpatients and community-based samples merits further investigation. It seems reasonable, however, that the severest cases are more likely to have a genetic contribution.

All the current published studies were performed on adult population. The possibility that suicidal adolescents may have different genetics cannot be ruled out. Another possibility is that adolescent suicide attempts differ phenotypically from attempts in adulthood. It is therefore difficult to assess the relationship between this report on adolescents and previous reports on adults.

It is concluded that the 5-HTTLPR polymorphism is unlikely to have major relevance to the etiology of adolescent suicidal behavior and other behavioral traits thought to be associated with suicidality (depression, impulsivity). However, violence and aggression as measured by the PFAVS seemed to differentiate between the LL and the LS genotype in our sample of suicidal adolescents. The PFAVS measures the severity of anger and violence and it is mainly a trait variable, hence the LL genotype may be associated with both aggressiveness and suicidal behavior or combination of both (i.e., aggressive suicidal behavior), a phenomenon that may be relevant to conduct disorder and/or borderline personality disorder, as we noted in a previous study [Apter et al., 1995]. It is noteworthy that a recent study has demonstrated an association between homozygosity for LL and increased hopelessness and suicide ideation in adult patients at risk for suicide, but no association of this polymorphism with suicidal behavior [Russ et al., 2000]. Larger-scale comparative studies on both adults and adolescents using family-based methods are needed to clarify the role of the serotonin transporter polymorphism genotype in vulnerability to violent traits and suicidal behavior.

**REFERENCES**


**TABLE III. Analysis of Variance of the Psychometric Characteristics Among the Three Genotypes (LL/LS/SS) With Bonferroni Posthoc Tests**

<table>
<thead>
<tr>
<th>Scale</th>
<th>Genotype</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>f</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFAVS</td>
<td>LL</td>
<td>8</td>
<td>9.50*</td>
<td>4.04</td>
<td>3.716</td>
<td>2,43</td>
<td>0.032b</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>22</td>
<td>5.36*</td>
<td>4.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>16</td>
<td>6.19</td>
<td>2.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>LL</td>
<td>8</td>
<td>37.00</td>
<td>4.60</td>
<td>1.032</td>
<td>2,45</td>
<td>0.365</td>
</tr>
<tr>
<td></td>
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<td>23</td>
<td>34.17</td>
<td>6.11</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>17</td>
<td>33.53</td>
<td>5.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>LL</td>
<td>8</td>
<td>34.38</td>
<td>3.78</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>LS</td>
<td>22</td>
<td>35.64</td>
<td>4.74</td>
<td>1.497</td>
<td>2,43</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>16</td>
<td>38.31</td>
<td>7.85</td>
<td></td>
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</tr>
<tr>
<td>BDI</td>
<td>LL</td>
<td>8</td>
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<td>0.782</td>
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<tr>
<td></td>
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<td>13.82</td>
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</tr>
<tr>
<td></td>
<td>SS</td>
<td>16</td>
<td>21.38</td>
<td>15.80</td>
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<td>LL</td>
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<tr>
<td></td>
<td>LS</td>
<td>22</td>
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<td>16</td>
<td>12.69</td>
<td>7.24</td>
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</tr>
</tbody>
</table>

*PFAVS = Past Feeling and Acts of Violence Scale; IS = Impulsivity Scale; SRS = Suicide Risk Scale; BDI = Beck Depression Inventory; BSIS = Beck Suicide Intent Scale.

*The mean difference between LL and LS is significant at 0.05 (Bonferroni, P = 0.029).

bSignificant at 0.05 level.


