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The biobehavioural effectiveness of spiritual-hypnosis-assisted therapy in PTSD with childhood trauma

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Abstract

Background: Posttraumatic Stress Disorder (PTSD) is a serious psychological trauma disorder. Treatment of psychological trauma tends to focus on patients' memory. Clinical Spiritual-Hypnosis Assisted Therapy is a culturally sensitive treatment that combines elements of psychodynamic hypnosis, cognitive—behavioral and humanistic therapies.

Methods: The current interventional single-blind randomized control study assessed the biobehavioural effectiveness of spiritual-hypnosis on cortisol and PTSD symptomatology in adults with childhood trauma. Participants were divided into spiritual hypnosis (n = 15) and a control group (n = 14) that received *fluoxetine*. This study used PCL-C & CTQ to screen the presence and severity of PTSD symptoms.

Results: Spiritual hypnosis was significantly better than fluoxetine at reducing PTSD symptoms, while both treatments had similar effects on cortisol modification.

Conclusions: Spiritual-Hypnosis Assisted Therapy for PTSD patients with childhood trauma appears to have a noteworthy effect in reducing PTSD clinical symptoms and results in a comparable to the pharmacological treatment modification of the HPA axis cortisol markers.

Keywords: Spiritual hypnosis, SHAT, Cortisol, Fluoxetine, PTSD, Childhood trauma

Background

Posttraumatic Stress Disorder (PTSD) was first included as a clinical taxon in the diagnostic guidelines of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) in 1980 [1]. PTSD is a serious mental disorder that results in a complex and often chronic mental, psychological dysfunction, especially if the individual is further exposed to severe life stressors. PTSD can develop from a prolonged response to catastrophic or frightening events or situations that cause stress (either brief or prolonged). The disorder can result from such stressors as natural or human-caused disasters, war, and severe accidents, witnessing a horrible death, being a victim of

torture, terrorism, rape, and other such crimes. Typical symptoms include episodes of repeated reliving of the trauma in intrusive memories ("flashbacks") or dreams, occurring against the persisting background of a sense of "numbness" and emotional blunting, detachment from other people, unresponsiveness to surroundings, anhedonia, and avoidance of activities and situations reminiscent of the trauma. There is usually a state of autonomic hyperarousal with hypervigilance, an enhanced startle reaction, and insomnia (World Health Organization [2]). Psychological trauma in childhood tends to have a powerful and long-term impact on the individual, and it can act as the main trigger of psychopathological dysfunction (Raab et al. [3]). In particular, psychological childhood trauma has been associated with depressive disorders, alcohol and substance abuse, generalized anxiety disorders, and posttraumatic stress disorder (PTSD)

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in adult life with long-term recovery [4–6]. In fact, literature reviews (Brown [7]), meta-analyses, epidemiological studies [8], and cross-cultural research [9, 10] tend to show a direct relationship between childhood psychological trauma and PTSD.

Psychological trauma has a stable and strong relationship with neurological changes in the hypothalamicpituitary-adrenal (HPA) axis. Cortisol (hydrocortisone) is a glucocorticoid steroid hormone that is considered one of the most powerful and stable biological reciprocal modulators of HPA axis neuronal behavior associated with stress [11]. Changes in plasma cortisol levels have been found in individuals that have experienced an early life trauma, with an adult diagnosis of PTSD or depression [12, 13], or even in the absence of a clinical psychological disorder [14]. Since cortisol markers show a strong association with childhood psychological trauma (Yehuda et al. [15]) they are ideal for assessing the biological effects of psychological or psychiatric treatment. Indeed, intervention strategies to modify cortisol levels are becoming some of the new focal approaches to PTSD treatment [16].

The principal goals of pharmacotherapy focus on the reduction of PTSD symptoms, the improvement of resilience to stress and quality of life, and the minimization of disability and comorbidity. Fluoxetine, one of the selective serotonin reuptake inhibitors (SSRIs), interacts with corticotropin-releasing hormone (CRH) and norepinephrine (NE) systems in coordinating affective and stress responses (Vermetten and Lanius [17]). It has shown efficacy in reducing symptom severity and in relapse prevention in PTSD patients [18, 19]. It can normalize hyperactivity of the HPA axis or decrease plasma cortisol [20]. These interactions further suggest that fluoxetine contributes to the reduction of the clinical symptoms of PTSD, including hypervigilance, increased startle, impulsivity, and intrusive memories, though the exact mechanisms involved in these contributions remain uncertain [21]. In the current study, fluoxetine was used as the control psychiatric pharmacological intervention.

Several psychological and psychiatric interventions (e.g., cognitive behavioral therapy, eye movement desensitization and reprocessing, hypnotherapy, etc.) have been developed, studied, and evaluated for their efficacy in the treatment of childhood psychological trauma (e.g., [22–25]). Most of these approaches are likely to require a rather long intervention period, and they tend to be commonly used in combination with psychiatric medications and carry a fairly high financial cost to both the patients and governmental subsidies.

Over the last 10 years, the clinical method of *Spirit-ual-Hypnosis Assisted Therapy* (SHAT) has been developed in Bali, Indonesia (for a detailed description and

procedure of this method) (Lesmana et al. [26]). SHAT has been used widely in clinical practice for the treatment of various psychiatric disorders, emotional distress (especially relating to loss and bereavement), sexual and relational problems, and PTSD [27-29]. The conceptual edifice of the approach centers on memory (re)framing and the unconscious, and particularly on the effects of (childhood) traumatic experiences on the development of psychological dysfunction. It combines spiritual and culturally sensitive elements, psychodynamic framing (especially during hypnotic states), cognitive behavioral therapy, and elements of humanistic therapy. SHAT caught the attention of the international psychotherapy community when a study by Lesmana et al. demonstrated the method's effectiveness in the treatment of psychological trauma in children exposed to the 2002 Bali bombing terrorist attacks.

The current study assessed the biobehavioural effectiveness of Spiritual-Hypnosis-Assisted Therapy on the modification of cortisol levels and PTSD symptom severity in adults with childhood trauma.

Methods

Participants and procedure

Spiritual-Hypnosis-Assisted Therapy (SHAT) is a psychotherapeutic approach focuses on memory and the unconscious mind and considers the effects of proto-experiences on the (dys)functional psychological development of the individual. It utilises spiritual, psychodynamic (in particular clinical hypnosis), CBT, and humanistic therapeutic elements.

The study followed an interventional single-blind randomized control design. A total of 475 adult out-patients (20 years and older) registered with a psychiatric clinic in Denpasar (Indonesia), who were not yet undertaking psychiatric, psychological, or pharmacological treatment, were screened for the presence and severity of PTSD symptoms (through the PCL-C) and childhood trauma (through the CTQ). Additional criteria for study inclusion were (1) a minimum education level of high school; (2) be Balinese Hindus, to minimize inter-religious and cross-cultural biases; (3) absence of a psychiatric diagnosis of concomitant psychosis, severe anxiety, organic mental disorder, or bipolar disorder; (4) absence of a medical diagnosis of cardiovascular disease, diabetes mellitus, or kidney disease. Of the above sampling frame, 35 (7.37%) individuals satisfied the study criteria and were all invited to participate. Of them, five individuals declined the offer, either because of the study length (approximately 10 weeks per participant) or because they were uncomfortable with having blood drawn from them. The remaining 30 individuals were randomly allocated into two treatment groups of equal membership,

viz. the experimental group that received SHAT (n=15) and a control group (n=15), who received fluoxetine 20 mg/day for 1 month. However, one participant from the control group failed to appear for the post-treatment data sampling and thus was excluded from the study, leaving the final sample at 29 participants. The demographic characteristics of the final sample are presented in Table 1. The two treatment groups did not differ in gender, marital status, or age, while a difference in levels of education was observed.

Participants were briefed about the general study process, while a full debriefing about the detailed purpose of the study was administered after the data-sampling period to minimize placebo or desirability effects. Informed consent was obtained at the initial pre-treatment assessment, followed by an in-person collection of questionnaire data, including the demographic background information. Both groups were evaluated on their behavioral and biological markers before the intervention and 1 month after the intervention. Blood plasma cortisol was collected twice, 1 day before treatment and 1 month after treatment, between 08.00 and 09.00 a.m. at a microbiology laboratory clinic in Denpasar, Indonesia. The CTQ was only administered pre-treatment, while the PCL-C was administered at both pre-and 1-month posttreatment sampling times. The CURSS was only administered to the SHAT treatment group prior to intervention. All participants in that group showed a high degree of responsiveness to hypnotizability. No adverse events occurred during the study.

The session began with the patient sit on a chair or lie on the couch. They are asked to feel comfortable and relaxed. After they relax, they are asked to focus only on the therapist's voice. Through this process, they are initially instructed to meditate with their eyes closed; after meditating for approximately 5 min, they are instructed to breathe deeply through their nose and slowly exhale through their mouth. These instructions are repeated five times. These deep breaths last for 1 min. Subsequently, the participants' breathing tends to remain constant and deep, without any further guidance from the therapist, at approximately five breaths per minute. At that stage, the therapist may wish to monitor certain behavioural cues on the patients, such as their arm rigidity and immobilisation, their eye catalepsy, and their inability to communicate, all of which are widely accepted indicators of a hypnotic state—which within the context of SHAT is referred to as a trance state.

When in trance, the patients are verbally guided through suggestion, to be sensitive to their personal spirit, and to go back to the memories of unhappy experience(s), where they felt angry, disappointed, ashamed, sad, scared, worried, or any specific situation of relevance to their problem, if it were clearly identified during the clinical interview. When they remember, feel, or see the experience(s), they are asked to express all unpleasant feelings by, for example, shouting, crying, or even hitting something. The purpose of this is for them to release the emotional burden that has been stored in their memory. They are led to their culturally familiar concept that the power of God would help them gain strength to cope with their emotions. These emotional discharges are different to abreactions, because of absence of catharsis experience, since the participants are in a hypnotic state, and unaware of experiencing any conflictual material. When they have finally expressed their emotions and returned to a calmer state, they are asked to see the experience(s) again, to help them understand

Table 1 Demographic data participant comparisons

| Demographics | Total <i>N</i> (%) | SHAT <i>n</i> (%) | Control n (%) | Statistics |
|--------------------|--------------------|-------------------|---------------|---------------------|
| Gender | | | | |
| Female | 16 (55.2) | 9 (60) | 7 (50) | $X^2(1, 29) = 0.29$ |
| Male | 13 (44.8) | 6 (40) | 7 (50) | p = 0.59, 2-tailed |
| Education level | | | | |
| High school | 9 (31) | 1 (6.7) | 8 (57.1) | $X^2(2, 29) = 8.67$ |
| Technical College | 4 (13.8) | 3 (20) | 1 (7.1) | p < 0.05, 2-tailed |
| University | 16 (55.2) | 11 (73.3) | 5 (35.7) | |
| Marital Status | | | | |
| Single/not married | 8 (27.6) | 4 (26.7) | 4 (28.6) | $X^2(2, 29) = 1.17$ |
| Married | 20 (69) | 11 (73.3) | 9 (64.3) | p = 0.56, 2-tailed |
| Divorced | 1 (3.4) | = | 1 (3.4) | |
| Age (in years) | | | | |
| M | 33.28 | 34.33 | 32.14 | t(27) = 1.04 |
| SD | 5.66 | 5.21 | 6.10 | p = 0.31, 2-tailed |

why they touched and affected them in such a way. They are then instructed to accept the experience(s) happening in their life and to understand that the those were important in developing them to be or become an adult, and that they enabled them to face new challenges in life.

In the last step, they need to accept that these experiences belong to the past, and it is made continually clear to them that their reality is their life in today, tomorrow, the day after, and so on. If they are not able to understand, or see the situation again, or are unable to reframe it into a positive context, they are asked to look deeply (even in a figurative sense) into the eyes of their mother, who, if possible, should be present at this stage and feel her eyes. In so doing, a positive meaning of the experience tends to emerge, and subsequently the patients tend not to be influenced by their old emotions. They reframe the experience into having a positive meaning and carrying positive emotions, and accept it as an important element of them becoming a fully psychologically developed individual.

Continuing in hypnosis, they may be asked to go to the past, when they were children, in order, once again, to reframe early life traumas or bad memories. They may feel memories of their mother, father, nuclear family, peers, friends, and environmental surroundings. Negative experiences are reframed to have a positive meaning. An example of a suggested reframing could be "Although you were angry (or scared) when your father hit you, he did it to help you become a better person". For them to feel safe, loved, and respected by their parents it is important to positively influence and guide their thoughts, emotions, behaviour, adaptation, and their ways of communicating and relating to others.

In the final part of the session, they are asked to find the way to happiness in future life—material and spiritual. Toward the end of the session, forgiveness and apology may be suggested. At the end of the session, the patient is guided back to their usual state of consciousness and may be given brief explanations to help them understand the psychological interpretation of the experience(s) expressed and felt while in trance.

The whole session is an individual and one time session with duration over an hour. For continuing results, the patients are instructed to meditate for 10–15 min, twice a day, morning and evening, or before going to sleep. Meditation helps with the maintenance of a psychophysiological homeostatic state, and as a result, it allows the self-modification of memories to work faster and be more efficient. The session was made by one of the author, Luh Ketut Suryani. Her training of hypnosis was based on Western concepts but the spiritual element were the concepts of Balinese culture and Hinduism, where every person consists of mind, body, and spirit,

which largely constitute one's biology and psychology, and are influenced by and in return affect, positively or negatively, one's sociocultural environment and religious/spiritual beliefs.

Measures

Cortisol. Plasma cortisol was measured by radioimmunoassay with the commercially available kit (Bayer ADVIA Centaur, Germany). The intra-assay and interassay CVs observed for quality assessment samples $(4.30-22.40~\mu g/dL)$ were less than 10%, respectively.

Posttraumatic Stress Disorder Checklist-Civilian version (PCL-C) (Weathers et al. 1991) is a widely used self-report measure that assesses PTSD symptoms following noncombat-related traumas. It consists of 17 items that assess PTSD symptomatology as defined by the ICD-10. Responses in each item are registered on a 5-point scale, ranging from (1) not at all to (5) extremely; higher scores are indicative of more severe PTSD symptomatology. The instrument also comprises three subscales that correspond to the ICD-10 symptom: re-experiencing, avoid-ance/numbing, and hyperarousal. Due to sample size restrictions, only the total PCL score was used in this study.

Childhood Trauma Questionnaire (CTQ) [30] is a 28-item retrospective self-report questionnaire designed to assess five dimensions of childhood maltreatment (five items in each dimension): physical abuse, emotional abuse, sexual abuse, physical neglect, and emotional neglect (plus a three-item minimization/denial scale—not used in the current study). Item response categories are scored from 1 (never true) to 5 (very often true) and are structured to reflect the frequency of maltreatment experiences.

Carleton University Responsiveness to Suggestions Scale (CURSS) (Spanos et al. [31]). This is a standardized scale of hypnotizability that consists of seven suggestions. The scale yields an objective score (CURSS: O) that reflects the number of suggestions to which subjects made the appropriate behavioral response and an involuntary score (CURSS: I) that reflects the intensity of their subjective responses to hypnotisability.

All the above psychometric instruments were backtranslated to Indonesian prior to admission. The internal consistency reliabilities of these instruments in the current study are shown in Table 2.

Results

Table 1 shows demographic data for this study. The mean age of the sample for this study is 33.28 years (SD = \pm 5.66). There are no statistically significant differences of the gender group with 55.2% female and 44.8% male (X^2 (1, 29) = 0.29). 60% of female and 40% of male

Table 2 Between-subjects ANCOVA, showing the effect of treatment (SHAT vs. Control) on total PTSD clinical symptom differences (PCL-C_{diff}) and cortisol level differences (Cortisol_{diff}), controlling for the effects of the CTQ factors

| | | Mean* | Std. error | F | df | P | Partial eta sqr | Power _{0.05} |
|-------------------------------|---------|----------------|------------|------|-------|------|-----------------|-----------------------|
| PCL-C _{diff} | SHAT | – 27.43 | 2.51 | 4.41 | 1, 24 | 0.04 | 0.15 | 0.52 |
| | Control | — 19.75 | 2.60 | | | | | |
| Cortisol _{diff} (µg/ | SHAT | - 0.43 | 1.36 | 0.18 | 1, 24 | 0.68 | 0.01 | 0.07 |
| dL) | Control | - 1.26 | 1.41 | | | | | |

^{*}Negative signs indicate a reduction in the levels of the respective variables

received the SHAT. The educational level showed 55.2% individuals with university education level, with 73.3% of them received SHAT. The other education level group, which are high school (31%) and technical college (13.8%) (X^2 (2, 29) = 8.67). In addition, as much as 69% individuals is married, where 73.3% of the married group received SHAT. There is also 27.6 individual who were single and 3.4% was divorced. 26.7% of the single marital status group received the SHAT.

Childhood trauma was measured using the Childhood Trauma Questionnaire (CTQ), a 28-item screening questionnaire intended to quantify self-reported childhood trauma history in the home. The CTQ measures childhood trauma using five subscales: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. The CTQ subscale scores have test-retest reliability coefficients ranging from. 79 to 86, and internal consistency coefficients ranging from. 66 to 92 across initial validation samples [30]. Responses are measured on a 5-point Likert scale (1 = never true, 2 = rarely true, 3=sometimes true, 4=often true, 5=very often true). Each subscale is represented by five questions with a score range from 5 to 25; scores fall into four categories: none to low trauma exposure (5-10), low to moderate trauma exposure (11-15), moderate to severe trauma exposure (16-20) and severe to extreme trauma exposure (21-25) for each scale. For example, young adults were asked to provide responses to the following statements: "People in my family look out for each other," "I thought that my parents wished I had never been born," and "I believe I was sexually abused." Some items are reverse coded. The CTQ also contains a minimization/denial scale (three questions), that screens for the likelihood of underreporting traumatic experiences.

Statistical analysis

There are no statistically significant differences between the two treatment groups on the three reliable factors of the CTQ (viz. physical abuse, sexual abuse, and emotional neglect; the factors of emotional abuse and physical neglect were not used in the analyses due to their

Table 3 Cronbach's alpha internal consistency reliabilities and descriptive statistics of the psychometric scales (unreliable alphas are in italic)

| Instrument | Factors | М | SD | а |
|---------------------------------|-------------------|-------|------|------|
| CTQ | Emotional abuse | 10 | 3.33 | 0.57 |
| | Emotional neglect | 11.41 | 4.03 | 0.62 |
| | Physical abuse | 7.03 | 2.76 | 0.80 |
| | Physical neglect | 9 | 3.23 | 0.57 |
| | Sexual abuse | 6.45 | 2.78 | 0.80 |
| PCL-C _{pre-treatment} | Total | 47.83 | 9.18 | 0.73 |
| | Avoidance | 3.55 | 2.08 | 0.73 |
| | Hyperarousal | 3.45 | 1.21 | 0.75 |
| | Re-experience | 2.45 | 1.40 | 0.71 |
| PCL-C _{post-treatment} | Total | 24.10 | 7.95 | 0.91 |
| | Avoidance | 0.59 | 1.02 | 0.77 |
| | Hyperarousal | 0.41 | 0.95 | 0.77 |
| | Re-experience | 0.38 | 0.98 | 0.85 |

rather unreliable alphas, see Table 3) were observed at both multivariate (Wilks' Lambda = 0.95, p = 0.73, partial eta squared=0.05, power $_{0.05}$ =0.13) and bivariate levels. This result suggests that individuals in both groups reported similar levels of childhood trauma. Furthermore, the two treatment groups had similar levels of pre-treatment plasma cortisol, t (27)=1.06, p = 0.30, two-tailed. Two one-way ANCOVAs were utilized (Table 3) to assess the effect of treatment (SHAT vs. Control) on the difference in the levels of PTSD clinical symptoms (post levels minus pre levels) and on the difference in cortisol levels (post levels minus pre levels), controlling for the effects of the three reliable factors of the CTQ. SHAT was statistically significantly better than the fluoxetine treatment at reducing the overall clinical symptoms of PTSD, F(1, 24) = 4.41, p < 0.05, partial eta squared = 0.15, power $_{0.05}$ = 0.52. No statistically significant effect differences between the two groups on cortisol levels were observed F(1, 24) = 0.18, p = 0.68, partial eta squared=0.01, power $_{0.05}$ =0.07, with both groups resulting in similar levels of cortisol reduction.

Discussion

Posttraumatic stress disorder

The culturally sensitive variation of clinical hypnosis therapy utilized in this study resulted in a larger and substantial decrease of the clinical symptoms of PTSD compared to the control pharmacological treatment. This result supports and extends previous empirical suggestions (Schottenbauer et al. 2008) that emphasise the importance of interventional elimination of the psychological effects of damaging experiences, such as irritations of discrimination, self-regulation, and dysfunctional cognitive integrations, in the treatment of traumatised individuals [32]. The therapeutic mechanisms of SHAT directly target pathogenic and pathoplastic traumatic memories and focus on positive reframing, while both respecting and utilizing relevant cultural beliefs and practices. Such an arguably holistic approach indeed appears to effectively minimise the adverse psychological effects of traumatic experiences and thus result in a significant improvement of the clinical symptoms of PTSD.

Cortisol

In relation to the modification of the biological marker of plasma cortisol, the study's results identified two rather interesting points. First, both treatment conditions resulted in a decrease in cortisol levels. This finding agrees with previous studies that have reported elevated levels of cortisol in PTSD patients compared to non-pathological norms (see study by Inslicht et al. in 2006) [33]. However, a number of studies have observed an inverse effect, with cortisol levels in PTSD patients being lower than those in normal controls (e.g., [34–36]). Finally, other studies simply identify abnormal cortisol fluctuations in traumatised individuals (e.g., [37-39]). This evidence inconsistency may be, at least partially, attributed to between-studies methodological variability, such as time of sampling (cortisol levels fluctuate diurnally), heterogeneity of samples, duration, type, or severity of trauma, sampled media (e.g., plasma, urinal, or salivary cortisol markers), absence of experimental control for possible lifestyle cortisol modifiers (e.g., participants' consumption of coffee, magnesium, or Omega-3 fatty acids), and possible presence of undiagnosed (subclinical) concomitant health problems, such as Addison's disease or Cushing's syndrome. Such absence of evidence clarity certainly warrants further research in this area, while it only permits the conclusion that in the current study the clinical spiritual-hypnosis intervention exhibited the same directionality in the modification of cortisol to the fluoxetine treatment.

The second point of interest in our findings is that clinical hypnosis had a similar effect on cortisol

modification to the pharmacological treatment. This is indeed an impressive, novel, and in earnest rather unexpected finding. Given that SHAT is a non-invasive, medication-free, rather easily practiced, and economic treatment, the current study's evidence suggests that it should be preferred to pharmacological interventions.

We found several limitations of this study. First of all, the therapeutic action of SSRIs may delay to 6 weeks or more; therefore, fluoxetine group may show more improvement in the following weeks. The other limitations of this study was that we did not have experimental control for possible lifestyle cortisol modifiers, such as participants' consumption of coffee, magnesium, or Omega-3 fatty acids level. Finally, the short time for follow-up of evaluation.

Recommendation for further research is needed, that should include immune-neuroendocrine interaction effect as biological markers, to fully appreciated and understand the therapeutic power of SHAT on the treatment of PTSD. The use of a more accurate and advanced means of measurement such as fMRI and PET scan will make it possible to observe the changes taking place in the central nervous system so that the changes taking place as a result of the SHAT application can be explained from the neuroimaging perspective.

Conclusions

Spiritual-Hypnosis Assisted Therapy for PTSD patients with childhood trauma appears to have a noteworthy effect in reducing PTSD clinical symptoms and results in a comparable to the pharmacological treatment modification of the HPA axis cortisol markers. Improved understanding of the behaviour of the neuroendocrine system in PTSD participants is important, as it should lead to the development of enhanced and more efficacious interventions. The current study's outcomes stimulate further research in culturally sensitive clinical hypnosis treatments for PTSD, assist with the understanding of the underlying relationships with the neuroendocrine system, and offer an alternative therapeutic solution to the prevention of biobehavioural dysregulation in traumatized individuals.

Abbreviations

CRH: Corticotropin-releasing hormone; CURSS: Carleton University Responsiveness to Suggestions Scale; CTQ: Childhood trauma questionnaire; DSM: Diagnostic and statistical manual of mental disorders; HPA: Hypothalamic-pituitary-adrenal; NE: Norepinephrine; PCL-C: Posttraumatic Stress Disorder Checklist-Civilian version; PTSD: Post traumatic stress disorder; SHAT: Spiritual-hypnosis assisted therapy; SSRIs: Selective serotonin reuptake inhibitors.

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Author contributions

CBJL and LKS was the first one to come up with the idea for this systematic review and was a major contributor in writing the manuscript. CBJL collect and analyze the data for the available publication and previous research regarding this topic also contribute in writing the manuscript. LKS and NT contributed to review the data analyzed. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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