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# Differentiating Nonsuicidal Self-Injury (NSSI) From Suicide Attempts in Adolescents: A Pilot Study

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## ABSTRACT

**Background:** Differentiating between nonsuicidal self-injury (NSSI) and suicide attempts (SA) in adolescents is crucial due to distinct underlying motivations, treatment needs, and potential outcomes associated with each behavior. This study explores biomarkers, specifically β-endorphin and endocannabinoids [anandamide (AEA), 2-arachidonoylglycerol (2-AG), N-palmitoylethanolamide (PEA), and oleoylethanolamide (OEA)], in adolescents admitted for NSSI and/or SA.

**Objectives:** We aimed to explore potential differences in different variables among adolescents hospitalized for NSSI, SA, or both.

**Method:** This pilot study enrolled 68 adolescents admitted for NSSI and/or SA at Puerta de Hierro University Hospital. Excluding those with endocrine issues, participants underwent clinical assessments, blood collection, and serum analysis for ACTH, cortisol, and β-endorphin, with endocannabinoids measured via LC–MS. Clinical tools, such as the MINI Kid and CTQ and statistical analyses via SPSS and Stata aimed to elucidate behavioral and biological differences between NSSI and SA groups.

**Results:** No sociodemographic factor differentiated the groups. Gestational diabetes and depression during pregnancy were more frequent in the NSSI group ($p = 0.047$ and $p = 0.003$, respectively). Abnormal presentation at birth was overrepresented in the SA group ($p = 0.001$). Familial antecedents of either suicide or SB were more frequently reported in the NSSI + SA group ($p = 0.027$ and $p = 0.035$, respectively). Blood PEA and β-endorphin were significantly elevated in the SA group ($p < 0.006$ and 0.014, respectively).

**Discussion:** This study identified shared risk factors across NSSI and SA, with distinct variables for each group, including β-endorphin and PEA biomarker elevations linked to SA.

**Highlights**

- Mental pain and all types of childhood maltreatment and/or negligence are frequent in adolescents hospitalized for suicide attempts (SA) and/or nonsuicidal self-injury (NSSI).
- Sociodemographic factors, mental pain, and childhood maltreatment and/or negligence do not differentiate between adolescents hospitalized for either SA or NSSI.
- Gestational diabetes and depression during pregnancy are more frequent in adolescents hospitalized for the NSSI group.
- Abnormal presentation at birth is more frequent in adolescents hospitalized for SA.
- Familial antecedents of either suicide or SB are more frequent in hospitalized adolescents for both NSSI and SA.
- Blood PEA and $\beta$-endorphin are higher in adolescents hospitalized for SA than those hospitalized by either NSSI or both NSSI and SA.

## 1 | Introduction

Differentiating between nonsuicidal self-injury (NSSI) and suicide attempts (SA) during adolescence is crucial due to distinct underlying motivations, treatment needs, and potential outcomes associated with each behavior. NSSI refers to deliberate self-harm without the intent to die, used as a maladaptive means of coping with emotional distress, according to the *Diagnostic and Statistical Manual of Mental Disorders (DSM)* [1]. In contrast, SA involves the explicit intent to die. In our study, SA was defined as "a self-destructive behavior with intent to end one's life independent of resulting damage" [2]. Accurately distinguishing between these behaviors is essential for developing specific therapeutic interventions: treatments for SAs often prioritize immediate life-saving measures, whereas interventions for NSSI focus on long-term coping strategies and emotional regulation [3]. However, this differentiation is complicated by overlapping features, such as the use of similar methods (e.g., cutting and overdosing), comorbid mental health conditions like depression and anxiety, and the possibility that repeated NSSI may escalate to SAs [4]. Furthermore, adolescents may find it difficult to clearly articulate their intent, complicating clinical assessments [5]. These challenges highlight the need for more refined diagnostic criteria and robust assessment tools to ensure accurate differentiation.

Current research highlights the complexity of both NSSI and SA, noting considerable overlap in their psychological and environmental risk factors. Studies have identified shared risk factors such as family dysfunction, a personal history of childhood trauma, depression, and anxiety, among others, all of which can contribute to both NSSI and SA [6]. Neurobiological research has also begun to elucidate underlying mechanisms, indicating that both behaviors may involve dysregulation of emotional processing, impulsivity, and altered pain perception [7]. Moreover, the frequency and severity of NSSI have been identified as predictors of future SA, indicating that NSSI may act as a precursor to more lethal forms of self-harm [8]. Despite these advancements, there remains a critical need for more precise biomarkers and predictive models to reliably differentiate between NSSI and suicidal intent [9, 10].

Our study seeks to address this gap by examining the potential utility of selected scales evaluating well-known suicidal risk factors and several biomarkers to differentiate between NSSI and SAs in adolescents. We studied the serum concentrations of ACTH (pg/mL), cortisol (μg/dL), $\beta$-endorphin (pg/mL), and four endogenous cannabinoids (endocannabinoids) within the endocannabinoid system (ECS) (pmol/mL)—anandamide (AEA), 2-arachidonoylglycerol (2-AG), N-palmitoylethanolamide (PEA), and oleoylethanolamide (OEA). The rationale for the selection of biomarkers is grounded in three models, namely, the stress–diathesis model of suicide formulated by John Mann [11], the pain model of SB [12], and the Blasco-Fontecilla's suicidal addiction model [13, 14]. According to the stress–diathesis formulation of suicide, dysregulation of stress-responsive neuroendocrine systems constitutes a diathesis that interacts with psychosocial stressors to increase the likelihood of suicidal acts; correspondingly, HPA-axis markers such as ACTH and cortisol have been repeatedly implicated in suicidal ideation and attempts and therefore provide a direct measure of the biological stress response relevant to Mann's model [11]. The seminal pain model of suicide postulated by Shneidman [12] stressed the role of mental pain (suffering) as the core of suicide. Not surprisingly, the endogenous opioid system has been proposed to mediate the affect-modulating, analgesic, and reinforcing consequences of self-harm, and empirical work has found altered $\beta$-endorphin levels in individuals with NSSI and in those characterized as "addicted" to suicidal behavior (SB), making it a mechanistically plausible marker for Blasco-Fontecilla's suicidal addiction hypothesis [13, 14]. $\beta$-endorphins, involved in pain modulation and emotional regulation, may differ in individuals engaging in NSSI compared with those with suicidal intent, reflecting differences in the neurobiological pathways activated during each behavior [15].

The addictive hypothesis of self-harm has gained some empirical support, and it is related to the concepts of major suicide repetition (suicide attempters with five or more suicide attempts) [16] and emptiness [17]. Finally, the ECS modulates stress reactivity, reward, affect regulation, and impulsivity—domains central to both stress–diathesis and addictive frameworks—and early clinical studies report altered circulating AEA and PEA in suicide attempters as well as broader ECS alterations in mood and stress-related disorders, justifying measurement of AEA, 2-AG, PEA, and OEA to probe putative ECS contributions to NSSI and SA [18, 19]. In other words, the ECS, which plays a key role in mood regulation and stress response, may display distinct patterns within these populations [20].

Taken together, these markers map onto converging neurobiological pathways (stress responsivity, pain/affect modulation, and reward/addiction circuitry) that are explicitly invoked by Mann's and Blasco-Fontecilla's models; measuring them in parallel allows (a) direct tests of HPA axis dysregulation as a diathesis, (b) assessment of opioid system involvement in reinforcement/relief processes hypothesized to sustain repetitive

self-harm, and (c) exploration of ECS alterations that may bridge stress and reward-based mechanisms implicated in both NSSI and SA.

By investigating these biomarkers, we aim to develop more objective, biologically based tools that can complement clinical assessments, ultimately improving the accuracy of diagnoses and the effectiveness of interventions. This approach could enhance understanding of the physiological underpinnings of NSSI and SA, paving the way for more targeted and personalized treatment strategies for at-risk adolescents. Given the complexity of differentiating between NSSI and SA in adolescence and the complex measurement of both β-endorphin and endocannabinoids, our study should be considered exploratory in nature.

## 2 | Methods

### 2.1 | Participants

A total of 68 adolescents, aged 12–17 years, were admitted to the emergency department or acute inpatient unit at Puerta de Hierro University Hospital (Majadahonda, Spain) for either NSSI and/or SA and were enrolled between February 25, 2021, and February 23, 2022. All participants were fluent in Spanish. Patients with endocrine disorders or those undergoing hormone treatments (except for oral contraceptives) were excluded. Participants and their legal guardians provided informed consent after receiving an explanation of the study, which was approved by the local ethics committee (IRB Number 82/20: February 23, 2021). The principal investigator (PI; HBF), together with another researcher (EW), assessed all participants to collect sociodemographic and clinical data. All biological assessments took place within 48 h of admission. All clinical diagnoses were made by clinical psychiatrists using DSM-5. Axis I diagnoses were determined through the Mini-international neuropsychiatric interview (MINI) [21]. Information was confirmed and supplemented through medical records. Childhood abuse and neglect were evaluated using the Spanish 28-item childhood trauma questionnaire (CTQ) [22]. The CTQ reliably measures emotional, physical, and sexual abuse, along with emotional and physical neglect. Each item is scored from 1 (never) to 5 (very often), producing trauma-specific scores from 5 to 25. Distinct cutoff scores were established for each trauma category, classifying maltreatment severity as none, low, moderate, or severe; these criteria have shown high specificity and sensitivity [23].

The protocol included self-reported NSSI and SA. An SA was defined as "self-destructive behavior with the intent to end one's life, independent of the resulting damage" [24, 25]. We used three scales to measure suicidal behavior (SB). First, the short form of the Personality and Life Event Scale (S-PLE) is a six-item instrument specifically developed to enhance the identification of individuals at risk for SB [26]. The full version of the PLE was developed by selecting the most discriminative variables from commonly used suicide risk assessment scales and sociodemographic factors (age and gender). The PLE demonstrated an area under the curve (AUC) of 0.92 (average accuracy: 86.4%, specificity: 89.6%, and sensitivity 80.8%) for identifying suicide attempters. The S-PLE demonstrated greater accuracy, sensitivity, and specificity in identifying individuals at risk than the original PLE [25]. Second, four questions from the *Self-Injurious Thoughts and Behaviors Interview* (SITBI) [5] were used, assessing the function of SA precipitants (Likert scale: 0–4): automatic negative reinforcement ("To stop bad feelings"), automatic positive reinforcement ("To feel something, because you felt numb or empty"), social negative reinforcement ("To avoid punishment"), and social positive reinforcement ("To get attention"). Finally, a short, psychometrically sound measure of unbearable psychache, known as the *unbearable psychache scale* (UP3), was employed [27]. The UP3 was developed by selecting three items from the original psychache scale (13-item) [28], focusing on the closest semantic parallels of "unbearable" or "intolerable" psychological pain.

### 2.2 | Blood Collection

Blood was collected from all participants to obtain serum samples between 7:30 and 8:30 a.m. The samples for biochemistry were two: a plasma tube with EDTA-K for ACTH determination and a serum tube with gel for cortisol determination. Immediately upon collection, a portion of the samples was processed to measure ACTH (pg/mL) and cortisol (μg/dL). The rest of the blood was collected in serum separator tubes and left to clot for 15–30 min at room temperature. The samples were then centrifuged at 3500 rpm for 10 min to separate the serum, which was then stored at −80°C for subsequent β-endorphin assays. Additionally, peripheral blood mononuclear cells (PBMCs) were isolated from the samples using a Ficoll-Hypaque gradient and stored at −196°C (in liquid nitrogen) until they were assayed for β-endorphin levels. Both serum and PBMCs β-endorphin levels were measured. Serum samples were anonymized to ensure that laboratory personnel were blind to clinical information.

### 2.3 | Assay Procedures for ACTH, Cortisol, β-Endorphin, and Endocannabinoids

ACTH (pg/mL) and cortisol (μg/dL) levels from serum collected between 7:30 a.m. and 8:30 a.m. were measured via chemiluminescence. Serum β-endorphin levels (pg/mL) were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit [Cloud-Clone Corp (CEA806Hu), range 12.35–1000 pg/mL] following the manufacturer's instructions. Endocannabinoid levels in serum (pmol/mL) were measured using high-resolution liquid chromatography with mass spectrometry (LC–MS).

### 2.4 | PBMC Isolation and LC–MS Analyses

PBMC samples for LC–MS analysis were prepared by protein precipitation with acetonitrile, followed by homogenization in methanol containing 5 μL (1 μg/mL) of deuterated internal standards (2-AG-d8, AEA-d8, OEA-d4, and PEA-d4) (Cayman Chemical). Lipid extraction was performed with chloroform/

methanol (2:1) with 0.1% formic acid. The organic phase was dried by a SpeedVac system at 55°C (Thermo Fisher Scientific), and the samples were subsequently reconstituted in methanol and analyzed using an Eksigent nano/microLC 425 system coupled to a TripleTOF 6600 plus mass spectrometer (SCIEX) with a Luna Omega 3 μm Polar C18 column (Phenomenex) at 30°C. Mobile phases consisted of 0.1% formic acid (Phase A) and acetonitrile (Phase B). Data were analyzed using SCIEX OS software, and individual signals were normalized using internal standards. The analytical method was validated by evaluating its calibration range, linearity, limit of detection, limit of quantification, carryover, accuracy, precision, selectivity, recovery, and matrix effect. Further information on endocannabinoid management can be found in Supporting Information S1.

### 2.5 | Statistical Analyses

A total of 68 patients were categorized into three groups: the NSSI group ($n = 11$) comprised adolescents with nonsuicidal self-injury but no history of SA; the SA group ($n = 5$), consisting of adolescents with SA but no history of NSSI; and the NSSI-SA group ($n = 52$), which included adolescents with both lifetime NSSI and SA. Descriptive statistics were calculated as absolute and relative frequencies for categorical data and mean (standard deviation) for numerical data. Univariable analyses employed chi-square or Fisher's exact tests for categorical variables and one-way ANOVA for numerical variables. Regarding the CTQ scale, in addition to subscale analyses, item-level analyses were implement to identify fine-grained differences that would not be detectable through aggregated scores. A significance level of $p < 0.05$ was considered statistically significant. All analyses were conducted using SPSS v.20 (Macintosh) and Stata/IC v.15.1. (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.).

## 3 | Results

### 3.1 | Sociodemographic Features

No single sociodemographic factor differentiated the three groups (see Table 1).

### 3.2 | Familial Antecedents, Obstetric History, and School Performance

Table 2 presents the familial antecedents, obstetric history, and school performance of the three groups. The NSSI group showed a higher frequency of certain pregnancy-related issues, including gestational diabetes and antenatal depression ($p = 0.047$ and $p = 0.003$, respectively). Conversely, abnormal presentation at birth was overrepresented in the SA group ($p = 0.001$). Notably, familial antecedents of either suicide or SB were more frequently reported in the NSSI + SA group ($p = 0.027$ and $p = 0.001$, respectively).

### 3.3 | Clinical and Personal Antecedents

Table 3 provides data on clinical features and personal antecedents. Other than the presence of a psychotic disorder, which was reported by one patient in the NSSI group, no other statistically significant differences emerged among the groups. However, most patients across all groups reported experiencing either adjustment or major depressive disorder.

### 3.4 | Child Maltreatment and Scales Measuring Self-Harm (NSSI and/or SA)

Table 4 presents result of the scales measuring child maltreatment and NSSI/SA. Two CTQ items were different between the subgroups: Item 2 ("Someone to take care of and protect me") was notably higher in the NSSI + SA group, indicating a less nurturing familial environment (as this item is reverse scored). Item 19 ("Family felt close to each other") was notably lower in the SA group. Overall, reliability was very good (Cronbach's alpha = 0.875).

### 3.5 | Biomarkers

Regarding biomarkers, two serum biomarkers, PEA and β-endorphin (pg/mL), showed statistically significant differences among the three groups (see Table 5 and Figures 1 and 2). Interestingly, both biomarkers were significantly elevated in the SA group without a history of NSSI.

## 4 | Discussion

This study provides valuable insights into clinical phenotypes of self-harm (NSSI and/or SA) in adolescents. Consistent with expectations, we found no clear distinction between the groups based on sociodemographic and clinical features, personal psychiatric histories, familial antecedents, or scales assessing SB. As expected, the scales measuring SB indicated a uniformly high level of emotional pain across all groups but did not differentiate between them. However, we identified statistically significant differences concerning familial antecedents, obstetric history, school performance, two CTQ items, and two biomarkers (serum PEA and β-endorphin). These results are presented separated by groups to better capture potential differences among them.

### 4.1 | Specific Risk Factors in Each Group

The NSSI group more frequently reported gestational diabetes and depression during pregnancy. Both conditions during pregnancy have been associated with future mental health issues in offspring [29–33]. Moreover, abnormal presentation at birth was overrepresented just in the SA group. Unfortunately, there is fewer literature linking pregnancy-related events with either NSSI or SA. In one study, several perinatal factors [higher maternal parity, younger maternal age (< 25 years), and low birth weight (< 2500 g)] were independently related to higher

**TABLE 1** | Sociodemographic features ($N = 68$).

| | NSSI $n = 11$ (%) | SA $n = 5$ (%) | NSSI and SA $n = 52$ (%) | Total $n$ (%) | $\chi^2$ | df | $p$-value |
|---|---|---|---|---|---|---|---|
| Sex | | | | | 0.86 | 2 | 0.65 |
| Female | 10 (90.9) | 4 (80) | 48 (92.3) | 62 (91.2) | | | |
| Adopted | | | | | 0.025 | 2 | 0.98 |
| No | 9 (81.8) | 4 (80) | 43 (82.7) | 56 (82.4) | | | |
| Living with | | | | | 0.647 | 2 | 0.724 |
| Family of origin | 9 (81.8) | 4 (80) | 37 (71.2) | 50 (73.5) | | | |
| Etnia | | | | | 0.709 | 2 | 0.702 |
| Caucasian | 8 (72.7) | 4 (80) | 39 (75) | 51 (75) | | | |
| Other | 3 (27.3) | 1 (20) | 13 (25) | 17 (25) | | | |
| Socioeconomic level (euros/month) | | | | | 4.59 | 2 | 0.100 |
| > 1500 | 6 (54.5) | 5 (100) | 26 (50.0) | 37 (54.4) | | | |
| Accompanied by | | | | | 2.49 | 4 | 0.646 |
| Mother | 6 (66.7) | 3 (60) | 33 (66.8) | 42 (67.7) | | | |
| Father | 1 (11.1) | 0 (0) | 1 (2.1) | 2 (3.2) | | | |
| Both parents | 2 (22.2) | 2 (40) | 14 (29.2) | 18 (29) | | | |
| Age | mean (Sd) | mean (Sd) | mean (Sd) | mean (Sd) | $F$ | df | $p$-value |
| | 14.55 (1.69) | 15.40 (0.89) | 14.90 (1.49) | 14.88 (1.49) | 0.58 | 2 | 0.563 |

Abbreviations: NSSI, nonsuicidal self-injury; SA, suicide attempt.

**TABLE 2** | Familial antecedents, obstetric and perinatal history, and school performance.

| | NSSI $n = 11$ (%) | SA $n = 5$ (%) | NSSI and SA $n = 52$ (%) | Total $n$ (%) | $\chi^2$ | df | $p$-value |
|---|---|---|---|---|---|---|---|
| Familial antecedents of suicide | 0 (0) | 0 (0) | 4 (14.8) | 4 (11.4) | 10.925 | 4 | **0.027** |
| Familial antecedents of suicidal behavior | 0 (0) | 0 (0) | 8 (28.6) | 8 (22.2) | 10.369 | 4 | **0.035** |
| Gestational diabetes | 3 (33.3) | 0 (0) | 3 (7) | 6 (10.5) | 6.134 | 2 | **0.047** |
| Depression during pregnancy | 3 (33.3) | 0 (0) | 1 (2.2) | 4 (6.8) | 11.883 | 2 | **0.003** |
| Urgent cesarean | 3 (30) | 1 (20) | 6 (13) | 10 (16.4) | 1.775 | 2 | 0.412 |
| Forceps | 1 (10) | 1 (20) | 10 (21.7) | 12 (19.7) | 0.717 | 2 | 0.699 |
| Abnormal presentation at birth | 0 (0) | 2 (40) | 1 (2.2) | 3 (4.9) | 14.418 | 2 | **0.001** |
| Birth weight < 2 kgs | 2 (20) | 2 (40) | 7 (15.2) | 11 (18) | 8.506 | 4 | 0.075 |
| First caring figure | | | | | 15.152 | 8 | 0.056 |
| Both parents | 7 (77.8) | 5 (100) | 32 (72.7) | 44 (75.9) | | | |
| Language development (first words before 2 years) | 7 (70) | 4 (100) | 40 (85.1) | 51 (83.6) | 2.952 | 4 | 0.566 |
| Motor development (first steps before 1 year) | 8 (80) | 3 (75) | 36 (76.6) | 47 (77) | 0.064 | 2 | 0.968 |
| Bladder control before 6 years | 8 (88.9) | 4 (100) | 35 (76.1) | 47 (79.7) | 3.816 | 4 | 0.431 |
| Fecal continence before 6 years | 9 (100) | 4 (100) | 37 (80.4) | 50 (84.7) | 3.001 | 4 | 0.558 |
| School failure (at least 1 year) | 0 (0) | 2 (40) | 21 (41.2) | 23 (34.8) | 18.939 | 4 | **0.001** |
| Special education needs | 1 (9.1) | 3 (60) | 20 (39.2) | 24 (35.8) | 9.347 | 4 | 0.053 |
| School measures for learning problems | 2 (18.2) | 3 (60) | 21 (41.2) | 26 (38.8) | 3.037 | 2 | 0.219 |
| Individualized education program (IEP) | 2 (18.2) | 0 (0) | 17 (33.3) | 19 (28.4) | 3.161 | 2 | 0.206 |
| Enrichment programs | 0 (0) | 1 (20) | 1 (2) | 2 (3) | 5.522 | 2 | 0.063 |
| Supplementary education | 2 (18.2) | 3 (60) | 14 (28.6) | 19 (29.2) | 2.948 | 2 | 0.229 |

Abbreviations: NSSI, nonsuicidal self-injury; SA, suicide attempt.

**TABLE 3** | Clinical features and personal antecedents.

| | NSSI $n = 11$ (%) | SA $n = 5$ (%) | NSSI and SA $n = 52$ (%) | Total $n$ (%) | $\chi^2$ | df | $p$-value |
|---|---|---|---|---|---|---|---|
| Medical antecedents | 7 (63.6) | 3 (60) | 30 (63.8) | 40 (63.5) | 0.029 | 2 | 0.986 |
| Taking any medication | 9 (81.8) | 2 (40) | 39 (81.2) | 50 (78.1) | 4.615 | 2 | 0.100 |
| Psychiatric antecedents | 9 (100) | 5 (100) | 45 (95.7) | 59 (96.7) | 0.616 | 2 | 0.735 |
| Previous medical or psychiatric drug | 9 (81.8) | 2 (40) | 39 (81.2) | 50 (78.1) | 4.615 | 2 | 0.100 |
| Psychotherapy and/or psychiatric treatment | | | | | 6.862 | 6 | 0.334 |
| Psychotherapy | 3 (27.3) | 1 (20) | 4 (9.3) | 8 (13.6) | | | |
| Drug therapy | 1 (9.1) | 0 (0) | 3 (7) | 4 (6.8) | | | |
| Both | 6 (54.5) | 3 (60) | 35 (81.4) | 44 (74.6) | | | |
| Previous psychiatric hospitalization | | | | | 2.781 | 4 | 0.595 |
| One | 1 (9.1) | 1 (20) | 7 (16.3) | 9 (15.3) | | | |
| Two or more | 0 (0) | 1 (20) | 6 (14) | 7 (11.9) | | | |
| Lifetime alcohol use | 4 (36.4) | 3 (60) | 16 (38.1) | 23 (39.7) | 0.957 | 2 | 0.620 |
| Lifetime cannabis use | 2 (18.2) | 2 (40) | 14 (33.3) | 18 (31) | 1.140 | 2 | 0.565 |
| **Main axis I diagnosis (index episode)** | **NSSI $n = 7$ (%)** | **SA $n = 3$ (%)** | **NSSI and SA $n = 43$ (%)** | **Total $n = 53$ (%)** | $\chi^2$ | **df** | **$p$-value** |
| ADHD | 1 (14.3) | 2 (66.7) | 5 (11.6) | 8 (15.1) | 1.333 | 2 | 0.513 |
| Learning disorder | 0 (0) | 0 (0) | 0 (0) | 0 (0) | | | |
| Generalized anxiety disorder | 0 (0) | 0 (0) | 0 (0) | 0 (0) | | | |
| Major depressive disorder | 5 (71.4) | 1 (33.3) | 25 (58.1) | 31 (58.5) | 1.267 | 2 | 0.531 |
| Eating disorder | 0 (0) | 0 (0) | 5 (11.6) | 5 (9.4) | 1.284 | 2 | 0.526 |
| Psychotic disorder (current) | 1 (14.3) | 0 (0) | 0 (0) | 1 (1.9) | 6.698 | 2 | **0.035** |
| Substance use disorder | 0 (0) | 0 (0) | 0 (0) | 0 (0) | | | |
| Adjustment disorder | 4 (66.7) | 3 (100) | 28 (68.3) | 35 (70) | 1.374 | 2 | 0.503 |

Abbreviations: NSSI, nonsuicidal self-injury; SA, suicide attempt.

suicide risk of offspring as young adults [34]. In another study, the risk of violent suicide attempts increased significantly (OR [95%] = 2.38[1.12–5.08]) in patients born prematurely [35]. A recent study demonstrated that after adjustment, "individuals exposed to maternal infections during pregnancy had higher risk of suicide attempt when compared to non-exposed (IRR 1.46 [1.36–1.56]), particularly those exposed in the second and third trimesters" [36]. The authors concluded that "maternal, but not paternal, infections were associated with later risk of suicide attempt in the offspring, pointing out to a possible role of the intra-uterine environment".

The SA group was the group in which we found more specific characteristics. They were characterized by a higher frequency of abnormal birth presentation, at least one year of school repetition, lower scores on CTQ Item 19 ("Family felt close to each other"), and elevated serum levels of PEA and $\beta$-endorphin. Although there is limited evidence linking abnormal birth presentation to long-term mental health consequences, school failure has been consistently associated with SB [37–42]. Additionally, family issues have been linked to SAs [43].

Perhaps, the most intriguing finding of our pilot study was the elevated serum levels of $\beta$-endorphin and PEA in the SA group. Our findings of an elevated serum $\beta$-endorphin level in SA is consistent with a previous study carried out by our group in adults following a similar methodology, as we also found an elevated serum $\beta$-endorphin level in adults hospitalized due to SA [44]. Others have reported lower levels of serum $\beta$-endorphin in adolescents with NSSI [45, 46] which is also consistent with our findings (patients with NSSI in our sample had lower levels of serum $\beta$-endorphin). Our findings may suggest that serum $\beta$-endorphin level may be a biomarker that allows for differentiating NSSI from SA, thus confirming our hypothesis. Accordingly, an elevated and low-serum $\beta$-endorphin may characterize SA and NSSI, respectively.

Regarding PEA, some authors have suggested that endocannabinoids are relevant in SB [47, 48]. Elevated levels may reflect a buffering effect, as evidence suggests PEA can alleviate pain-related conditions [49]. Recent research in murine models has also shown that PEA improves anxiety and depression symptoms related to reduced monoamine levels [50]. Furthermore, PEA has demonstrated efficacy in treating major depression and bipolar disorder [51]. Moreover, we also found elevated levels of PEA in hospitalized adult suicide attempters after controlling for cannabis use compared with psychiatric controls [19].

**TABLE 4** | Scales evaluating childhood maltreatment and suicidal behavior.

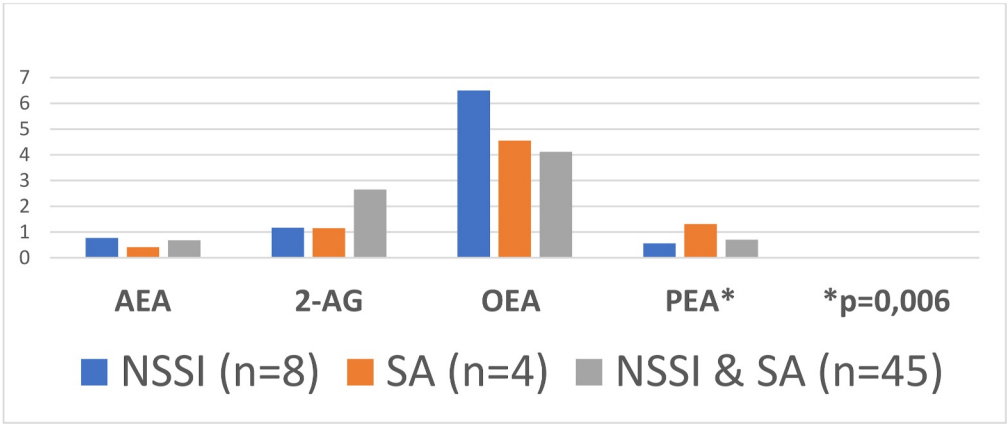
| | NSSI mean (Sd) | SA mean (Sd) | NSSI and SA mean (Sd) | Total mean (Sd) | *F* | df | *p*-value |
|---|---|---|---|---|---|---|---|
| CTQ item 2[a] («someone to take care of and protect me») ($n = 54$) | 0.29 (0.76) | 0.25 (0.50) | **0.40 (1)** | 0.37 (0.94) | 12.893 | 2 | **0.021** |
| CTQ item 19 («family felt close to each other») ($n = 54$) | 1.57 (1.13) | **0.50 (0.58)** | 1.70 (1.60) | 1.59 (1.51) | 15.849 | 2 | **0.027** |
| CTQ emotional abuse ($n = 41$) | 8.67 (5.50) | 7.25 (3.77) | 8.71 (5.72) | 8.56 (5.45) | 7.627 | 2 | 0.885 |
| CTQ physical abuse ($n = 41$) | 3.33 (4.72) | 0.25 (0.50) | 2.26 (4.91) | 2.22 (4.62) | 23.006 | 2 | 0.596 |
| CTQ sexual abuse ($n = 41$) | 3 (5.51) | 0 (0) | 3.84 (5.52) | 3.34 (5.29) | 53.026 | 2 | 0.398 |
| CTQ emotional negligence ($n = 42$) | 7.67 (2.25) | 4.50 (1.73) | 7.22 (5.65) | 8.56 (5.45) | 29.174 | 2 | 0.579 |
| CTQ physical negligence ($n = 43$) | 1.50 (1.38) | 1.25 (1.26) | 3.18 (2.69) | 2.77 (2.53) | 24.515 | 2 | 0.149 |
| S-PLE score ($n = 51$) | 2.99 (0.37) | 2.76 (0.32) | 2.89 (0.69) | 2.89 (0.61) | 0.172 | 2 | 0.801 |
| **S-PLE questions** | **NSSI mean (Sd)** | **SA mean (Sd)** | **NSSI and SA mean (Sd)** | **TOTAL mean (Sd)** | ***t*** | **df** | ***p*-value** |
| I often feel empty inside ($n = 64$) | 10 (100) | 5 (100) | 46 (93.9) | 61 (93.3) | 0.964 | 2 | 0.618 |
| Previous self-harm ($n = 57$) | 10 (100) | 4 (80) | 36 (85.7) | 50 (87.7) | 1.833 | 2 | 0.400 |
| I have been the victim of unfair attacks on my character or reputation ($n = 62$) | 8 (80) | 3 (75) | 34 (70.8) | 45 (72.6) | 0.553 | 4 | 0.968 |
| I think my partner (or lover) may be unfaithful to me ($n = 62$) | 2 (20) | 0 (0) | 8 (67.7) | 10 (16.1) | 2.974 | 6 | 0.812 |
| I can't decide what kind of person I want to be ($n = 64$) | 7 (70) | 4 (80) | 34 (69.4) | 45 (70.3) | 2.422 | 6 | 0.877 |
| I have tantrums or angry outbursts ($n = 64$) | 9 (90) | 4 (80) | 37 (75.5) | 50 (78.1) | 1.031 | 2 | 0.597 |
| **Psychological pain unbearable psychache scale (UP3)** | **NSSI mean (Sd)** | **SA mean (Sd)** | **NSSI and SA mean (Sd)** | **Total mean (Sd)** | ***F*** | **df** | ***p*-value** |
| I can't take my pain any more | 3.67 (1.37) | 3 (1.41) | 3.84 (1.46) | 3.75 (1.44) | 0.622 | 52 | 0.541 |
| Because of my pain, my situation is impossible | 3.17 (1.72) | 2.75 (1.26) | 3.65 (1.46) | 3.53 (1.48) | 0.882 | 52 | 0.420 |
| My pain is making me fall apart | 3.83 (1.17) | 3.75 (0.96) | 4.12 (1.48) | 4.06 (1.40) | 0.203 | 52 | 0.617 |
| **Reported function (0–4 scale) ($n = 42$)** | **NSSI ($n = 6$) mean (Sd)** | **SA ($n = 4$) mean (Sd)** | **NSSI and SA ($n = 43$) mean (Sd)** | **TOTAL ($n = 53$) mean (Sd)** | ***F*** | **df** | ***p*-value** |
| Automatic negative reinforcement ("To stop bad feelings") | 2.83 (1.17) | 3.50 (0.58) | 3.16 (1.07) | 3.15 (1.04) | 0.493 | 52 | 0.614 |
| Automatic positive reinforcement ("To feel something, because you felt numb or empty") | 3 (1.09) | 1.75 (1.71) | 2.79 (1.30) | 2.74 (1.32) | 1.291 | 52 | 0.284 |
| Social negative reinforcement ("To avoid doing something you don't want to do") | 2.33 (1.63) | 3.75 (0.50) | 2.95 (1.30) | 2.94 (1.32) | 1.407 | 52 | 0.254 |
| Social positive reinforcement ("To communicate with someone or get his/her attention) | 0.33 (0.82) | 0.75 (0.96) | 1 (1.37) | 0.91 (1.29) | 0.727 | 52 | 0.488 |

[a]The item has reversed scoring; Automatic negative reinforcement: "To stop bad feelings"; Automatic positive reinforcement: "To feel something, because you felt numb or empty"; Social negative reinforcement: "To avoid doing something you don't want to do"; Social positive reinforcement: "To communicate with someone or get his/her attention".

Finally, the NSSI + SA group more frequently reported familial antecedents of SB, completed suicide, and school repetition and scored higher on CTQ Item 2 ("Someone to take care of and protect me"). This reversed-scored item indicates a perception of inadequate familial care and protection. Familial antecedents of SB or suicide may suggest that self-harming behaviors are influenced by familial factors, potentially reflecting greater severity in this group. A meta-analysis indeed found that adolescents

**TABLE 5** | Serum biomarkers.

| | NSSI ($n = 8$) mean (Sd) | SA ($n = 4$) mean (Sd) | NSSI and SA ($n = 45$) mean (Sd) | Total ($n = 57$) mean (Sd) | *F* | df | *p*-value |
|---|---|---|---|---|---|---|---|
| AEA | 0.77 (0.50) | 0.41 (0.46) | 0.68 (0.75) | 0.67 (0.70) | 0.169 | 56 | 0.717 |
| 2-AG | 1.17 (0.83) | 1.15 (0.29) | 2.65 (2.37) | 2.34 (2.21) | 10.508 | 54 | 0.116 |
| OEA | 6.50 (5.04) | 4.55 (1.54) | 4.12 (3.47) | 4.41 (3.50) | 10.035 | 38 | 0.885 |
| PEA | 0.56 (0.21) | 1.31 (0.87) | 0.70 (0.34) | 0.73 (0.40) | 0.801 | 55 | **0.006** |
| ACTH (pg/mL) | 25.45 (8.92) | 29.8 (14.43) | 28.29 (15.62) | 28.08 (14.78) | 27.618 | 53 | 0.885 |
| Cortisol (blood) (μg/dL) | 15.34 (7.38) | 15.87 (6.29) | 15.10 (6.07) | 15.19 (6.11) | 1.162 | 53 | 0.800 |
| β-endorphin (pg/mL) | 197.47 (30.74) | 289.12 (56.49) | 201.92 (58.70) | 2.77 (2.53) | 14362.064 | 52 | **0.014** |



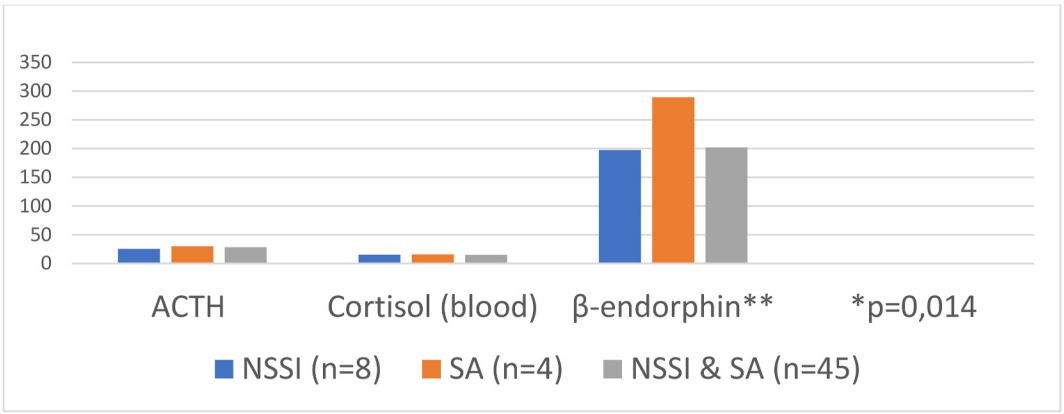




**FIGURE 1** | Serum endocannabinoids (pmol/mL), ACTH (pg/mL), cortisol (μg/dL), and β-endorphin (pg/mL).

presenting both NSSI and SA exhibit greater psychopathological severity [52]. Academic failure has been identified as a significant risk factor for self-harm in young people [53].

## 4.2 | Strengths and Limitations

A major strength of this study is the evaluation of different serum biomarkers and scales under similar conditions, allowing for comparison across the three groups. However, our study also faced limitations inherent to retrospective studies. A key limitation of this study is the small sample size and the imbalance in group sizes (e.g., 5 vs. 52) that may also affect the robustness and interpretability of the statistical analyses. Furthermore, this limitation not only restricted our ability to match groups by age and sex for critical analyses but also reduced our statistical power to detect significant differences.

Given that the complexity of measuring either β-endorphin or endocannabinoids in patients with self-harm is provided, most research to date is hampered by the small sample size. Indeed, our study is probably one of the studies offering a higher sample size in self-harming adolescents. In any case, this limitation also hampered our ability to control for potential confounding

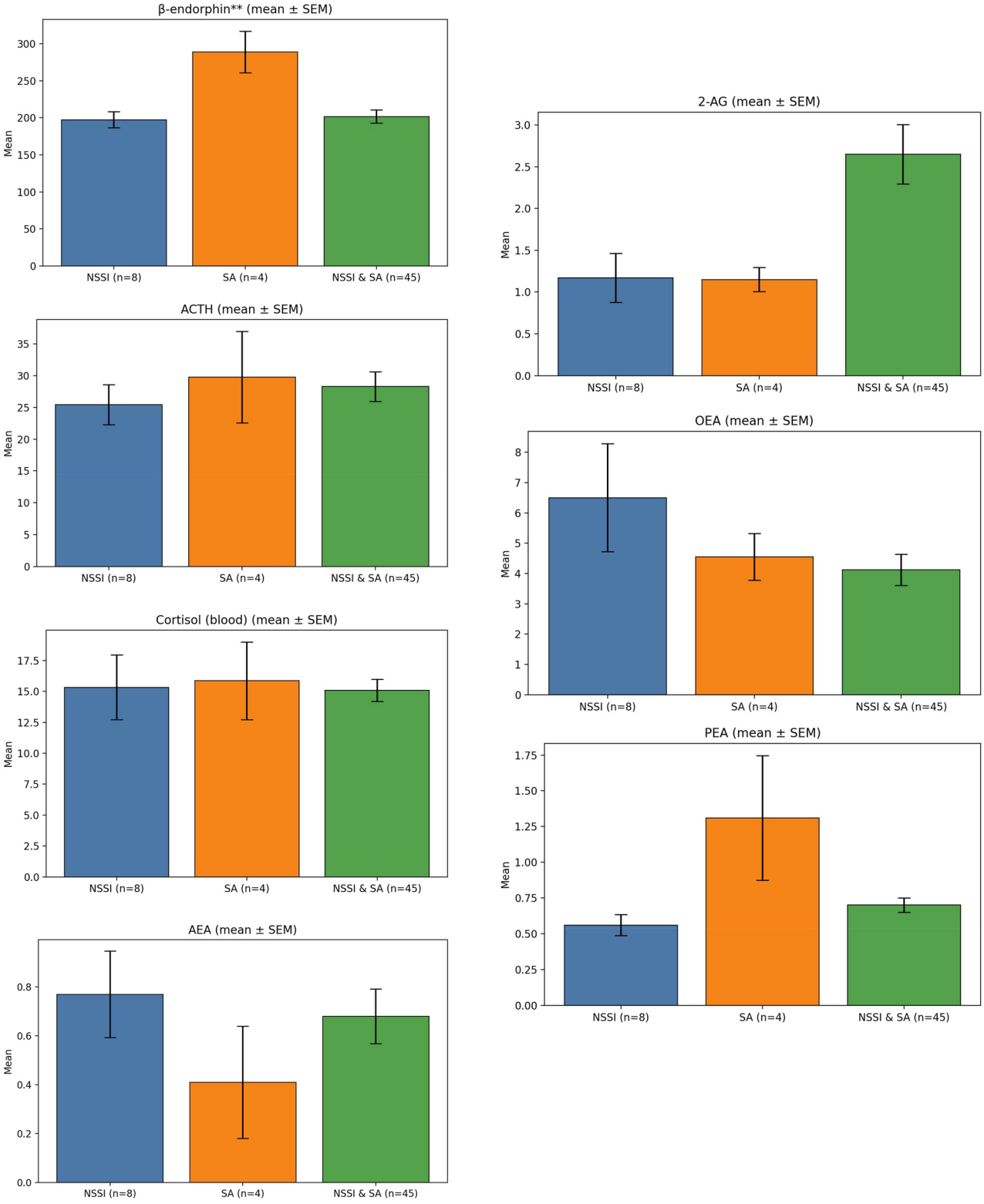



**FIGURE 2** | Serum biomarkers with add error bars (e.g., SEM) [endocannabinoids (pmol/mL), ACTH (pg/mL), cortisol (μg/dL), and $\beta$-Endorphin (pg/mL)].

factors. To sum up, caution about the limited power of these comparisons should be borne in mind, and there is a need for replication in larger samples. Another limitation was that we analyzed single CTQ items. This approach involves multiple statistical tests and may increase the risk of Type I error. As stressed before, our pilot study is by definition exploratory in nature; replication of our results is mandatory. Furthermore, because neuroendocrine systems are developing in adolescents;

some of our findings regarding serum biomarkers may be explained just by age and development issues. Another limitation was that the S-PLE scale is not validated for adolescents. Finally, the absence of healthy controls results in a lack of evidence regarding the specificity of these biomarkers for NSSI and for SA patients.

## 4.3 | Conclusions

NSSI and SA often co-occur in adolescence. Adolescents displaying NSSI alone, SA alone, and both NSSI and SA share common risk factors, including childhood maltreatment and neglect, as well as high levels of unbearable mental pain. Although identifying distinct risk factors that clearly differentiate the groups is challenging, we found specific variables more frequently present in each group, many of which are supported by existing literature. The most interesting finding was the elevation of two serum biomarkers ($\beta$-endorphin and PEA). Thus, serum PEA and $\beta$-endorphin levels may serve as potential biomarkers for SA without NSSI, providing a basis for further research into the biological underpinnings of these behaviors. Our findings may be influenced by the acute situational context in which SA and NSSI were evaluated. Given these limitations, our results should be interpreted with caution. The preliminary nature of this work underscores the need for further investigation with a larger sample size.

### Author Contributions

**María Posada-Ayala:** conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, validation, writing – original draft, writing – review and editing. **Ping Wang:** methodology, writing – review and editing. **Gemma Barroso-Garcia:** methodology, writing – review and editing. **Encarnación Donoso-Navarro:** conceptualization, data curation, methodology, writing – review and editing. **Elena Hernandez-Alvarez:** data curation, writing – review and editing. **María Gil-Ligero:** data curation, writing – review and editing. **Julián Romero:** conceptualization, funding acquisition, investigation, writing – review and editing. **Silvia Rosado-Garcia:** data curation, writing – review and editing. **Antonio José Sánchez-López:** conceptualization, funding acquisition, investigation, project administration, resources, validation. **Hilario Blasco-Fontecilla:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing – original draft, writing – review and editing, software, validation.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### Ethics Statement

This study was approved by the Research Ethics Committee of Hospital Universitario Puerta de Hierro Majadahonda (IRB Number 82/20; February 23, 2021). All participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki.

### Conflicts of Interest

In the last 2 years, H.B.F. has received lecture fees from Laboratorios Rubio and Takeda. He received funding from AB BioTek HNH. He was the principal investigator (PI) during an IPFIS research contract (www.isciii.es; IFI16/00039), the SINCRONIA and DISCRONIA projects (funded by Bitsphi, www.bitsphi.com), and co-PI of an MINECO research grant (RTI2018-101857-B-I00). He was also recipient of (1) an FIPSE Grant and (2) an IDIPHIPSA intensification grant. He was involved in two clinical trials (NEWROFEED Study, ESKETSUI2002). He is co-founder of Haglaia Solutions (www.haglaia.com). The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

### Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available due to restrictions related to patient confidentiality and ethical approval but may be made available from the corresponding author upon reasonable request.

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**Supporting Information**

Additional supporting information can be found online in the Supporting Information section.
**Supporting Information S1:** mdb270030-sup-0001-suppl-data.docx.