Depression, psychosocial factors, and laboratory routine testing in people living with HIV in Northeastern Mexico: prevalence, correlations, and its associations

Depressão, fatores psicossociais e exames laboratoriais de rotina em pessoas vivendo com HIV no nordeste do México: prevalência, correlações e associações

Depresión, factores psicosociales y pruebas de laboratorio de rutina en personas que viven con VIH en el noreste de México: prevalencia, correlaciones y sus asociaciones

Manuel Ángel Bermúdez Barrera - ORCID

Ruth Cruz Santos - ORCID

Abdías Alonso González - ORCID

Juan Antonio Escobar Félix - ORCID

ABSTRACT

Introduction: Depression is up to five times more prevalent in people living with HIV (PLWHIV). There are neurohormonal, virological and psychosocial factors involved and it is associated with antiretroviral treatment non-adherence, decreased life expectancy, faster progression to AIDS and premature death. Studies support that with lower CD4 levels, and the higher viral load (VL), depression increases. Objective: To establish whether there is a correlation and association between the VL and CD4 count with depression and its symptoms in PLWHIV. Method: Under follow-up in the Infectious Disease outpatient clinic a survey of sociodemographic variables, and a Beck's Depression Inventory (BDI) was applied to the study subjects, whose results were analyzed using Rho Spearman (rs) and Chi Squared test (X2) with VL and CD4 levels. Results: 137 individuals were included, from 18-73 years, which 97 (70.8%) were male assigned at birth. The prevalence of depression reported, was 25.5%. A positive correlation was found between VL and BDI score, and a negative correlation between BDI score and CD4. In addition, correlation was found between VL, and depressive symptoms such as guilt, discouragement and self-image perception. These symptoms
were strongly associated with death wishes, previous suicide attempts, and treatment non-adherence. **Conclusion:** VL may have implication in depression and its symptoms in this population, so their control is extremely important to prevent depressive episodes and suicidal behavior and prolong treatment adherence.

**Keywords:** depression, HIV, viral load, CD4 cell count

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**RESUMO**

**Introdução:** A depressão é até cinco vezes mais prevalente em pessoas vivendo com HIV (PVHIV). Existem fatores neuro-hormonais, virológicos e psicossociais envolvidos, e está associada à falta de adesão ao tratamento antirretroviral, diminuição da expectativa de vida, progressão mais rápida para a SIDA e morte prematura. Estudos apoiam que, à medida que os níveis de CD4 diminuem e a carga viral (CV) aumenta, a depressão aumenta. **Objetivo:** Estabelecer se existe uma correlação e associação entre a CV e a contagem de CD4 com a depressão e seus sintomas em PVHIV. **Método:** Na clínica de doenças infecciosas, foi aplicado um questionário de variáveis sociodemográficas e o inventário de depressão de Beck (BDI), cujos resultados foram analisados utilizando o teste de Spearman (rs) e Qui-quadrado (X2) com os níveis de CV e CD4. **Resultados:** Foram incluídos 137 indivíduos, com idades entre 18 e 73 anos, dos quais 97 (70,8%) foram designados como homens ao nascer. A prevalência relatada de depressão foi de 25,5%. Foi encontrada uma correlação positiva entre a carga viral (CV) e a pontuação do BDI, e uma correlação negativa entre a pontuação do BDI e o CD4. Além disso, foi encontrada correlação entre a CV e sintomas depressivos, como culpa, desânimo e percepção da autoimagem. Esses sintomas estavam fortemente associados a desejos de morte, tentativas de suicídio anteriores e falta de adesão ao tratamento. **Conclusão:** A CV pode ter implicações na depressão e seus sintomas nessa população, sendo seu controle extremamente importante para prevenir episódios depressivos, comportamentos suicidas e prolongar a adesão ao tratamento.

**Palavras-chave:** depressão, HIV, carga viral, contagem de células CD4

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**RESUMEN**

**Introducción:** La depresión es hasta cinco veces más prevalente en las personas viviendo con VIH (PVVIH). Existen factores neurohormonales, virológicos y psicossociales involucrados, y está asociada con falta de...
adherencia antirretroviral, disminución en la esperanza de vida, progresión más rápida a SIDA y muerte prematura. Estudios respaldan que a medida que disminuyen los CD4 y aumenta la carga viral (CV), acrecienta la depresión. **Objetivo:** Establecer si existe una correlación y asociación entre CV y recuento de CD4 con la depresión y sus síntomas en las PVVIH. **Métodos:** En la consulta de infectología, se aplicó una encuesta de variables sociodemográficas y el inventario de depresión de Beck (BDI), cuyos resultados se analizaron utilizando la prueba de Rho de Spearman (rs) de Chi cuadrado (X2) con los niveles de CV y CD4. **Resultados:** Se incluyeron 137 individuos, con edades entre 18 y 73 años, de los cuales 97 (70.8%) de sexo masculino asignado al nacer. La prevalencia de la depresión reportada fue del 25.5%. Se encontró una correlación positiva entre CV y la puntuación del BDI, y una correlación negativa entre la puntuación del BDI y el CD4. Además, se encontró correlación entre CV y síntomas depresivos como la culpa, el desánimo y la percepción de la autoimagen. Estos síntomas estaban fuertemente asociados con deseos de muerte, intentos de suicidio previos y falta de adherencia al tratamiento. **Conclusión:** La CV puede tener implicaciones en la depresión y sus síntomas en esta población, por lo que su control es extremadamente importante para prevenir episodios depresivos, comportamientos suicidas y prolongar la adherencia al tratamiento.

**Palabras clave:** depresión, VIH, carga viral, recuento de células CD4


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Depression in people with HIV in Mexico

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Bermúdez Barrera MA [1, 2, 3, 6, 7, 8, 11, 12, 13, 14], Cruz Santos R [2, 3, 5, 6, 10, 14], Alonso González A [2, 5, 8, 11, 14], Escobar Félix Já [3, 12, 13, 14]

Introduction
Depression in people living with HIV is four to five times more prevalent than general population and it is associated with treatment non-adherence, faster progression to AIDS, decreased quality of life, and premature death [1, 2]. Depression could be a risk factor for having sexually transmitted HIV infection, facilitate transmission, but can also be a consequence of HIV infection [3, 4]. In Mexico, prevalence of depression in people living with HIV (PLWHIV) is reported in 27%, while in Spain it is reported in 35.4%, and USA 22-36%, and it is well known that less developed countries present higher rates of depression. There is no data available, in the present, about depression in PLWHIV in Northeastern México [5].

Suicide is a catastrophic consequence of depression, and in the last decades, there has been a well-established correlation between HIV/AIDS and suicide. Suicide rates in men living with HIV in USA and Canada have reported 3 times higher rate than adult men without a history of HIV [6]. Global grouped incidence of consumed suicide in PLWHIV has a rate of one hundred times greater than people without HIV [7].

The presence of the HIV virus in the central nervous system has been associated with neurobiological changes [8, 9]. Several direct (viral action) and indirect (psychological stress) mechanisms have been related in an induction of a chronic state of stress and malfunction of hypothalamic pituitary adrenal axis, and immune response associated with the biological etiology of depression [8, 10, 11].

Viral load (VL) is defined as the number of circulating viruses in one milliliter (mL) of blood and is measured by RNA circulating copies of HIV in one mL. CD4 count is the number of T-lymphocytes in one cubic millimeter (mm3) of blood. The use of VL and CD4 count, has a very important role in the diagnosis, treatment, and management of HIV. VL functions as a biological marker for initial and sustained response to antiretroviral therapy, while CD4 helps us differentiate HIV infection from AIDS. These laboratory tests that can and should be done in most places of the world, according to World Health Organization [12].

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Given the lack of time in medical consultation and few physicians specialized in this population on this region of the world, we must prioritize time and attention costs, and in the last years a new challenge appeared. Most of the physicians with infectious disease specialty, both in outpatient or inpatient clinics had to support the health system by dedicating most of their time to COVID hospitalized patients, in which PLWHIV had consequences in appointments and treatment refill delay [13, 14].

Diagnosing depression in PLWHIV requires a very meticulous medical or psychiatric interrogation. The use of tools in PLWHIV and depression have been questioned in advance stages of HIV, people can present depression-like symptoms that intersect with clinical manifestations of depression. Beck Depression Inventory is a validated clinometric tool for Mexican Population living with HIV, that received a Cronbach´s alfa measurement of 0.91, in 2021. BDI considers the somatic symptoms that could intersect in advance stages of HIV [15, 16].

There are multiple cross-sectional studies that have reported positive correlation between VL and depression, and negative correlation between depression and CD4 levels. Some others have found statistical association between these three variables [17 – 21]. Also, studies have reported association between certain depression symptoms, such as sleep, guilt, and image self-perception with HIV, VL and CD4 count [21, 22]. However, there is limited data about the use of VL and CD4, both routine biomarkers for HIV treatment, as predictors of occurrence and severity of depression in PLWHIV in Northeastern Mexico.

Since HIV infection chronicity, number of copies of HIV in blood, and the diagnosis of AIDS are important factors associated with neuro-immunobiology of depression, our study aims to examine if there is a positive correlation between VL and depression symptoms, and a negative correlation between CD4 count and depression symptoms in PLWHIV in the public sector in Northeastern Mexico. Psychosocial factors were also included.

Methods
We conducted a cross-sectional study between December 2022 and May 2023 at Hospital de Especialidades No. 25, Centro Médico del Noreste, in Monterrey Nuevo León. This is a third level hospital from the Mexican Institute of Social Security (IMSS) that oversees Inpatient and Outpatient
follow-up of multiple specialties. About what it concerns to PLWHIV, it receives patients from four Northeastern States of Mexico.

Using the finite population equation, considering a confidence level of 95% and a margin of error of 5%, and an expected proportion of 0.5, a sample size of 137 individuals living with HIV was calculated. This calculation considered a total population of 212 individuals living with HIV under continuous follow-up at the Infectious Disease outpatient clinic of the facility in that period.

During the study stage, PLWHIV who came to the outpatient follow-up each day were invited to participate. Only subjects older than 18 years were included, and patients with history of psychosis, bipolar disorder, or autoimmune disease were excluded. A verbal explanation describing the aims of the study was given, and patients who agreed to cooperate were admitted after signing an informed consent form (previously approved by the bioethics and investigation committee of the hospital and institution). Only two individuals met exclusion criteria, one of them with history of previous acute psychosis, and the other with ankylosing spondylitis.

A sociodemographic characteristics questionnaire was administered individually, for us to obtain information about relevant personal history (age, gender, orientation, marital status), infection history (time of diagnosis, and time in treatment), mental, and psychosocial factors involved (antiretroviral treatment non-adherence, death wishes, previous suicide attempt, and perceived discrimination). The assessment of the sociodemographic variables was carried out through closed questions with dichotomous answers of yes and no.

A BDI was handed to evaluate depression in a quantitative way. BDI provides a score ranging from 0-63. A score from 0-13 is reported as not depressed, and from 14-63 it is positive for depression. As for levels of depression the cut-off score ranges were from 14-19 for mild depression, 20-28 for moderate depression, and more than 28 as severe depression. Also, each one of the questions in BDI was taken a single variable symptom for analysis. The values for BDI items are scored from 0-3. A score value of 0 in the item was taken as symptom not presented, and a value of 1-3 represents the presence of the studied symptom.
VL and CD4 data from each patient was collected from the last laboratory records (patients are submitted to these tests every 3-6 months). VL in our laboratory is reported as number of RNA HIV copies in one milliliter of blood (copies/mL), and CD4 count is reported in cubic milimeters (mm3).

Data obtained from the questionnaire, BDI, and lab test results, were introduced into a database in Jamovi Statistical Software 2.3 Version 2022 for its analysis. We estimated prevalence of depression, and depression severity. Frequencies of sociodemographic and BDI variables were described.

Rho Spearman coefficient was used for the correlational statistical analysis between CD4 levels and HIV VL with Depression Score. Similarly, this coefficient was employed to perform correlational analysis between the score of each Beck symptom with viral load and CD4.

For the associative statistical analysis of VL with depression and its symptoms, the chi-square statistical test was used. This tool was also conducted between depression symptoms (items in BDI) and sociodemographic variables. The cutoff point for viral load was 500 copies per milliliter, and for BDI score was 14. A p-value of <0.05 was considered significant, with a confidence level of 95% and a margin of error of 0.5.

**Results**

The study included 137 individuals, with ages from 18 to 73 years (mean 42.8, SD± 13.5), 70.8% (97/137) of whom where male assigned at birth, and 53.3% (73/137) of all subjects were single. Regarding gender identity, only 1 person identified as a trans person, and 27% (37/137) preferred not to respond the gender item. 21.9% of the patients involved reported being infected with HIV less than one year. The rest of the personal history is reported in [Table 1].

Almost all the subjects comprised (134/137) were receiving antiretroviral drugs, 82.8% with BIC/FTC/TAF as their therapeutic scheme, and those receiving ARV treatment had from one to 407 months receiving ARV therapy (mean 77.1 months, SD ±76.9).

From all the subjects studied, 25.5% (35/137) scored positive to depression in BDI, 15.3% (21/137) as mild depression, 5.8% (8/137) as moderate depression and 4.4% (6/137) as severe depression. Frequencies of depression by sexual orientation, 30.8% (4/13) of all bisexual oriented
individuals presented depression, 30.0% (15/50) for heterosexual oriented, and 17.4% (8/46) for homosexual oriented, of those who decided not no answer orientation item, 28.5% scored positive for depression. Cisgender women presented higher frequencies of depression reporting 36.7% being depressed. VL mean for the total of the individuals was reported in 13,890 copies/mL, SD± 72,606, min 0, max 693,000 copies/mL. CD4 count mean score was 431 cels/mm3, median 396, SD± 248, min 29, max 1118 cels/mm3.

Correlation for VL and BDI score reported a positive result (Rho Spearman 0.212 P=0.006), and correlation for CD4 and BDI score reported a low negative correlation (Rho Spearman -0.144 P=0.047). In the case of VL and each one of the items from BDI, a positive correlation was found between VL and discouragement guilt, self-image perception and level of effort. With a cut point at 500 cels/mL, we found association between VL and self-image perception, VL and guilt, and VL and discouragement. [Table 2] presents Rho Spearman correlation and chi-squared values with P values.

As for CD4 and the items on BDI, we found a low negative correlation for self-image perception (Rho Spearman=-0.188 p=0.014), and low libido (Rho Spearman=-0.169 p=0.024). Finally, we did correlational analysis between the variable age and the variable depression, reporting a negative correlation (R Pearson=-0.269 p=.<.001).

Contingency tables with chi squared test were made in variables that presented positive correlation between VL and BDI symptoms. An association was found for the item guilt with treatment non-adherence, perceived discrimination, and previous suicide attempt. The item discouragement had an association with treatment non-adherence, previous death wishes and previous suicide attempt. In the case of self-image perception and BDI symptoms, an association was found for: death wishes, previous suicide attempt, and treatment non-adherence [Table 3]. We also found difference in frequencies for depression and antiretroviral treatment non-adherence (X2=19.2 p<.001).

Discussion
To our knowledge, this is the first study in Northeastern Mexico to report depression in PLWHIV. The prevalence of depression was 25.5%, which are similar rates of depression of PLWHIV on Mexican population reported in a
global metanalysis [5]. The distribution of sociodemographic characteristics found among our study population were comparable to others described in another study on Mexican population. One of these factors include higher risk of depression among cisgender women living with HIV, while in our investigation, one third of cisgender women participating presented positive scores for depression [23].

We found some differences regarding sociodemographic depression factors in PLWHIV. Firstly, in our study, most subjects showed cooperativeness and even interest in filling up questionnaires and complying to follow ups, differing from results in research by Gutiérrez-Veililla and collaborators [14]. Another difference found in our investigation from others, is that there’s a well-documented association of age progression and the development of depressive episodes in PLWHIV, due to psychosocial, virulent and neurohormonal factors [23]. However, in our study, this association was not found, and to the contrary, negative correlation was found. To evaluate the highlighted differences reported in other studies, we saw that these studies were from Africa or other Latin American countries [24]. We believe there may be cultural, economic, and other social factors that may explain these differences. Future studies should aim at the understanding and explaining these factors to establish if there’s any correlation and a potential protective factor for depression in older PLWHIV in our population.

Although it has been very well described that members of the LGBTQ+ population have a higher risk to develop depressive episodes, in our study population, rates differed, as 30.8% of bisexual oriented patients, 30% of heterosexual oriented patients, and only 17.4% homosexual oriented patients, fulfilled criteria for major depressive disorder. We consider this is a remarkable phenomenon, considering that as a less developed country, it could be theorized factors such as less social support and more perceived discrimination would be the cause for higher rates of depression among homosexual oriented patients in our population, but this hypothesis is not fulfilled in our study.

Future studies should consider psychosocial characteristics and other sociodemographic factors that may be related to the complication of major depression in homosexual, heterosexual and bisexual PLWHIV, having in mind that disparities on sexual orientation and gender in the access, information, and use of HIV services have been reported previously [25].
VL was positively correlated with BDI score, and an inverse correlation between CD4 count and BDI score. Having in mind financial issues, lack of time and medical personnel in our country, and that fewer than half of depression cases in PLWHIV get a clinical diagnosis, and that only 18% gets treated, and only 5% achieve remission, VL may orient, in our healthcare environment, if a patient needs a reference for a mental health screening consultation [26, 27].

We found a positive correlation between VL and discouragement, guilt, self-image perception, and level of effort. We hypothesize that these results could be the outcome of many factors. Firstly, of neurotrophic and neurohormonal effects in which HIV can be a direct cause of depressive symptoms. Secondly, that these reflect the consequence of psychological burden of PLWHIV who fail to achieve remission early. Thirdly, it may as well be related to the need of more aggressive ART drugs, and the affliction of side effects or feeling as though they have a more aggressive form of the infection may be a reason for distress. Future studies should investigate these factors, so that healthcare personnel can provide a more enriched mental-health oriented attention.

CD4 and depression reported a low negative correlation, that contrasts with other investigations that had found higher negative correlation. We consider important to highlight that our study used BDI proven in our population of interest for depression in PLWHIV, in contrast to the other studies [17 – 20].

Depression has a relation with treatment non-adherence behavior [23]. This was also seen in our results. A relationship between guilt and treatment non-adherence was also stated, this interesting finding deserves a more careful review, as it may be linked to self-blame, unworthiness, or perhaps feelings or thoughts of deserving punishment, which would also explain the relation between guilt and perceived discrimination we located on our study. The importance of this finding would be in understanding the state of mind or personality traits of PLWHIV, and maybe being able to predict depression or even suicide attempts more successfully.

This study also reported how guilt, discourage and self-image perception has association with suicidal behavior such as death wishes, and previous suicide attempt. Previous studies have reported guilt and self-image perception as relevant symptoms in natural history of HIV infection, and
association with CD4 levels. Although in our study, these symptoms were evaluated from the BDI, it would be worth using specific tools for measuring these items and emphasize on this symptomatology [22, 28].

Finally, this investigation, only reports results from one hospital on the third level of attention, so results should be managed with caution, and should not be used to determine causality. New similar studies need to be done in the future to evaluate if the same correlations and associations are reported in other HIV attention centers.

Conclusions
Our results show new points of interest, on account of, we found differences between depression and psychosocial factors in our population and global literature. Specifically, the differences in age and sexual orientation. We encourage future research should explore the differences between Latinx LGBTQIA+ and Latinx elder PLWHIV in other parts of the world, and how those cultural and psychosocial differences relate to depression.

The study outcomes demonstrate that VL has correlation with depression by itself and is associated with some of depression symptoms like guilt, discourage, and self-image perception. At the same time, these last symptoms enlisted had strong association with death wishes and suicide attempts. Being able to control VL may not only help in a physical immune matter in PLWHIV, but also, may have a potential benefit in stopping the endless HIV-infection-depression cycle, and may reduce the catastrophic number of consumed suicides. This stop in the endless cycle may also help in progression to AIDS, and premature death. This finding would also mean to try to include mental health services more frequently in the evaluation and treatment of PLWHIV.

Considering that a small sample size was conducted and only one public hospital clinic was involved in this study, we encourage more thorough research to be done, regarding this topic, in which larger samples are used for these conclusions to be powerful enough to make changes in the way we treat and assess mental health in PLWHIV. And in the future, perhaps setting a threshold in VL in which it would be important in meaning to reassess mental health issues in PLWHIV.
References


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### Table 1. Relevant Personal History

<table>
<thead>
<tr>
<th>Assigned birth sex</th>
<th>Count (%)</th>
<th>Gender</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>97 (70.8%)</td>
<td>Cis man</td>
<td>68 (49.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>37 (27.0%)</td>
<td>Cis woman</td>
<td>30 (21.9%)</td>
</tr>
<tr>
<td>I prefer not to respond</td>
<td>3 (2.2%)</td>
<td>Trans woman</td>
<td>1 (0.7%)</td>
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<td></td>
<td></td>
<td>Other</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td></td>
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<td>I prefer not to respond</td>
<td>37 (27.0%)</td>
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<thead>
<tr>
<th>Orientation</th>
<th>Count (%)</th>
<th>Occupation</th>
<th>Count (%)</th>
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<tbody>
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<td>Heterosexual</td>
<td>50 (36.5%)</td>
<td>Employed</td>
<td>96 (70.0%)</td>
</tr>
<tr>
<td>Homosexual</td>
<td>46 (33.6%)</td>
<td>Unemployed</td>
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</tr>
<tr>
<td>Bisexual</td>
<td>13 (9.5%)</td>
<td>Student</td>
<td>4 (2.9%)</td>
</tr>
<tr>
<td>I prefer not to respond</td>
<td>28 (20.4%)</td>
<td>Other</td>
<td>106 (77.4%)</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Count (%)</th>
<th>Time of diagnosis</th>
<th>Count (%)</th>
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<tr>
<td>Single</td>
<td>73 (53.3%)</td>
<td>Less than 1 year</td>
<td>30 (21.9%)</td>
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<tr>
<td>Married</td>
<td>48 (35.0%)</td>
<td>1-4 years</td>
<td>51 (37.2%)</td>
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<tr>
<td>Divorced</td>
<td>16 (11.7%)</td>
<td>5-9 years</td>
<td>14 (10.2%)</td>
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<td></td>
<td>More than 10 years</td>
<td>42 (30.7%)</td>
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<tr>
<th>ARV Treatment Non-Adherence</th>
<th>Count (%)</th>
<th>Death Wishes</th>
<th>Count (%)</th>
</tr>
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<tr>
<td>Yes</td>
<td>20 (14.6%)</td>
<td>Yes</td>
<td>31 (22.6%)</td>
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<td>No</td>
<td>117 (85.4%)</td>
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<table>
<thead>
<tr>
<th>Previous Suicide Attempt</th>
<th>Count (%)</th>
<th>Perceived Discrimination</th>
<th>Count (%)</th>
</tr>
</thead>
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<tr>
<td>Yes</td>
<td>8 (5.8%)</td>
<td>Yes</td>
<td>42 (30.7%)</td>
</tr>
<tr>
<td>No</td>
<td>129 (94.2%)</td>
<td>No</td>
<td>95 (69.3%)</td>
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</table>
### Table 2. Correlation and association between VL and BDI symptom items

<table>
<thead>
<tr>
<th></th>
<th>Viral Load</th>
<th>Self-Image Perception</th>
<th>Guilt</th>
<th>Discouragement</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Spearman</td>
<td>- P – value</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chi-squared</td>
<td>- P – value</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spearman</td>
<td>- P – value</td>
<td>0.006**</td>
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<td></td>
<td></td>
<td>Chi-squared</td>
<td>- P – value</td>
<td>19.3</td>
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* p < .05, ** p < .01

### Table 3. Association between psych history and BDI symptom items

<table>
<thead>
<tr>
<th></th>
<th>Guilt</th>
<th>Discouragement</th>
<th>Self-image perception</th>
</tr>
</thead>
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<tr>
<td>Treatment non-adherence</td>
<td>$X^2$</td>
<td>8.35</td>
<td>19.2</td>
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<tr>
<td></td>
<td>$P$</td>
<td>0.004</td>
<td>&lt;.001**</td>
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<tr>
<td>Death wishes</td>
<td>$X^2$</td>
<td>15.6</td>
<td>16.1</td>
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<tr>
<td></td>
<td>$P$</td>
<td>&lt;.001**</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td>Previous suicide attempt</td>
<td>$X^2$</td>
<td>7.17</td>
<td>6.1</td>
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<tr>
<td></td>
<td>$P$</td>
<td>0.007</td>
<td>&lt;.001**</td>
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<tr>
<td>Perceived discrimination</td>
<td>$X^2$</td>
<td>8.88</td>
<td>3.29</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.003</td>
<td>0.07</td>
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** p < .01