Universidade do Minho
Escola de Ciências da Saúde

Wilza Beatriz Filipe Fumo

Effect of HIV infection on suicidal risk among heroin users

O efeito da infeção pelo VIH no risco suicidário nos usuários de heroína

maio de 2014

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Dissertação de Mestrado
Mestrado em Ciências da Saúde

Trabalho efetuado sob a orientação da
Profª. Doutora Margarida Correia-Neves
e co-orientação do
Professor Doutor António Pacheco Palha

maio de 2014

DECLARAÇÃO

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Número do Bilhete de Identidade: AF087177

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A minha querida mãe

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ABSTRACT

Suicide is an important cause of death worldwide. HIV^+ patients and heroin users are groups with elevated risk of suicide when separately compared to general population. Suicide has been also associated with psychiatric disorders as well as high cortisol levels. Still, suicide is hardly ever predicted and biological correlates to suicide are lacking. We aimed to investigate the effect of HIV infection on suicidal risk among heroin users in treatment with methadone, through a comparative study. Additionally, we assessed factors known to influence suicidal risk like stress (based on both cortisol levels and self-reported stress), anxiety and depression among HIV^+ heroin users. A cross-sectional study was performed. Thirty-seven (37) HIV^+ and fifty-two (52) HIV^- heroin users participated in our study. Psychological evaluation was performed using the scales for depression (Hospital Depression Scale - HDS), anxiety (Hospital Anxiety Scale - HAS), perceived stress scale (PSS-10) and Beck Scale of Suicidal Ideation (BSS). Simultaneously, we measured salivary cortisol due to its correlation with stress. Our results revealed that the HIV^+ heroin users had higher total suicidal ideation scores compared to HIV^- heroin users. No statistically significant differences across groups were detected in neither perceived stress scores, nor in anxiety and depression frequencies. Among HIV^+ patients, the higher the total self-reported scores reported by participants, the higher the total suicidal ideation scores obtained. Likewise, salivary cortisol levels correlated positively with total suicidal ideation scores. Individuals classified as presenting depression, as well as those classified as presenting anxiety showed higher total suicidal ideation scores when compared to those who were classified as presenting "no" depression or presenting "no" anxiety, respectively. These results suggest an additional effect of HIV infection on the risk of suicide among heroin users. Parallel to this, our findings highlighted the relevance of screening psychiatric disorders among heroin users in order to prevent suicide. Since a correlation between the cortisol level and suicidal ideation scores was detected, and cortisol level was shown to be a good predictor of suicidal ideation in a regression model of suicide made, it might be further explored as predictor of suicide risk.

RESUMO

O suicídio é uma causa importante de morte a nível mundial. Pacientes VIH$^+$ e usuários de heroína são grupos com elevado risco de suicídio quando isoladamente comparados com a população geral. O suicídio tem sido igualmente associado a perturbações psiquiátricas bem como a níveis elevados de cortisol. Entretanto, é difícil prever o suicídio e marcadores biológicos que se associem ao suicídio estão em falta. O presente estudo teve como objetivo investigar o efeito da infeção pelo VIH no risco de suicídio entre usuários de heroína em tratamento com metadona, através de um estudo comparativo. Adicionalmente avaliaram-se fatores conhecidos como tendo influência na ideação suicida como o stresse (baseados nos níveis de cortisol e no stresse percebido), ansiedade e depressão em usuários de heroína VIH$^+$. Foi feito um estudo transversal. Trinta e sete (37) usuários de heroína VIH$^+$ e cinquenta e dois (52) usuários de heroína VIH$^-$ participaram no nosso estudo. A avaliação psicológica foi feita através de escalas de depressão (Escala Hospitalar de Depressão), ansiedade (Escala Hospitalar de Ansiedade), Escala de Stresse Percebido (PSS-10) e Escala de Ideação Suicida de *Beck* (BSS). Em simultâneo, mediram-se os níveis de cortisol dos participantes devido à sua correlação com o estresse. Os resultados revelaram que os usuários de heroína VIH$^+$ tiveram pontuações totais mais altas de ideação suicida do que os usuários de heroína VIH$^-$. Não foram encontradas diferenças estatisticamente significativas entre os dois grupos nas pontuações de stresse percebido nem na frequência de ansiedade e depressão. Entre os pacientes usuários de heroína VIH$^+$, quanto mais altas as pontuações de stresse percebido mais altos os valores totais de ideação suicida obtidos. Da mesma forma, os níveis de cortisol salivar tiveram uma correlação positiva com as pontuações totais de ideação suicida. Por outro lado, os participantes classificados como tendo depressão ou tendo ansiedade tiveram valores mais altos de ideação suicida do que os que foram classificados como não tendo depressão ou não tendo ansiedade, respetivamente. Estes resultados sugerem um efeito adicional da infeção pelo VIH no risco de suicídio entre pacientes usuários de heroína. Paralelamente, os nossos resultados salientam a importância do rastreio de patologias psiquiátricas entre usuários de heroína tendo em vista a prevenção do suicídio. Uma vez que o nível de cortisol correlacionou-se com a pontuação de ideação suicida e que no modelo de regressão linear feito o nível de cortisol revelou-se um bom preditor de ideação suicida, poderá ser mais explorado como preditor de risco suicidário.

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ART	Anti- Retroviral Therapy
AZT	Zidovudine
BSS	Beck Suicidal Ideation Scale
CRI	*Centro de Respostas Integradas*
DA	Dopamine
DICAD	*Departamento de Intervenção nos Comportamentos Aditivos e Toxicodependências*
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunoassay
HAART	Highly Active Anti-Retroviral Therapy
HADS	Hospital Anxiety and Depression Scale
HAS	Hospital Anxiety Scale
HDS	Hospital Depression Scale
HIV	Human Immunodeficiency Virus
HIV^+	Human Immunodeficiency Virus positive
HIV^-	Human Immunodeficiency Virus negative
HPA axis	Hypothalamic-Pituitary-Adrenal axis
5-HT	Serotonin
MSM	Man having Sex with Men
NE	Norepinephrine
NF-ƙB	Nuclear Factor kappa B
NK	Natural Killer
PML	Progressive Multifocal Leucoencephalopathy
PSS-10	Perceived Stress Scale with 10 items
RNA	Ribonucleic Acid
SPSS	Statistical Package for Social Sciences
TMB	3, 3', 5, 5'- tetra-methyl benzidine
TNF	Tumor Necrosis Factor
VL	Viral Load
WHO	World Health Organization

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1. INTRODUCTION

1.1. General background

Human Immunodeficiency Virus (HIV) is a lentivirus that causes a progressive immunodeficiency leading to AIDS (Acquired Immunodeficiency Syndrome) (Doitsh et al., 2014). Although the first cases of the syndrome were reported in men having sex with men (MSM) near 1981, it was in 1983 that the virus was firstly identified (UNAIDS & WHO, 2003). Since then, the global prevalence increased from about 8 million people infected with HIV in 1990 (WHO, UNAIDS, & Unicef, 2011) to approximately 33.2 million people, with an incidence estimated of 2.7 million people in 2007 (Getahun, Gunneberg, Granich, & Nunn, 2010). By the end of 2012, the estimated prevalence of HIV infection worldwide was of 35.3 million people, with an incidence of 2.3 million and nearly 1.6 million people had died of AIDS (UNAIDS, 2013). Evidences suggest a decrease in HIV epidemics all around the world, except in specific groups such as injecting drug users in both Central Asia and Eastern Europe and young women in Sub-Saharan Africa (Beyrer, Baral, & Griensven, 2013). As for men having sex with men, infections in HIV are still increasing all through most countries (Wallace, Li, & McDaid, 2014) (Beyrer et al., 2013).

HIV/AIDS prevalence in Portugal in 2012 was of 42 580 people, with an incidence of 776 people (Departamento De Doencas, Infecciosas, & Sida, 2013) which represents a concern compared to other European countries, highlighting the need to intensify counseling and testing (Yazdanpanah et al., 2013). The transmission of HIV occurs through sexual and perinatal routes, blood transfusion or use of infected needles (Hessol, Gandhi, & Introduction, 2005). The risk behaviors mostly associated with the disease are non-protected sexual anal intercourse (Beyrer et al., 2013), multiple sexual partners (Vuylsteke et al., 2012), and injecting drug use (Choopanya et al., 2013) .

The HIV binds to its receptor on the surface of cells from the immune system. The most important being the CD4, on the surface of $CD4^+$ T lymphocytes. The HIV binds to the host cells making use of one of its glycoproteins – the gp120 (Adamson & Freed, 2011). Upon this interaction, the virus membrane fuses with the cell membrane and the replication cycle within the cell is initiated (Citron, Brouilette, & Beckett, 2005) If the patient does not receive appropriate anti-retroviral treatment, the viral replication progresses followed by destruction of the $CD4^+$ T lymphocytes, with advent of more severe diseases until death (Vlahov et al., 1998). $CD4^+$ T lymphocytes counts and blood viral load measures are extremely useful to determine the progression of the disease (Li et al., 1998) .

Immediately after the discovery of the disease, there was no treatment available (CDC, 2011). The first drug approved as Anti-Retroviral Therapy (ART) was zidovudine (AZT) in 1987, still with high mortality rates (UNAIDS, 2013). Only in 1996 the advantages of giving triple combined drugs were highlighted with significant decrease in mortality and morbidity (CDC, 2011), being ART nowadays usually referred to as Highly Active Anti-Retroviral Therapy (HAART) (Vercauteren et al., 2013).

1.2. HIV and central nervous system

Besides the effects on the immune system, it is well-established that HIV infects the Central Nervous system (CNS) at early stages and replicates in the brain parenchyma targeting the microglial cells (Ellis, Calero, & Stockin, 2009). It has been suggested that HIV reenters the brain parenchyma during periods of high viral load, causing neuronal deterioration and death (Kaul, Zheng, Okamoto, Gendelman, & Lipton, 2005). This neuronal dysfunction and death occur most probably through indirect mechanisms involving inflammatory cytokines from the host cells than direct HIV neuronal damage (Kaul, Garden, & Lipton, 2001)Nevertheless, the symptoms of neurologic deterioration do not seem to appear during the early stages being more frequently associated with late stages of the disease (Ellis et al., 2009). In the final stages of the disease, the neurologic symptoms may be associated with the infection of the central nervous system by the HIV and/or due to opportunistic infections and/or secondary malignancies (Kranick & Nath, 2012). Neurocognitive and neurological syndromes associated with AIDS include peripheral neuropathy, Progressive Multifocal Leukoencephalopathy (PML), meningoencephalitis, cerebral toxoplasmosis and so forth (Citron et al., 2005).

Considerable interest has been given to cognitive alterations associated with HIV, being a syndrome usually found due to AIDS in the absence of opportunistic diseases (Yeh et al., 2000) the so called HIV Associated Dementia complex (HAD, which affects cognitive function, leads to motor impairment and behavioral alterations (Kaul et al., 2005) a subject of much research investigation (Valcour, Sithinamsuwan, Letendre, & Ances, 2011)

1.3. HIV and psychiatric disorders

In addition to the physical and neurological abnormalities due to the disease, increasing research has described some emotional and behavioral phenomena occurring more frequently in the group of people infected with HIV (Carrico et al., 2007). Since its discovery, due to the incurability and the general awareness of the disease as a terminal illness, there is a perception of risk associated with the disease that may lead to a variety of negative feelings of fear, fatalism and impotence (Keiser et al., 2010). Together with this, the sexual context of the disease, since it was primarily described as a disease found in man having sex with men (MSM) (Beyrer et al., 2013) and sexual promiscuity brought up social discrimination as well as perception of stigma and feelings of guilt and shame to the individuals infected (Hemmati Sabet, Khalatbari, Abbas Ghorbani, Haghighi, & Ahmadpanah, 2013). Some models describe that these factors that might be present from the diagnosis and throughout the evolution of the infection might act as important ongoing psychosocial stressors (Carrico et al., 2007) - that can be defined as outward factors that endanger one´s welfare (Pearlin, Menaghan, & Lieberman, 2009). Additionally, these psychiatric stressors may lead to psychiatric disorders like depression, anxiety and suicidal ideations (Carrico et al., 2007). However, since then people have been reacting differently to a HIV infection diagnosis Social support (Yi et al., 2006) religiosity (Yi et al., 2006), individuals appraisal of the event are some factors that play an important role in reducing the negative impact of the psychosocial stress associated to the disease (Tuck, McCain, & Elsiwick, 2008) and strategies that emphasize the involvement of family and friends as well as cognitive behavioral therapy have been benefiting the quality of life of people infected with HIV (Hemmati Sabet et al., 2013).

Interestingly, despite the knowledge of the HIV infection and disease evolution mechanisms, better treatments (HAART) developed and the resulting increased life expectancy, the perceived stress and psychological distress conditions (like experiencing anxiety and depression symptoms) (Drapeau, Marchand, & Beaulieu-prévost, 2007) are still more prevalent in HIV positive patients compared to negative controls (Morrison et al., 2002). Indeed, in the era of HAART, anxiety, depression and stress have shown to impair some important aspects of the treatment and transmission, such as the adherence and sexual risk behavior that have been shown to improve with the management of the disorders (Hemmati Sabet et al., 2013).

1.4. HIV, suicide and injecting drug users

Suicide that is the act of willfully killing oneself (OECD, 2013), is a public health burden in many countries all around the world (OMS, 2000) (Jin et al., 2014) (OECD, 2013). It is a complex and often multifactorial phenomenon, which is explained usually combining several variables (Minayo, Cavalcante, & Souza, 2006). 2011). Events of life (Currier & Mann, 2009)(Pereira, 2011), personality traits (Maloney, Degenhardt, Darke, & Elliot, 2010) as well as genetic predisposition (Pereira, 2011) may also be associated to its occurrence. The WHO (OMS, 2000)described the frequency of suicide as being higher in young men, people with alcoholism, drug users and people with chronic diseases with poor prognosis.

Some authors found that the risk of suicide is higher in the group of risk of HIV infection both in infected and not infected people. That is, people at risk of committing suicide share some characteristics of people at risk of getting infected with HIV, hence it is unclear whether this event is a result of disease or is part of the vulnerability of the group (Starace, 1993)(Kelly et al., 1998). As for injecting drug use, it is a practice commonly intersected with HIV infection and may independently constitute a risk factor for suicide (Jin et al., 2014).

Patients infected with HIV have higher suicide rates than general population (Bragança & Palha, 2011). The suicidal ideations are commonly found anytime throughout the disease (Carrico et al., 2007) though some studies suggest that they are more prominent during disclosure, when the first symptoms and signals of the disease appear or in association with morphological alterations and other side effects of medication (Citron et al., 2005). Another interesting aspect is that factors that may independently lead to suicide are more pronounced in HIV^+ people: psychiatric comorbidities are increased among HIV^+ individuals, as aforementioned, highlighting the importance of treating these disorders in order to reduce suicidal risk. This evidence is demonstrated by Keiser et al, 2010 wherein among HIV^+ individuals who died by suicide about 23% were not receiving any psychiatric treatment. Similarly, psychiatry disorders are frequently found in the history of injecting drug users who attempt and commit suicide (Kuramoto, Chilcoat, & Ko, 2012).

Regarding biological aspects, prolonged heroin use has been associated with a cognitive impairment and altered behavior through alterations in the opioid system (Ersche & Sahakian, 2013). Meanwhile, some literature postulates that the metabolism of tryptophan, the precursor of serotonin that is a neurotransmitter associated to mood disorders might be altered due to HIV infection (Widner, Laich, Sperner-Unterweger, Ledochowski, & Fuchs, 2002). This is supported by Zangerle et al, wherein not only

HIV infection was suggested to increase the degradation of tryptophan, but also this effect was partially reversed by HAART. Since there is a strong correlation with neuropsychiatry abnormalities through immune activation, the metabolism of tryptophan gave insight of possible mechanisms through which the immune system and the Central Nervous System may interact (Widner et al., 2002).

1.5. Neurobiology of stress, depression and anxiety in relation with immunity

There are evidences of a bidirectional interaction between the immune system and the central nervous system (Dantzer, 2004). The mechanism by which this interaction occurs is more often appointed to the Hypothalamic-Pituitary-Adrenal (HPA) axis, in that, an excess of circulating glucocorticoids – the final product of the activation of the HPA system – is responsible by an altered immune response (Sternberg, 2007). In situations of chronic stress, the excess of glucocorticoids resulting from HPA activation, leads to a reduced response of glucocorticoids receptors through negative feedback, which might result in stimulated release of pro inflammatory cytokines (which is inhibited by genes activated by glucocorticoids receptors in normal functioning) (Raison, Capuron, & Miller, 2006). In the brain, the pro-inflammatory cytokines have been suggested to interfere with the metabolism of neurotransmitters such as serotonin (5HT), dopamine (DA) norepinephrine (NE) in different neuro circuitries involved in mood, anxiety, and motor activity namely amygdala, basal ganglia, prefrontal cortex that may explain the psychiatric disorders (Miller, Maletic, & Raison, 2009) (Raison, Capuron, & Miller, 2012), as illustrated in Figure 1.

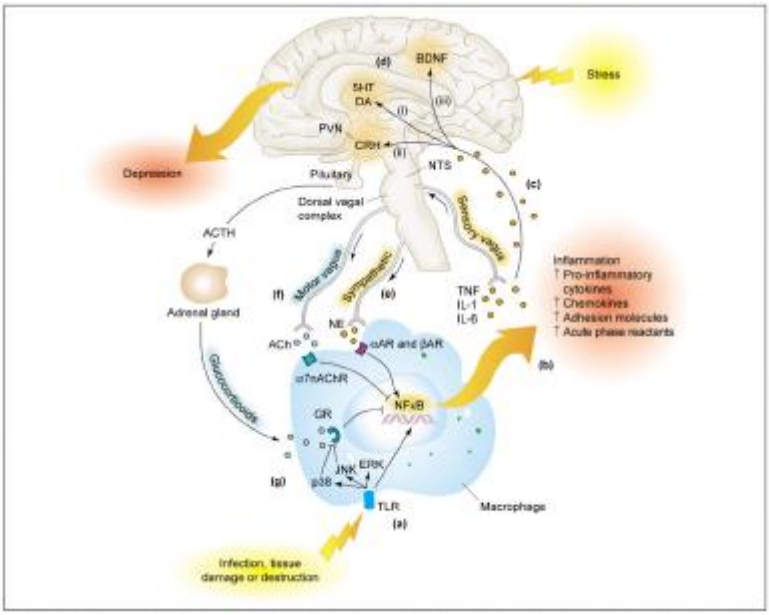

Figure 1. *Psychosocial stress inducing immune response activation (from Raison et al, 2006).*

The evidence of the interaction between the central nervous system and the immune system was primarily given by changes in the behavior during acute infectious diseases, like social avoidance, anhedonia, fatigue, alterations in sleep and appetite patterns and so forth, referred to as sickness behavior (Dantzer, 2004). These behavior alterations were initially considered to be important by reducing the spread of the disease as well as to save energy resources to other metabolic processes from the disease (Dantzer, 2004) (Miller et al., 2009). Further, depression has been associated with increased pro-inflammatory profile (Raison et al., 2012) in that some studies showed an improve in depressive symptoms in patients treated with Tumor Necrosis Factor (TNF) antagonist (Raison et al., 2012) as well as modulation of behavior by manipulating cytokines in experimental animal models (Mesquita et al., 2008).

As for psychological distress conditions like anxiety symptoms and depressive symptoms, the measures of cellular immunity might be found altered although with controversial findings, some showed association between depression and anxiety with low $CD4^+$ T lymphocyte counts and progression to AIDS (Leserman, 2008) and others found no association (Kinyanda, Hoskins, Nakku, Nawaz, & Patel, 2011). Whilst, some studies further indicate other measures of cellular immunity that could better clarify the association wherein, a lower Natural Killer (NK) activity and higher activated $CD8^+$ T lymphocyte counts and HIV viral load are appointed to be associated with major depression and anxiety symptoms (Evans et al., 2002). As

potential mechanisms underneath, models of interaction between elements of innate immunity such as NK and cytotoxic T cell activation mediating higher levels of psychological distress and impaired HIV disease severity are used to explain association (Greeson et al., 2008), as represented in Figure 2.

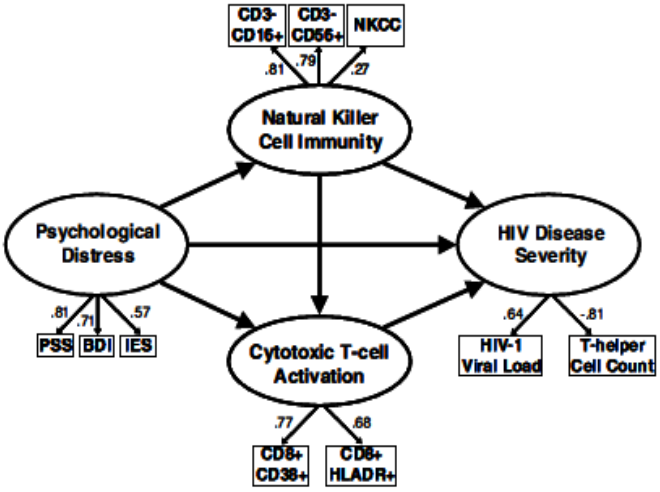

Figure 2. Hypothesized model of interaction between psychological distress and HIV disease mediated by elements of natural immunity (from Greeson et al, 2008).

1.6. Cortisol and suicide

Increasing evidence of association between cortisol and suicide has been suggested (Brunner et al., 2002) ;(Jokinen et al., 2007); (Sequeira et al., 2012) and abnormalities in the serotonergic system often correlate with suicide attempts (Mann, Brent, & Arango, 2001). In a study by Chatzittofis et al, 2013 on suicide attempters, a correlation between suicide and high cortisol concentrations was found. Again the dysregulation of the HPA-axis together with dysfunction of the central serotonin system, as shown by Ghaziuddin et al, 2014, seems to be implicated in suicidal ideation and complete suicide. These findings are in line with evidence of an association between high cortisol levels and depression and anxiety (Sequeira et al., 2012), that are well-established risk factors for suicide, though studies showing cortisol levels independently predicting suicide are lacking (Dwivedi, 2012).

The strategies being used to date in some countries, such as counseling and group meetings created for people to share their "living with HIV" experiences have been shown to be important in the quality of life of the individuals, yet they might not be enough in preventing suicide that requires skills from professionals and depends very much on one's temper to be willing to talk about his or her fears and intents. Moreover, people appraise and react differently even facing similar situations. By understanding the factors underneath this variation, one could get closer to those who need more attention. If it is shown that there is a biological and available resource associated to it, it would be valuable to the services to benefit of it as additional tool to at least more quickly identify individuals that would more likely attempt suicide.

2. AIMS

The study aims to:

1) Examine suicidal ideation in HIV^+ patients that are heroin users in comparison with HIV^- heroin users.

2) Investigate associations between suicidal ideation and the following parameters: stress levels (based on both perceived stress scale - PSS-10 - scores and cortisol levels), anxiety and depression (Hospital Scale for Depression and Anxiety- HADS) among HIV^+ patients who are concomitantly heroin users.

3) Access intrinsic factors associated with the occurrence of suicide in HIV^+ heroin users, namely history of psychiatric diagnosis, familiar history of suicide, past suicide attempts, time since HIV disclosure, and so forth.

Results will provide additional tools to draw interventional plans more focused on risk factors, increasing the efficiency in screening suicidal risk within this group.

3. METHODOLOGY

3.1. Study area and sample size determination

The cross-sectional study was carried out in DICAD ("*Departamento de Intervenção nos Comportamentos Aditivos e nas Dependências*") in Braga.

The sample included in this study is composed of heroin users with a positive diagnosis of HIV infection who are in the program of substitution of drugs by methadone in an outpatient regime. Enrolment took place from November 2013 to April 2014.

Excluding criteria

- Patients younger than 18 years and older than 55.
- Severe psychiatric disorders and/or cognitive impairment (clinically accessed).
- Severe diseases of the Central Nervous System (clinically accessed).
- Pregnant women.
- Patients treated with corticosteroids in the previous semester.
- Patients taking oral anti-conceptive.
- Patients with oral infections.
- Patients not able to provide informed consent.

A control group of patients that are also heroin users in treatment with methadone, with a negative result of HIV infection in the previous two years and without declared risk behavior was enrolled at the same time.

Procedure

Patients were approached after taking methadone and asked to participate. For those who accepted to be enrolled in the study after an explanation of the procedure and purposes of the study, a small interview was driven in order to fill the form for socio-demographic data as well as the scales and saliva sample was collected.

We asked for C.R.I ("*Centro de Respostas Integradas"*) ethic committee permission and obtained informed consent of each volunteer.

3.2. Data collection

3.2.1. Socio-demographic data and neuropsychological access

A questionnaire about socio-demographic information was used to access individual's personal data and lifestyle, namely: age, gender, marital status, residence, occupation, years of education, smoking habits, alcohol consumption, use of drugs and intake of oral anti-conceptive.

This form also enquired about clinical data: intake of medication to treat psychiatric disorders, psychiatric medical history, previous suicide attempts, comorbidities, sexual orientation, and length of time from disclosure, details concerning HAART.

The HADS (Hospital Anxiety and Depression Scale) was used to screen for depression and anxiety (Snaith, 2003). HADS is a form composed of 14 questions - 7 for depression and 7 for anxiety - each one rated from 0 to 3 with a final total cut point scoring of 8 meaning the presence of the disorder in both subscales (Álvaro et al., 2007).

A Portuguese version of the PSS-10 (Perceived Stress Scale) - a psychological measure of the extent to which events in one's life are recognized as excessive demands to be tolerated(Cohen, Kamarck, & Mermelstein, 1983) – was applied. It is composed by 10 queries about perception of uncontrollability and unpredictability concerning to different aspects of life during the previous month. Answers are given in form of frequency varying from never (0) to very often (4), with a minimum total score of 0 and a maximum of 40. Scores are calculated by summing reversing responses depending on the positivity meaning of the item. As general interpretation, those who scored more are appraising life more stressfully.

The BSS (Beck Scale for Suicidal Ideation) was provided to participants to evaluate suicidal risk assessment. The BSI is a scale with 19 items with answers organized from less to more serious condition in each item concerning attitudes and wishes about suicide, simultaneously looking for one individual's ability to consummate the intent when detected as well as for opportunities. There are no cut points for the final scores - higher scores obtained are interpreted like more severity of ideation and the participant is more likely to attempt suicide - (Cochrane-Brink, Lofchy, & Sakinofsky, 2000).

3.2.2. ACCESS OF CORTISOL CONCENTRATION IN THE SALIVA

Samples of saliva were used to access salivary cortisol levels (Hellhammer, Wüst, & Kudielka, 2009). The concentration of cortisol in the saliva is about 1/3 inferior to that in the blood. Nevertheless, the evaluation of cortisol in the saliva is useful since its level is strongly correlated with that in the blood when evaluated in response to stress and saliva is much easier to collect (Petrowski, Wintermann, Schaarschmidt, Bornstein, & Kirschbaum, 2013).

Small tubes with a piece of cotton inside (*salivettes*) for salivary collection were used. They were distributed after an explanation of its use, between 9 and 12 a.m., regarding the recommendations about the time between meals and collection procedures: patients were advised not to smoke or eat 30 min before collection. They should open the tube until access the cotton, put it in mouth keeping 1-2 min under the tongue or chewing it. Then return it back to the tube, avoiding manual contact. The procedure was done under supervision. After collection, *salivettes* were stored at -20˚C.

In average after 1 week, the small tubes were centrifuged at 2000g during 2 min, distributed in aliquots with the minimum required of 0.5 ml and stored at -80 ˚C until analysis.

Salivary cortisol levels were determined by competitive ELISA (enzyme-Linked Immunoassay) , following the instructions indicated by the manufacturer, with an analytical sensitivity of 0.007 µg/dl and a coefficient of variation intra-assay and inter-assay of 4.6% and 6%, respectively.

The protocol to perform the competitive ELISA to quantify the cortisol is briefly described in Annex 1.

Materials provided by supplier

- Microtiter plate;
- Enzyme conjugate;
- Standard (6 dilution of cortisol);
- Controls (2 solutions with known cortisol concentrations);
- Substrate solution (TMB);
- Stop solution (TMB);
- Wash buffer concentrate;
- Non-specific binding wells.

Materials used not provided by supplier

- Micropippettes;
- Plate rotator;
- Vortex mixer;
- Multichannel micropipette;
- Microtiter plate reader with capacity to read absorbance at 450nm;
- Deionised water;
- Paper towels;
- Pipette tips;
- Reagent reservoirs;
- Serological pipette (to deliver up to 24 ml);
- Timer.

The following steps were performed according to Cortisol ELISA manufacture instructions.

- 1X wash buffer was prepared by diluting wash buffer concentrate 10-fold with deionized water (50 ml of 10X wash buffer concentrate to 450 ml of deionized water).
- 1:1600 dilution of cortisol enzyme conjugate was made by adding 15 µl of the enzyme conjugate to 24 ml of assay diluent.
- 25 µl of each standard (containing 3, 1, 0.333, 0.111, 0.037 and 0.012 µg/dl), Control (control high and low), and doubled sample were pipetted into respective wells of the microtiter paper.
- 200 µl of the diluted enzyme conjugate was pipetted into each well. The plate was shacked carefully after covered and incubated at room temperature on an orbital shaker during 60 minutes. It was washed with the diluted wash buffer and added 200 µl of substrate solution into well and incubated during 30 minutes at room temperature, on an orbital shaker, covered.
- The reaction was stopped by adding 50 µl of stop solution into well.
- Results of cortisol levels were obtained using the optic density of standard curve, using an automated method (Bio Rad; model 680 microplate reader). The interpretation was done using software of microplate manager 5.2.1.

Results were processed using ELISA based on competition (Inder, Dimeski, & Russell, 2012).

3.3. Statistical analysis

To analyze data, Statistical Package for Social Sciences (SPSS) version 22 was used. The alpha level of statistical significance was set at 0.05. Student´s t test for independent samples, Levene's and Chi-square test were performed to compare medians between HIV^+ and HIV^- groups, variances and frequencies, respectively. To see whether the effect of belonging to one group or other on suicidal ideations could change whenever some variables of interest were kept constant, a sequential model of regression including socio-demographic and psychological variables of interest was drawn. Among HIV^+ patients, student´s t test for independent samples was performed to compare medians between groups presenting depression and "no" depression, and between those presenting anxiety and "no" anxiety. Pearson correlation coefficient was computed to examine the correlation between both perceived stress and cortisol levels with suicidal ideations, separately. A model of linear multiple regression including variables found to be associated with suicide ideations in HIV^+ patients was performed to access how accurately each variable could predict suicidality.

4. RESULTS

4.1. Characterization of the study population

The characterization of the study population is summarized in Table 1. Although 89 heroin drug users accepted to participate in the study, 3 dropped out, 6 did not meet the inclusion criteria and 7 did not complete the study protocol. Thus, the study population was composed by 80 individuals: 37 HIV^+ and 43 HIV^- negative, wherein 73 completed the study protocol. The mean age between the two groups was statistically different (HIV^+ and HIV^- groups mean age was 42 ± 4 and 39±7 respectively; [t (78) = 2.33; p = 0.02; Cohen´s "d" = 0.53]. Both groups were comparable in gender [χ^2 (1) = 0.36; p = 0.56; phi = -0.07], being the sample mostly composed by men. There was no difference on the number of years of education between the HIV^+ patients (7.6 ± 2.7) and HIV^- patients (8.1 ± 3.3); [t (77) = 0.54; p = 0.54; Cohen´s d = 0.17]. Both HIV^+ and HIV^- patients were predominantly unemployed, with no statically significant differences in occupation between the two groups, though the group of HIV+ had a trend to have higher frequency of unemployed individuals [χ^2 (3) = 6.74; p=0.08; Cramer´s "V" = 0.29]. The group of HIV^+ patients was composed predominantly by injecting drug users, whereas the group of HIV^- used other ways of administration rather than injection, with a statistically significant difference between both [χ^2 (1) = 0.807; p = 0.01; Cramer's "V" = - 0.30]. Hepatitis C was the most frequent comorbidity in both groups, being more frequently found in the group of HIV^+ patients, with a statistically significant variation in its frequency between the groups [χ^2(4) = 10.741; p = 0.03; Cramer's "V" = - 0.10]. The HIV^+ group showed a statistically significant difference in the body mass index mean (21.0 ± 2.1) compared to HIV^- (22.7 ± 3.4); [t (70.4) = -2.69 p = 0.01, Cohen's "d" = -0.60]. No statistically significant differences in sexual identity were detected across groups [χ^2 (1) = 1.154; p = 0.28; Cramer's "V" = 0.12].

Regarding HIV^+ group, the study population was composed predominantly by men (81.1%) and single individuals (70.3%). 97.2% of the patients described themselves as heterosexual. Nearly 1/2 of participants reported to have children (43.2%) and 81.1% were currently living with either a partner or a relative. As for routes of transmission of the HIV, 22.2% did not know how they got infected, 19.4% reported to have been infected through unprotected sexual intercourse and 58.3% by sharing needles. The disclosure of the HIV infection was made for an average time of 11.2 years prior to interview and all participants were taking HAART for a mean time of 7.4 years, as shown in Table 2.

4.2. Comparison of the frequencies of psychiatric disorders between HIV+ and HIV- patients

In respect to frequencies of psychiatric disorders in our study population, although the HIV^{+} patients showed a trend to have higher self-reported scores of PSS-10 (20.1± 7.1) compared to HIV^{-} (18.7 ± 7.2), no statistically significant differences were found between the two groups [t (71) = 0.85, P = 0.40 d = 0.21]. Regarding depressive symptoms based on HDS scores, the frequency were higher in the group of patients infected with HIV compared to HIV^{-}, 37.8% and 22.2% respectively, but no statistically significant difference was found [χ^2 (1) = 2.1; p = 0.20; phi = -0.17]. Similarly, the frequency of symptoms of anxiety based on HAS scores (45.9% in HIV^{+} patients and 41.6% in HIV^{-} patients were not statistically significant different across groups [χ^2 (1) = 0.14.; P = 0.81 Cohen´s "d" = -0.04], as represented in Table 1.

Table 1. *Characterization of the study population.*

Characteristic	HIV +	HIV -	p-value	Cohen´s "d"/ phi
Age M (SD)	42 (4)	39 (7)	0.02*	0.53
Male/Female N	30/7	37/6	n.s.	-0.07
Years of education M (SD)	7.6 (±2.7)	8.1 (±3.3)	n.s.	0.17
Unemployed N (%)	32 (86.4%)	28 (66.7%)	n.s.	0.29
Smoker N (%)	36 (97.2%)	39 (92.8%)	n.s.	0.37
Alcohol ingestion N (%)	15 (40.5%)	14 (33.3%)	n.s.	-0.07
Injecting drug user N (%)	36 (97.2%)	32 (76.1%)	0.007*	-0.3
Psychiatric disease N (%)	11 (42.3%)	12 (28.5%)	n.s.	-0.30
Previous suicide attempt N (%)	15 (40.5%)	13 (30.9%)	n.s.	-0.01
Hepatitis C antibody positive N (%)	16 (43.2%)	10 (24.3%)	0.03*	-0.10
Body Mass Index M (SD)	21.0 (±2.1)	22.7 (±3.4)	0.009*	0.60
Perceived stress score M (SD)	20.1 (±7.1)	18.7 (±6.2)	n.s.	0.21
Anxious N (%)	17 (45.9%)	5 (41.6%)	n.s	-0.04
Depressive N (%)	14 (37.8%)	8 (22.2%)	n.s.	-0.17

4.3. Comparison of suicidal ideation (BSS scores) between HIV+ patients and control

In order to investigate whether there was a difference in suicidal ideation between HIV^+ and HIV^- heroin users, student´s t test for independent samples was performed. The total suicidal ideation scores based on BSS were significantly higher in the group of HIV^+ patients (8.5 ± 9.0) compared to HIV^- (2.7 ± 5.9) [t (71) = 3; p=0.002; Cohen´s "d" = 0.76], as shown in Figure 3.

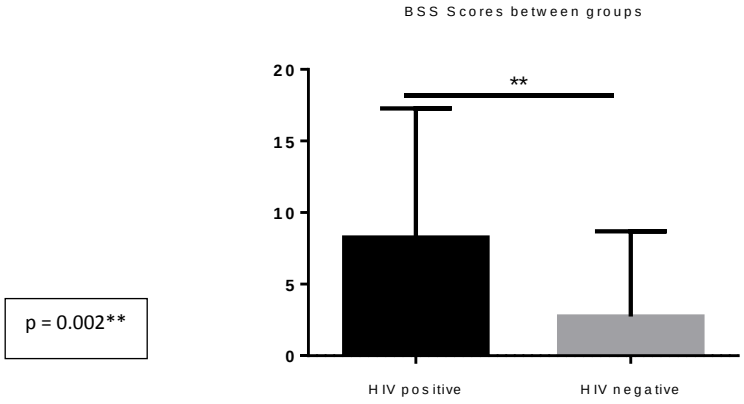

Figure 3. BSS scores between groups of HIV+ and HIV- patients.

4.4. Sequential linear multiple regression model of suicidal ideation scores and variables commonly associated with suicide in HIV+ and HIV- heroin users

Since the total suicidal ideation scores were statistically different between HIV^+ and HIV^- patients, a sequential linear multiple regression was made to access whether of belonging to one group or other could have a statistically meaningful influence on suicidal ideations if some variables usually associated to suicide in literature were maintained constant. Firstly, results showed that belonging to one group or other was statistically significant (β = - 0.36; P = 0.002), as shown in Table 2.

Table 2. Linear regression model of the HIV+ and HIV- groups and suicidal ideation scores.

	B (C.I.: 95%)	Beta	t	p
Study group	-5.82 (-9.38; -2.25)	-0.36	-3.25	0.00

Model: R = 0.36; R^2 = 0.13; R^2 adjusted = 0.12; p <0.05.

Since differences in age, gender and occupational state have been shown to influence the suicidal ideation in general population, these variables were added to the model in order to analyze if belonging to one group or other would still be statistically significant. Results revealed that even if the age, gender and occupational state (being employed or unemployed) were kept constant, being HIV^+ or HIV^- would still be statistically significant to the gravidity of suicidal ideations (β = - 0. 32; p = 0.009), as represented in Table 3.

Table 3. *Multiple linear regression model of socio-demographic variables and suicidal ideation scores in HIV+ and HIV- patients*.

	B (C.I.: 95%)	Beta	t	p
Study group	-5.24 (-9.11; -1.37)	-0.32	-2.70	0.01
Age	0.01 (-0.29; 0.31)	0.01	0.07	0.94
Gender	0.32 (-4.41; 5.05)	0.01	0.14	0.89
Occupational status	2.40 (-1.98; 6.78)	0.13	1.09	0.28

Model: R = 0.38; R^2 = 0. 14; R^2adjusted: 0.09; p <0.05.

Besides, since stress, depression and anxiety are well-established correlates of suicide, the psychological measures of perceived stress, depression and anxiety were also added to the model to examine if the scores of perceived stress and frequencies of anxiety and depression were maintained constant, the fact of belong to the HIV^+ group or HIV^- group could statistically and significantly interfere with suicidal ideations scores. Results found, showed that even if the perceived stress and frequencies of anxiety and depression were the same in our study group, the group to which one individual would belong would still be statistically significant to the severity of suicidal ideations (β = - 0.23; P = 0.02), as demonstrated in Table 4.

Table 4. *Multiple linear regression model of psychological parameters and suicidal ideation scores between HIV+ and HIV- patients.*

	B (C.I.: 95%)	Beta	t	p
Study group	-3.80 (-7.00; -.60)	-0.23	-2.37	0.02
Age	0.10 (-.015; 0.35)	0.08	0.80	0.42
Gender	1.14 (-2.77; 5.04)	0.05	0.58	0.56
Occupational status	1.80 (-1.80; 5.40)	0.09	0.99	0.32
PSS-10 scores	-0.03 (-0.30;	-0.02	-0.21	0.83
HAS scores	0.25)			
HDS scores	4.89 (1.51; 8.27)	0.30	2.89	0.00
	7.26 (3.50;	0.41	3.86	0.00
	11.02)			

Model: R = 0.68; R^2 = 0.48; R^2 adjusted = 0.40; p <0.05.

4.5. Clinical and demographic description of the group of HIV+ patients in relation with suicidal ideations (BSS scores)

Since there was a significant difference on suicidal ideations between HIV^+ and HIV^- heroin users, it was further investigated intrinsic factors of HIV^+ patients correlates of suicide described in literature (respectively to general population) in order to better identify risk factors in HIV^+ heroin users. Single individuals, younger people, men and injecting drug users have been showing higher suicidal risk; hence one way ANOVA, student´s t test and Pearson correlation were conducted in order to see the influence of these factors on suicidal ideations. No statistically significant effects of gender, marital state, age or routes of transmission of HIV in the suicidal ideations gathered through BSS scores were detected in HIV^+ heroin users, as shown in Table 5.

4.6. Psychiatric diseases, lifetime history of suicide and suicidal ideations in HIV+ patients

In order to examine the influence of psychiatric disorders and previous suicide attempts ideation that have been associated to suicide in suicidal ideation among HIV^+ patients, frequencies of psychiatric disorders and student´s t test of HIV^+ patients with a history of psychiatric disorders and those with no history of psychiatric disorders were computed. 42.3% had a diagnosis of a psychiatric disease throughout their lives. 24% of the patients with psychiatric disorders were taking anti-depressants. However, no statistically

significant differences in suicidal ideation scores between individuals with a psychiatric disease reported (11.9 ± 10.2) compared to those with no psychiatric disease reported (7.0 ± 8.2) were found; [t (35) = -1.53; p = 0.13; Cohen´s "d" = 0.53]. Nevertheless, a significant difference in current suicidal ideation scores between individuals with one previous suicide attempt (13.7 ± 9.2) and those with no past suicide history (4.9 ± 7.1) was detected [t (35) =- 3.298. p = 0.002; Cohen "d" = 1.1], as shown in Figure 4.

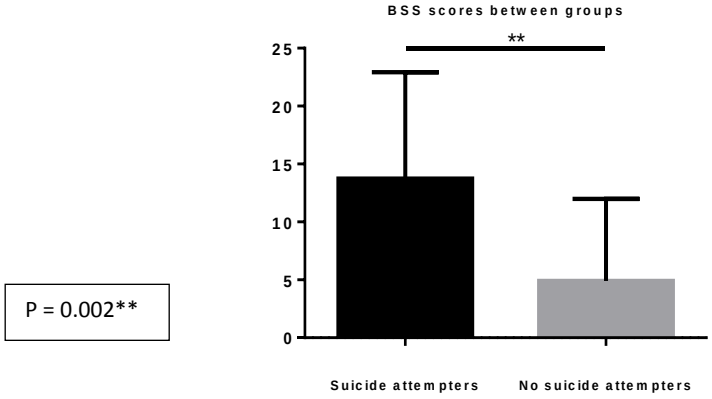

Figure 4. *BSS scores between participants with and without previous suicide attempt in HIV+ patients.*

Table 5. Demographic and clinical description of the HIV+ patients.

Variable	N/ (%/SD)	Cohen's "d"/ Pearson´s "r"	p-value of differences between groups in (or correlation with) BSS scores
Male	30 (81.1%)	0.36	n.s
Age	42 (±4)	-0.25	n.s.
Marital status			
Single	26 (70.3%)		
Widower	1 (2.7%)	0.98	n.s.
Divorced	9 (24.3%)		
Married	1 (2.7%)		
Have children			
Yes	16 (43.2%)	0.41	n.s.
No	21 (56.8%)		
Live alone			
Yes	7 (18.9%)		
No	30 (81.1%)	0.51	n.s.
Time since disclosure	11.2 (±5.8)	0.02	n.s
Heterossexual	36 (97.2%)	-	n.a
Previous suicide attempt			
Yes	15 (40.5%)	1.01	0.00*
No	22 (59.5%)		
Route of transmission			
Shared needles	21 (58.3%)		
Sexual	7 (19.4%)	0.14	n.s.
Unknown	8 (22.2%)		
HAART	37 (100%)	-	n.a
Time on HAART	7.4 (±5.9)	-0.39	0.02*

4.7. Perceived stress scores and suicidal ideation in HIV+ heroin users

In order to explore a relation between self –reported stress (PSS-10) and suicidal ideation (BSS) in HIV heroin users since perceived stress might be associated to suicidality and simultaneously has been found as being associated to HIV infection, a Pearson correlation coefficient was computed. A positive correlation between both was found, meaning that the higher the patients perceived stress, the higher the frequency of suicidal ideation found in this group ($r = 0.37$; $p = 0.02$), as shown in Figure 5.

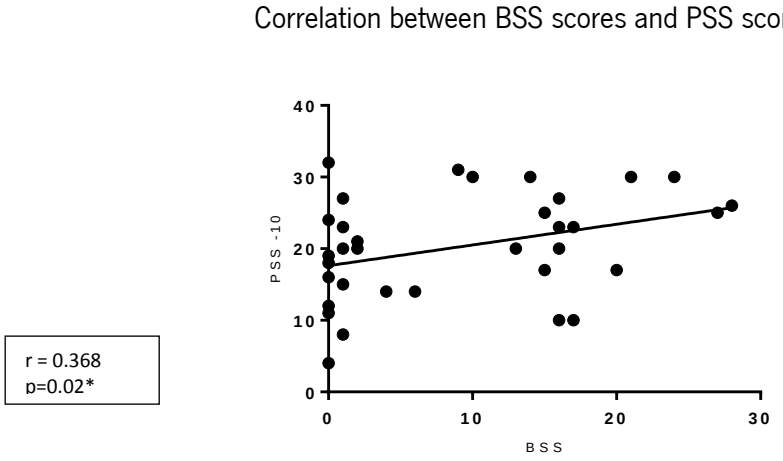

Figure 5. *Correlation between BSS scores and PSS-10 scores.*

4.8. Comparison of suicidal ideation in HIV+ heroin users presenting depression or presenting "no" depression

Since depression has been correlated with suicide in literature and showed high frequency (38.7%) in HIV^+ group in the present study, it was performed student´s t test for those who were classified as presenting depression and those who had scores compatible with "no" depression in HDS in order to examine the effect of depression on suicidal ideation on HIV^+ heroin users. Results showed a significant difference in suicidal ideation scores between those with depression (15.1 ± 8.3) compared to those with "no" depression (4.5 ±6.9), in that higher suicidal ideation scores were found in the group of depression [t (35) = -4.2; p=0.000 Cohen "d" = 1.39], as represented in Figure 6.

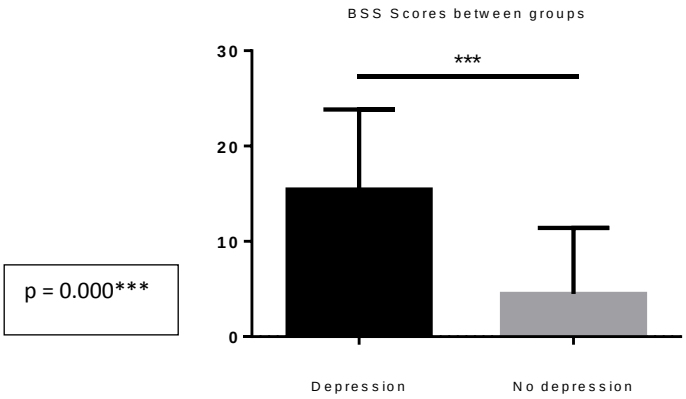

Figure 6. *BSS scores between individuals classified as with or without depressive symptoms (HDS) in HIV+ patients.*

4.9. Comparison of suicidal ideation in HIV+ heroin users presenting anxiety or presenting "no" anxiety

Similarly, individuals were categorized as "yes" or "no" anxious based on HAS so as to examine the effect of anxiety on suicidal ideation in HIV^+ heroin users. Results revealed that those participants classified as anxious had statistically significantly higher suicidal ideations scores (13.6 ± 9.0) compared to those with "no" anxiety (4.1 ± 6.4); [t (35) = -3.7; p= 0.001; Cohen's "d" = 1.21], as seen in Figure 7.

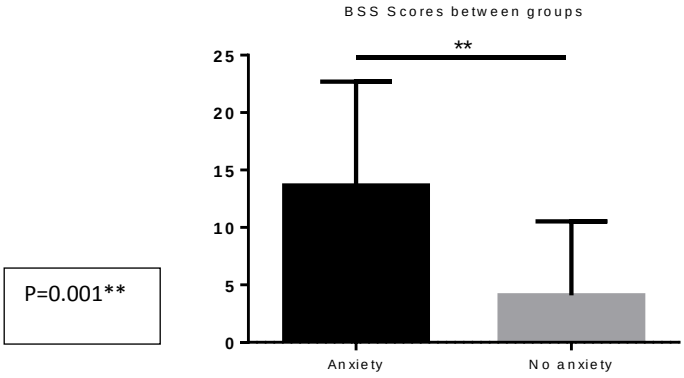

Figure 7. *BSS scores between individuals classified as with or without anxiety symptoms (HAS) among HIV+ group.*

4.10. Relation between salivary cortisol concentration and suicidal ideation scores among HIV+ heroin users

Since stress is a risk factor of suicide described in literature in both HIV+ patients and injecting drug users and cortisol levels have been showing association with suicidality is some studies, it was examined if there was an association of cortisol levels and suicidal ideations in HIV+ heroin users. To explore a possible relation between cortisol levels and suicidal ideation, a Pearson correlation was performed. It was found a positive correlation between both ($r = 0.42$; $p = 0.01$), as shown in Figure 8.

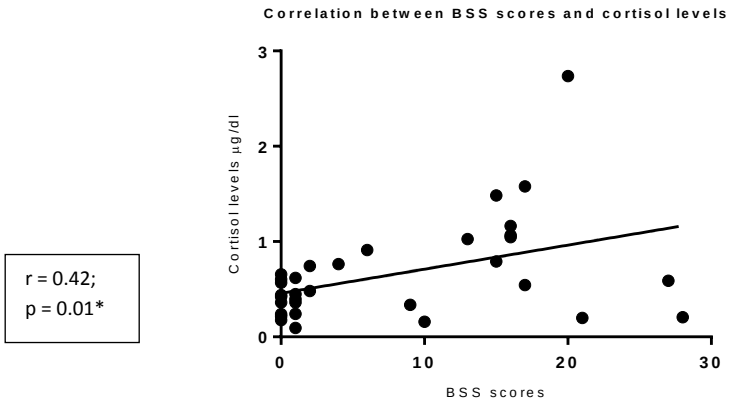

Figure 8. Correlation between salivary cortisol levels and BSS scores.

4.11. Multiple linear regression model of predictors for suicide among HIV+ heroin users

Based on variables that showed relation with suicidal ideation scores among HIV+ patients in the present study, we made a multiple linear regression, wherein we found three parameters that could significantly predict suicide ideations measured by BSS scores, namely: previous suicide attempt ($\beta = 0.29$, $p = 0.03$), salivary cortisol levels ($\beta = 0.44$, $p = 0.00$) and HAD ($\beta = 0.45$, $p = 0.00$). Taken together, the model drawn from variables predicts a significant increase in the scores of suicidal ideation, as represented in Table 6.

Table 6. Multiple linear regression model of variables related to suicide in HIV+ patients.

BSS	B (C.I.: 95%)	Beta	t	p
HAS	3.38 (-2.15; 8.90)	0.19	1.25	0.22
HDS	8.19 (2.59; 13.80)	0.45	3.00	0.00
Cortisol	7.91 (3.19; 12.63)	0.44	3.44	0.00
PSS-10	-0.07 (-0.57; 0.38)	-0.05	-0.31	0.76
Previous suicide attempt	5.25 (0.57; 9.92)	0.29	2.30	0.03

Model: R= 0.80; R^2 = 0.64; R^2 adjusted = 0.58. P <0.05.

5. DISCUSSION

5.1. Comparison of suicidal ideation between HIV+ and HIV- heroin users

We aimed to investigate suicidality among HIV^+ heroin users through a comparative study with HIV^- heroin users. Statistically significant higher suicidal ideation scores were found in the group of HIV^+ compared to HIV^- patients. This result is not in agreement with a similar study by Jin et al 2013, addressing suicide across heroin users in the context of HIV infection, wherein no differences in suicidal ideations were found by comparing HIV^+ patients with HIV^- patients. Nevertheless, the higher rates of suicidal ideations in the group of HIV+ patients in the present study suggests that not only does HIV infection increase the risk of suicide compared to general population from different settings as reported in previous studies (Aldaz et al., 2011) (Jin et al., 2006) (Govender & Schlebusch, 2012), but it may also represent additional risk of suicide among injecting drug users. Notwithstanding, the sequential multiple linear regression model made in the present study predicting suicidal ideation from socio-demographic and psychological variables usually associated with suicide, showed that when socio-demographic variables like age, gender, occupational status were taken into account the difference on suicidal ideation between HIV^+ and HIV^- individuals was still present. This means meaning that the difference on suicidal ideation between the two populations was not due to the differences on the socio-demographic variables between the two study populations. Interestingly, in the same sequential multiple linear regression model, when the psychological parameters usually correlated to suicide were considered, the significance of the group to which one individual in the study population belonged was maintained. Apparently, in our study population neither perceived stress nor depression or anxiety were relevant factors behind the influence of being HIV^+ or HIV^- heroin user on suicidal ideation.

5.3. Comparison of social, demographic and clinical factors associated to suicide between HIV+ and HIV- heroin users

The sequential regression model made showed that if age, gender and occupational status were homogeneous between the two groups, the HIV status would still be a significant predictor of suicidal ideation. We compared these variables and others also previously reported to be associated to suicide in our study population to investigate if relevant differences were present between the two groups. No statistically significant differences were found in other social factors usually associated to predisposition to psychosocial stress across HIV^+ and HIV^- patients, such as marital status, alcoholic or smoking habits.

Similarly, no statistically significant differences in clinical factors commonly found in individuals with records of suicide attempts such as psychiatric disorder and familiar history of suicide between two groups were detected. None-the- less, no statistically significant differences were found either in the frequency of anxiety or depression symptoms (based on HADS scores) across the two groups. These findings were not parallel to others reported regarding depression (Kelly et al., 1998), or anxiety (Morrison et al., 2002) as well as in the self-reported stress wherein these parameters were found as being higher in HIV^+ patients when they are compared to general population. However, considering that the study population is composed of drug users, these results are in line with others studies that show high frequency of psychiatric conditions in both HIV^+ patients and drug users (Grassi et al., 2001), though the type of injected drug used was not discriminated. This finding is also supported by other authors that postulate that some situations experienced by both injecting drug users and HIV^+ patients might render them more likely to develop psychiatric disorders (Sarin, Singh, Samson, & Sweat, 2013), namely: absence of social support, unemployment, homelessness in the first group against social isolation, discrimination and occupational difficulties due to HIV infection (Kelly et al., 1998) in the second. Indeed, in the present study, study population was mostly composed of unemployed, single men irrespectively to diagnosis of HIV. However, the absence of differences in psychological measures and socio-demographic across groups, suggests that the additional effect of the HIV infection increasing suicidal risk among injecting drug users might eventually be explained by alterations related with the HIV and the immune response to the virus.

5.2. Comparison of frequencies of past suicide attempt between HIV+ and HIV- heroin users

Since in previous studies individuals with a past history of suicide were found to be more likely for new attempts of suicide (Kelly et al., 1998), we examined whether or not there could be differences on history of suicide attempt between groups. Our results showed that among heroin users, 40.5% of HIV^+ and 30.9% of HIV^- had attempted suicide at least once in their lifetime and the variation was not statistically significant. This result is in accordance with results reported by Malbegiere & De Andrade, 2001 wherein by analyzing suicidality between HIV^+ and HIV^- drug users, no statistically significant differences were found. But still, these high frequencies in both groups show that heroin users are very likely to attempt suicide regardless HIV diagnosis, what is consistent with literature that describes injecting drugs use as an important risk factor for suicide (Sarin et al., 2013) and is replicated in the study by Jin et al 2013 that also included a

healthy control and found statistically significant differences in suicidal ideation scores among the two groups of drug users (both HIV^+ and HIV^-) when compared to healthy individuals. Withal, the absence of statistically significant differences in the frequency of previous suicide attempt in the present study may arise from the fact that the attempt in this study was considered during lifetime history, meaning that it is possible that the attempt might have occurred before the HIV infection when groups analyzed would have been in the same condition of HIV status.

5.4. Association between psychological measures (anxiety and depression scores), previous suicide attempts and suicide ideation scores among HIV+ heroin users

Although there were no differences in clinical and psychological measures between HIV^+ and HIV^- patients, we further analyzed the influence of these variables on suicidal ideation in HIV^+ patients, in order to obtain information that could be of relevance to draw interventional plans to reduce the risk of suicide in HIV^+ heroin users. Among patients infected with HIV when analyzed separately, the results found revealed that those participants classified as presenting depression in HDS scores had statistically significant higher suicidal ideation scores compared to those classified as presenting "no" depression, what is consistent with the literature that shows an important role of depression increasing the risk of suicide across patients infected with HIV (Bragança & Palha, 2011). Suicidal ideation scores were found higher in individuals classified as presenting anxiety as well, after dichotomized in "yes" or "no" anxious based on HAS scores. These results show that anxiety and depression symptoms are a concern among HIV^+ patients and play an important role increasing suicidal risk in HIV^+ patients who are concomitantly heroin users.

Participants HIV^+ showed a difference in the scores of suicidal ideation relatively to past suicide attempts that was significant, in which those with a lifetime history of suicide had higher suicidal ideation scores compared to those who had never attempted, suggesting that mechanisms that lead to suicide may be enduring and constitute a factor of vulnerability to individuals. This is consistent with several evidences showing people who attempted suicide once, are more likely to attempt again compared to those with no past history of suicide (Kinyanda et al., 2011) .

5.4. Correlation between risk and chronologic factors of HIV infection and suicide among HIV+ heroin users

Regarding risk behavior associated to HIV infection, the most frequently route of transmission of HIV reported by participants was by sharing needles. Even though the route of transmission was not associated with suicidality in the present study, this result highlights the need of intensifying the mechanisms to reduce this practice among people with risk behavior. No correlation was found between time of disclosure of HIV infection and time since suicide attempt, what differs of what is reported in WHO 2000, pointing suicide attempts as more frequently occurring by the time of diagnosis or a few months later. Curiously, it supports the study by Haastrecht et al, 1995 that found no independent effect of disclosure of HIV infection inducing suicide, as far as HIV^+ injecting drug users are concerned. By reacting differently to a disclosure of HIV infection than general population, this result suggests that the appraisal of threat associated to HIV infection might differ in injecting drug users compared to the general population. However, we only considered the time of last attempt of suicide and it is possible that the individuals might have tried to kill themselves several times after disclosure; thus, if we had considered the time of first attempt of suicide rather than the time of last attempt of suicide, it could have been more informative. No correlation was found with time of disclosure and suicidal ideation as well. Meanwhile, a negative correlation was found between time on HAART and current suicidal ideation ($r = -0.40$; $p = 0.02$; $df = 32$). That is, patients that initiated HAART more recently have more thoughts about suicide. If we consider a low immunity as criteria for initiating HAART, one may speculate that low levels of $CD4^+$ T lymphocytes might be related to suicidal ideation. But still, an adaptation to treatment is another aspect to be considered.

5.5. Correlation between stress (cortisol levels and PSS-10 scale scores) and suicidal ideations among HIV+ heroin users

Even though the psychological parameters were associated to suicide ideation among HIV^+ group, they might not explain all the effect on suicidal ideation since no differences in psychological measures were found between them and HIV^- patients, as aforementioned. Since stress has been associated to suicide and cortisol level is an available biological marker of stress, we further explored if there was a relation between cortisol level and suicide ideation among HIV^+ heroin users. To examine a possible relation between salivary cortisol level and suicidal ideation, a Pearson correlation was performed. Our results revealed that cortisol levels had a positive correlation with BSS scores in HIV^+ heroin users. This finding is consistent with the

study by Chatzifittofis 2013 that found higher cortisol levels in suicide attempters, compared with healthy volunteers. Yet to this state of knowledge studies addressing cortisol levels in suicide, either in the context of heroin users or HIV+ patients, are lacking, hence comparison with studies in groups with similar characteristics could not be made. Equally, self-reported stress scores correlated positively with suicidal ideation scores. That is, participants who appraised their lives as stressful during previous month had more thoughts of suicide what is in line with literature that described high perceived stress scores among HIV+ patients (Su et al., 2013), still the high scores were associated to other psychological measures but suicide. Actually, existing studies related to perceived stress and suicide either used different scales to measure perceived stress, or evaluated a different psychological parameter that might in turn be associated to suicide. In addition to this, again studies applying the PSS-10 scale to analyze suicide in HIV+ heroin users are lacking and these comparisons are not considering the specific characteristics of this group.

Cortisol levels were not associated neither with anxiety scores [t (31) = -1.27; p = 0.21; df = 0.42], nor with depression scores [t (31) = 0.60; p = 0.55; df = 0.21]. This finding is not in agreement with published evidence showing that depression is correlated with cortisol levels. Still, these correlations were found in patients with other psychiatric disorders, such as women with major depression (Geoffroy, Hertzman, Li, & Power, 2013) or bipolar disorder (Kamali, Saunders, Prossin, & Brucksch, 2013) and none of them included HIV+ patients concomitantly using injected drugs. Moreover, cortisol levels did not correlate with perceived stress as well. Apparently, although participants appraise their lives as stressful it does not correlate with the cortisol release. This is consistent with other studies wherein no correlation between the two variables was found (Faresjö et al., 2013). One possible explanation to this absence of correlation would be that because of the effects of both heroin and HIV in the Central Nervous System, one may think that the appraisal of the individuals might be affected by alterations in cognition. However, to better clarify it would be necessary to screen altered cognition but no measures were applied to this purpose in the present study.

Study limitations

This study had some limitations that should be acknowledged. Firstly, our study population was recruited by convenience. Secondly, a longitudinal study could be more conclusive in the context of causality. Third,

some information that we intended to include in analysis regarding clinic variables was missing. Finally, study was restricted to heroin users, thus results might not be applied neither to other drugs users nor to other HIV^+ patients. Still, by working with a more homogeneous group, the effects of variability between groups to consider in analysis were reduced.

6. CONCLUSIONS

Our findings revealed that besides being a population with several risk factors accounting for suicide, HIV infection increased the risk compared to HIV^- heroin users. Higher scores of anxiety and depression were associated with higher suicidal ideation scores among HIV^+ patients. These variables together with perceived stress showed to play an important role in suicidal ideation among HIV^+ heroin users, highlighting the importance of screening and treating psychiatric disorders in order to reduce the risk of suicide and suffering among these patients. Previous suicide attempt was associated with suicidal ideation, what is consistent with other literature addressing correlates to suicidal ideation in general population. Once no statistically significant differences in past suicide history were found across HIV^+ and HIV^- participants, the finding emphasizes the need of intensifying suicidal screening among heroin users irrespectively to HIV infection. Together with psychiatric disorders and aforementioned results, in the model of regression made, cortisol levels showed to be a good predictor of suicide ideation among HIV^+ patients that are heroin drug users, replicating results of other published studies, though in a study population with different settings. This finding suggests that cortisol might be a potential variable to further explore as a biological tool to be either accessed or manipulated in the management of suicidality.

7. FINAL REMARKS AND FUTURE RESEARCH PERSPECTIVES

This work was thought to contribute to emphasize the need of more efficient interventional plans focused on screening suicide ideation among HIV^+ heroin users. While suicide is unpredictable, people might benefit of any reduction in psychiatric disorders prevalence to both partially control the suicide risk and reduce suffering associated to diseases. Notwithstanding, important aspects of treatment such as adherence to HAART and prevention of transmission of HIV infection might be improved by reducing the frequency of psychiatric disorders as well.

Replicate findings in more heterogeneous groups from different settings may amplify the utility of the results and simultaneously increase their reliability.

Moreover, respectively to HIV infection, some immunologic parameters are suggested to be associated to psychiatric disorders such as CD4+ T lymphocyte counts, $CD8^+$ T lymphocyte and the RNA viral load (VL). Since literature suggests an association between the HPA axis dysregulation and immune activation as influencing behavior, analyzing these parameters in association with the cortisol levels could hence be worthy to investigate other mechanisms through which HIV infection could be influencing suicidal risk.

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9. ATTACHMENTS

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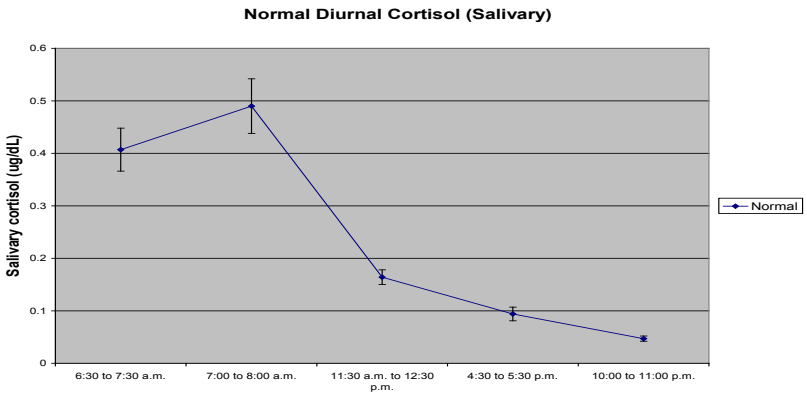
Introduction

Cortisol (hydrocortisone, Compound F) is the major glucocorticoid produced in the adrenal cortex. (1) Cortisol production has a circadian rhythm, (2,3) with levels peaking in the early morning and dropping to lowest values at night. (4,5) Levels rise independently of circadian rhythm in response to stress. (6)

In blood, only about 5-10% of cortisol is in its unbound or biologically active form. The remaining cortisol is bound to serum proteins. (7) Unbound serum cortisol enters saliva via intracellular mechanisms; in saliva, the majority of cortisol remains unbound to protein. Salivary cortisol levels are unaffected by salivary flow rate and are relatively resistant to degradation from enzymes or freeze-thaw cycles. (8,9)

Studies consistently report high correlations between serum and salivary cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels. (10-12)



(Internal Salimetrics Data, n=26. Time of cortisol peak will vary in individuals relative to their normal wake-up time.)

Test Principle

A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present. (13)

pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Cortisol values from samples with a pH ≤ 3.5 or ≥ 9.0 may be artificially inflated or lowered. (14)

Storage

All components of this kit are stable at 2-8°C until the kit's expiration date.

Safety Precautions

- Liquid stop is a 3-molar solution of sulfuric acid. This solution is caustic; use with care.
- See "Material Safety Information" at the end of procedure.
- A safety data sheet is available on request.

Materials Needed But Not Supplied

- Precision pipette to deliver 15 and 25 µL
- Precision multichannel pipette to deliver 50 µL and 200 µL
- Vortex
- Plate rotator with 0.08-0.17 inch orbit capable of 500 rpm (if unavailable, tap plate to mix)
- Plate reader with a 450 nm filter
- Log-linear graph paper or computer software for data reduction
- Deionized water
- Reagent reservoirs
- One disposable polypropylene tube to hold at least 24 mL
- Pipette tips
- Serological pipette to deliver up to 24 mL

Materials Supplied with Single Kit

	Item	Quantity/Size
1	**Microtitre Plate** Coated with monoclonal anti-cortisol antibodies.	1/96-well
2	**Cortisol Standards** In a saliva-like matrix. Ready to use, traceable to NIST standard: 3.0, 1.0, 0.333, 0.111, 0.037, 0.012 µg/dL (82.77, 27.59, 9.19, 3.06, 1.02, 0.33 nmol/L). Contain: cortisol, buffer, preservative.	6 vials/500 µL each
3	**Cortisol Controls** High, Low, in a saliva-like matrix. Ready to use. Contain: cortisol, buffer, preservative.	2 vials/500 µL each
4	**Wash Buffer Concentrate (10X)** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle/100 mL
5	**Assay Diluent** Contains: phosphate buffer, pH indicator, preservative.	1 bottle/60 mL
6	**Cortisol Enzyme Conjugate** Concentrate. Dilute before use with assay diluent. (See step 5 of Procedure.) Contains: cortisol conjugated to HRP, preservative.	1 vial/50 µL
7	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle/25 mL
8	**3 M Stop Solution** Contains: sulfuric acid.	1 bottle/12.5 mL
9	**Non-Specific Binding (NSB) Wells** Do not contain anti-cortisol antibody. Break off and insert as blanks (optional) where needed.	1 strip

Specimen Collection

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Bovine hormones normally present in dairy products can cross-react with anti-cortisol antibodies and cause false results. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Donors may collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, and then passing the saliva through the Saliva Collection Aid, Item No. 5016.02, into a polypropylene vial. Collection protocols are available upon request or online at www.salimetrics.com. Samples from adults and from children ages 6 and above may also be collected using the SalivaBio Oral Swab (SOS), Item No. 5001.02. Samples from children under the age of 6 may be collected with the SalivaBio Children's Swab (SCS), Item No. 5001.06. The SalivaBio Infant's Swab (SIS), Item No. 5001.08, is available for use with children under the age of 6 months.

Samples visibly contaminated with blood should be recollected. We recommend that samples be screened for possible blood contamination (15,16) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Item Nos. 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.

It is important to record the time and date of specimen collection when samples are obtained due to the diurnal variation in cortisol levels.

Sample Handling and Preparation

After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate samples within 30 minutes, and freeze at or below -20°C within 4 hours after collection. (Samples may be stored at -20°C or lower for long-term storage.)

Do not add sodium azide to saliva samples as a preservative, as it may cause interference in the immunoassay.

Freezing saliva samples will precipitate mucins. On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding, leading to falsely elevated results. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Re-freeze saliva samples as soon as possible after adding to the assay plate. Centrifuge/re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

General Kit Use Advice

- This kit uses break-apart microtitre strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the sealed foil pouch with desiccant and used in the frame provided.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volumes of wash buffer and conjugate prepared for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
- Do not mix components from different lots of kits.
- When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.
- The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68-74ºF (20-23.3ºC). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.

- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.

- Routine calibration of pipettes is critical for the best possible assay performance.

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1.5 hours is necessary for the 24 mL of assay diluent used in Step 5 (conjugate dilution) to come to room temperature.

- Bring microtitre plate to room temperature before use. ***It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***

- Prepare 1X wash buffer by diluting wash buffer concentrate 10-fold with room-temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H_2O). ***Dilute only enough for current day's use, and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay.)

Procedure

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.000 Std	3.000 Std	Ctrl-H	Ctrl-H								
B	1.000 Std	1.000 Std	Ctrl-L	Ctrl-L								
C	0.333 Std	0.333 Std	Unk-1	Unk-1								
D	0.111 Std	0.111 Std	Unk-2	Unk-2								
E	0.037 Std	0.037 Std	Unk-3	Unk-3								
F	0.012 Std	0.012 Std	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB*	NSB*	Unk-6	Unk-6								

*NSB = Non-specific binding wells. These may serve as blanks. Use is optional.

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock foil pouch containing unused wells and desiccant. Store at 2-8°C.

Cautions: 1. Extra NSB wells should not be used for determination of standards, controls or unknowns.

2. Do not insert wells from one plate into a different plate.

Step 3: Pipette 24 mL of assay diluent into a disposable tube. Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 25 µL of assay diluent into 2 wells to serve as the zero value.
- Pipette 25 µL of assay diluent into each NSB well.

Step 5: Make a 1:1600 dilution of the conjugate by adding 15 µL of the conjugate to the 24 mL of assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Conjugate tube may be centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately mix the diluted conjugate solution and pipette 200 µL into each well using a multichannel pipette. Make note of any wells with dark yellow or purple pH indicator changes (see description of pH indicator, p. 3).

Step 6: Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 55 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well, and then discarding the liquid by inverting the plate over a sink. After each wash, the plate should be thoroughly

blotted on paper towels before being turned upright. ***If using a plate washer, blotting is still recommended after the last wash, just before the addition of the TMB.***

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix).

  Caution: Spillage may occur if mixing speed exceeds 600 rpm.

- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.

- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 nm is desirable.)

Quality Control

The Salimetrics' high and low salivary cortisol controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

1. Compute the average optical density (OD) for all duplicate wells.

2. Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.

3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo). (The zero is not a point on the standard curve.)

4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter non-linear regression curve fit.

5. Samples with cortisol values greater than 3.0 µg/dL (82.77 nmol/L) should be diluted with assay diluent and rerun for accurate results. If a dilution of the sample is used, multiply the results by the dilution factor.

   When running multiple plates, or multiple sets of strips, a standard curve must be run with each individual plate and/or set of strips.

Assay Summary

1. Bring all reagents to room temperature and mix before use.
2. Bring plate to room temperature and prepare for use with NSB wells. (Use of NSB wells is optional.)
3. Prepare 1X wash buffer.
4. Prepare tube with 24 mL of assay diluent for conjugate dilution, which will be made later.
5. Pipette 25 µL of standards, controls, and unknowns into appropriate wells.
6. Pipette 25 µL of assay diluent into zero and NSB wells.
7. Make 1:1600 dilution of conjugate (15 µL into 24 mL assay diluent), mix,

and immediately pipette 200 µL into each well. Note any pH indicator color changes.

8. Mix plate for 5 minutes at 500 rpm. Incubate for an additional 55 minutes at room temperature.

9. Wash plate 4 times with 1X wash buffer. Blot.

10. Add 200 µL TMB solution to each well.

11. Mix plate for 5 minutes at 500 rpm. Incubate in dark at room temperature for 25 additional minutes.

12. Add 50 µL stop solution to each well. Mix for 3 minutes at 500 rpm.

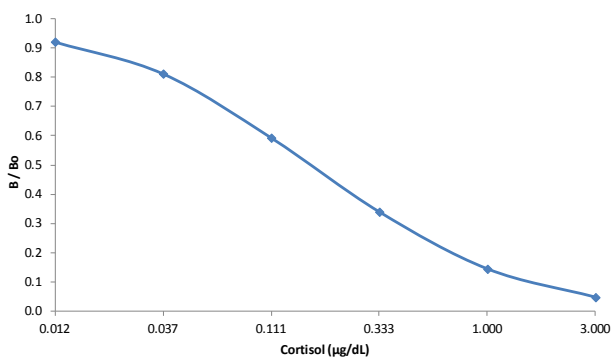
13. Wipe plate bottom clean and read within 10 minutes of adding stop.

Typical Results

The following chart and graph are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	B	B/Bo	Cortisol (µg/dL)
A1,A2	S1	0.094	0.071	0.048	3.000
B1,B2	S2	0.236	0.213	0.145	1.000
C1,C2	S3	0.524	0.501	0.340	0.333
D1,D2	S4	0.897	0.874	0.593	0.111
E1,E2	S5	1.219	1.196	0.812	0.037
F1,F2	S6	1.379	1.356	0.921	0.012
G1,G2	Bo	1.496	1.473	NA	NA
H1,H2	NSB	0.023	NA	NA	NA

Example: HS Cortisol 4-Parameter Curve Fit



Material Safety Information*

Hazardous Ingredients

Liquid stop solution is caustic; use with care. We recommend the procedures listed below for all kit reagents. MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

HS Salivary Cortisol EIA Kit Performance Characteristics

Sample Dilution Recovery

Four saliva samples were diluted with assay diluent and assayed.

Sample	Dilution Factor	Expected (µg/dL)	Observed (µg/dL)	Recovery (%)
S1	undiluted	N/A	0.73	N/A
	1:2	0.37	0.39	107
	1:4	0.18	0.20	111
	1:8	0.09	0.10	111
	1:16	0.05	0.05	105
S2	undiluted	N/A	0.80	N/A
	1:2	0.40	0.40	101
	1:4	0.20	0.19	97
	1:8	0.10	0.09	94
	1:16	0.05	0.05	110
S3	undiluted	N/A	0.61	N/A
	1:2	0.31	0.30	98
	1:4	0.15	0.15	101
	1:8	0.08	0.08	108
	1:16	0.04	0.04	108
S4	undiluted	N/A	2.89	N/A
	1:2	1.45	1.53	105
	1:4	0.72	0.77	106
	1:8	0.36	0.42	115
	1:16	0.18	0.2	108

Linearity of Assay

Sample	Samples		Avg Observed	Expected	Recovery
	Low	High			
a (Low)	100%	0%	0.07	0.07	N/A
b	90%	10%	0.36	0.34	108
c	80%	20%	0.63	0.61	104
d	70%	30%	0.93	0.88	106
e	60%	40%	1.13	1.15	98
f	50%	50%	1.45	1.42	102
g	40%	60%	1.64	1.69	97
h	30%	70%	1.88	1.96	96
i	20%	80%	2.27	2.23	102
j	10%	90%	2.49	2.50	99
k (High)	0%	100%	2.77	2.77	N/A

Analytical Sensitivity

The lower limit of sensitivity was determined by interpolating the mean optical density minus 2 SDs of 10 sets of duplicates at the 0 µg/dL level. The minimal concentration of cortisol that can be distinguished from 0 is 0.007 µg/dL.

Precision

The intra-assay precision was determined from the mean of 20 replicates each.

Sample	N	Mean (µg/dL)	Standard Deviation (µg/dL)	Coefficient of Variation (%)
PS1	20	2.07	0.08	4
PS2	20	1.14	0.05	4
PS3	20	0.42	0.01	3
PS4	20	0.16	0.01	5
PS5	20	0.06	0.00	7

The inter-assay precision was determined from the mean of average duplicates for 20 separate runs.

Sample	N	Mean (µg/dL)	Standard Deviation (µg/dL)	Coefficient of Variation (%)
PS1	20	1.99	0.05	3
PS2	20	1.16	0.05	4
PS3	20	0.43	0.01	3
PS4	20	0.18	0.01	9
PS5	20	0.06	0.01	11

Recovery

Five saliva samples containing different levels of endogenous cortisol were spiked with known quantities of cortisol and assayed.

Sample	Endogenous (µg/dL)	Added (µg/dL)	Expected (µg/dL)	Observed (µg/dL)	Recovery (%)
1	0.071	2.00	2.13	2.20	103
2	0.071	0.20	0.27	0.28	104
3	0.071	0.04	0.11	0.11	98
4	0.078	2.33	2.25	2.33	103
5	0.078	0.20	0.28	0.31	113
6	0.080	0.04	0.12	0.12	103
7	0.86	0.20	1.06	1.16	109
8	0.89	0.04	0.93	1.02	109

Correlation with Serum

The correlation between serum and saliva cortisol was determined by assaying 49 matched samples using the Diagnostic Systems Laboratories serum Cortisol EIA and the Salimetrics HS Salivary Cortisol EIA.

The correlation between saliva and serum was highly significant, $\underline{r}\,(47) = 0.91$, $\underline{p} < 0.0001$.

Specificity of Antiserum

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Cortisol EIA
Prednisolone	100	0.568
Prednisone	1000	ND
Cortisone	1000	0.130
11-Deoxycortisol	500	0.156
21-Deoxycortisol	1000	0.041
17α-Hydroxyprogesterone	1000	ND
Dexamethasone	1000	19.2
Triamcinolone	1000	0.086
Corticosterone	10,000	0.214
Progesterone	1000	0.015
17β-Estradiol	10	ND
DHEA	10,000	ND
Testosterone	10,000	0.006
Transferrin	66,000	ND
Aldosterone	10,000	ND

ND = None detected (<0.004)

Salivary Cortisol Example Ranges

Each laboratory should establish its own range of expected values. The following values have been reported for salivary cortisol.

Group	Number	Overall Range (µg/dL)
Children, neonatal	275	ND - 3.417
Children, age 6 months	165	ND - 2.734

Group	Number	AM Range (µg/dL)	PM Range (µg/dL)
Children, ages 2.5-5.5	112	0.034 - 0.645	0.053 - 0.607
Children, ages 8-11	285	0.084 - 0.839	ND - 0.215
Adolescents, ages 12-18	403	0.021 - 0.883	ND - 0.259
Adult males, ages 21-30	26	0.112 - 0.743	ND - 0.308
Adult females, ages 21-30	20	0.272 - 1.348	ND - 0.359
Adult males, ages 31-50	67	0.122 - 1.551	ND - 0.359
Adult females, ages 31-50	31	0.094 - 1.515	ND - 0.181
Adult males, ages 51-70	28	0.112 - 0.812	ND - 0.228
Adult females, ages 51-70	23	0.149 - 0.739	0.022 - 0.254
All adults	192	0.094 - 1.551	ND - 0.359

Group	Number	2300h (ug/dL)
Normal subjects	19	0.007 – 0.115
Cushing's subjects	21	0.130 – 2.972

ND = None detected

Expected ranges for neonates to 5.5 years were derived using the Salimetrics Salivary Cortisol Immunoassay Kit.

Expected ranges for 8 to 18 years were reported from an unpublished manuscript, Pennsylvania State University's Behavioral Endocrinology Laboratory. Adult ranges were obtained from published literature. (7)

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Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in writing. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Considerando a "Declaração de Helsínquia" da Associação Médica Mundial

(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)

Nome do Estudo: O efeito da infeção por VIH nos usuários de heroína

Chamo-me Wilza Beatriz Filipe Fumo, sou médica generalista moçambicana que no momento estou a fazer Mestrado em Ciências de Saúde na Universidade do Minho que inclui a realização de um projeto de tese.

Gostaria de convidar o Senhor (a) a participar neste projeto de investigação que se intitula: **Efeitos do cortisol, colesterol, grau de imunodeficiência, ansiedade e depressão na avaliação do Risco Suicidário nos Pacientes VIH positivos.**

1 – Introdução

O suicídio é considerado uma causa importante de morte pela Organização Mundial Da Saúde (OMS). A sua ocorrência é maior na presença de doenças crónicas, como é o caso da infeção pelo Vírus de Imunodeficiência Humana (VIH), em pessoas jovens e usuárias de drogas.

Estudos têm associado algumas perturbações psiquiátricas, nomeadamente a depressão e ansiedade, bem como baixas concentrações de colesterol e altas concentrações de cortisol com um aumento na sua frequência.

Por outro lado, as células T CD4 sofrem alterações durante a infeção pelo VIH, com tendência a reduzir as suas concentrações no seu decorrer pelo que são utilizadas para controlar a progressão da doença. Contudo, falta perceber se existe alguma relação entre os seus níveis e a tendência aumentada para cometer suicídio neste grupo.

O objetivo do presente estudo é investigar o efeito da infeção por VIH nos usuários de heroína. Adicionalmente, procurar associações entre as perturbações psiquiátricas, os níveis do cortisol e o risco de suicídio entre as pessoas infetadas pelo VIH, procurando identificar em que fase da infeção este risco predomina como forma de desenvolver estratégias ou melhorar as já existentes para controlá-lo.

Para tal, os participantes irão responder a um questionário para apurar dados sociodemográficos bem como informação clínica relevante que já tenha sido implicada no aumento do risco suicidário.

Para rastreio das perturbações neuropsiquiátricas, serão usadas escalas para a medição da ansiedade, da depressão e do stresse percebido.

O risco de suicídio será obtido através do preenchimento de uma escala de ideação suicida.

Paralelamente iremos colher amostras de saliva para daí medir a concentração do cortisol (que é uma hormona usada para quantificar o stress), que serão codificadas e conservadas em condições adequadas com fins estritamente de investigação no Instituto de Ciências da Vida e Saúde (ICVS) da Universidade do Minho.

Informação relativa às concentrações das células T CD4 serão extraídas dos processos clínicos dos participantes.

Em nenhuma fase do estudo serão divulgados dados confidenciais do doente. Durante o processo de análise não será possível saber a identificação dos doentes envolvidos no estudo.

No entanto, os dados poderão ser observados por um comité de Ética e podem ser publicados em jornais científicos ou em outros lugares sem que a sua identidade seja revelada.

Este estudo conta com a colaboração da Universidade do Minho, na Escola de Ciências de Saúde/ICVS, e contará com a orientação científica da Profª. Doutora Margarida Correia Neves, Professora e investigadora no ICVS e do Professor Doutor António Pacheco Palha.

O estudo teve a aprovação pelo Centro de Respostas Integradas de acordo com a lei vigente.

A realização dos testes por si só não tem influência direta na sua doença nem acarreta quaisquer riscos. No entanto, alguns testes poderão tornar-se "cansativos" pelo que tem todo o direito de interrompê-los ou recusar realizá-los.

Você é livre para optar por participar no estudo. Se recusar participar, não perderá quaisquer benefícios relativos à orientação da sua doença. Uma vez integrado no estudo pode também a qualquer momento abandoná-lo, sem qualquer prejuízo para o seu futuro acompanhamento clínico.

Muito obrigada pela sua atenção!

O investigador

Wilza Fumo

2- Autorização

Eu, abaixo assinado, (nome completo do participante)_____

_____,

Compreendi a explicação que me foi fornecida, por escrito e verbalmente, da investigação que se tenciona realizar, para qual é pedida a minha participação.

Foi-me dada a oportunidade de fazer as perguntas que julguei necessárias, e para todas obtive resposta satisfatória.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação que me foi prestada versou os objetivos, os métodos, os benefícios previstos, os riscos potenciais e o eventual desconforto. Além disso, foi-me afirmado que tenho o direito de decidir livremente aceitar ou recusar a todo o tempo a minha participação no estudo. Sei que se recusar não haverá qualquer prejuízo na assistência que me é prestada.

Foi-me dado todo o tempo de que necessitei para refletir sobre esta proposta de participação.

Nestas circunstâncias, decido livremente aceitar participar neste projeto de investigação, tal como me foi apresentado pelo investigador (a).

Nome do Participante

_____

Assinatura do Participante

_____
___

Data _____/_____/_____

Assinatura do Coordenador do estudo

_____
___

Data _____/_____/_____

Assinatura da pessoa que obteve o consentimento

_____

Data _____/_____/_____

QUESTIONÁRIO – RISCO SUICIDÁRIO E VIH

Número de inquérito: _____ Data:___/_____/_____

1.Identificação

1.1.Nome (iniciais) _____ 1. 2.Número do processo_____

1.3. Sexo 0. Masculino 1. Feminino (_____) 1.4 Idade_____

1.4. Estado Civil 1.Solteiro 2. Casado 3. Divorciado 4. Viúvo (_____)

1.5.Residência 1.Urbano 2. Semiurbano 3. Rural (_____)

1.6. Nível educacional (anos de estudo)_____ _____

1.7. Ocupação 0. Empregado 1. Desempregado 2. Estudante (_____)

2. Hábitos de vida

2.1. Tabágicos 0. Não_____ 1. Sim_____

2.1.1. Se SIM, quantificar (U.M.A.)_____

2.2 Hábitos alcoólicos 0. Não_____ 1. Sim_____

2.2.1 Há quanto tempo não ingere álcool (dias)?_____

2.2.1 Se sim, quantificar (g/dia)_____

2.3. Uso de drogas endovenosas 0. Não_____ 1. Sim _____

2.3.1 Tipo de droga 0. Morfina 1. Heroína 2. Cannabis 3. Barbitúricos 4. Outro (_____)

2.3.2 Há quanto tempo está abstinente (dias)?_____

2.3.2 Tratamento actual 0. Metadona 1. Buprenorfina 2. Nenhum 3. Outro (_____)

2.4. Uso de anticonceptivos orais 0. Não___ 1.sim_____

2.5. Data da última menstruação (D.U.M) _____/_____/_____

2.6 Tem filhos? 0. Não___ 1. Sim_____

2.7 Com quem vive? 0. Sozinho 2. Acompanhado (____)

3. Doença psiquiátrica concomitante_____

4. Tratamento psiquiátrico 0. Não___ 1. Sim_____

4.1 Grupo farmacológico

1. Antidepressivos 2. Antipsicóticos 3. Estabilizadores do humor 4. Ansiolíticos 5. Outros (____)

5. Antecedentes de suicídio_____

6. Tempo desde a última tentativa (meses)_____

5. História familiar psiquiátrica_____

6. História familiar de tentativa de suicídio_____

7. Comorbilidades

1. HTA 2. DM 3. Epilepsia 4. Cancro 5.Perturbação da personalidade 6.Outra (_____)

7. Duração da confirmação de diagnóstico (meses) _____

8. Via de transmissão

1. Sexual 2. Partilha de seringas 3 Desconhece (_____)

9. Orientação sexual

1. Heterossexual 2. Homossexual 3. Bissexual (_____)

8. Em TAR 0. Não ____ 1.Sim_____

8.1. Há quanto tempo? (anos)_____

8.2. Regime terapêutico _____

9. Dados antropométricos

10.1 Altura (metros)

10.2 Peso (quilos)

10.3 Índice de massa corporal (IMC) (kg/m²)

10. Exames complementares

11.1Colesterol (mmol/L)_____

11.2Cortisol salivar (µg/dL)_____

11.3CD4 (número de células/microlitro)_____

11.4 Carga viral (cópias/mL)_____

11.5 Monócitos (número de células/mm³ de sangue)_____

BSS

Nome _____ Estado Civil _____ Idade _____ Sexo __

Data ___/___/___ N.º Processo _____/___

INSTRUÇÕES: Por favor leia cuidadosamente cada grupo de afirmações abaixo. Assinale a questão, em cada grupo, que melhor descreve como se tem sentido na última semana, incluindo hoje. Tenha a certeza de ler todas as afirmações em cada grupo antes de fazer uma escolha.

Parte 1

1. 0 Eu tenho um desejo moderado a forte de viver 1 Eu tenho um desejo fraco de viver 2 Eu não tenho qualquer desejo de viver **2.** 0 Eu não tenho qualquer desejo de morrer 1 Eu tenho um desejo fraco de morrer 2 Eu tenho um desejo moderado a forte de morrer **3.** 0 As minhas razões para viver superam as minhas razões para morrer 1 As minhas razões para viver e morrer são ambas iguais 2 As minhas razões para morrer superam as minhas razões para viver	**4.** 0 Eu não tenho qualquer desejo de me matar 1 Eu tenho um desejo fraco de me matar 2 Eu tenho um desejo moderado a forte de me matar **5.** 0 Eu tentaria salvar a minha vida se me encontrasse numa situação de ameaça de vida 1 Eu deixaria ao acaso viver ou morrer se me encontrasse numa situação de ameaça de vida 2 Eu não tomaria os passos necessários para evitar a morte se me encontrasse numa situação de ameaça de vida **Se assinalou as afirmações zero em ambos os grupos 4 e 5 acima, avance para o grupo 20. Se marcou um 1 ou 2, quer no grupo 4 quer no 5, então vire a página e vá para o grupo 6.**

20. 0 Eu nunca tentei o suicídio 1 Eu tentei o suicídio uma vez 2 Eu tentei o suicídio duas ou mais vezes
Se anteriormente tentou o suicídio, por favor continue com o próximo grupo de afirmações.
21. 0 O meu desejo de morrer, durante a última tentativa de suicídio era baixo 1 O meu desejo de morrer, durante a última tentativa de suicídio era moderado 2 O meu desejo de morrer, durante a última tentativa de suicídio era elevado

_____ **SUBTOTAL PARTE 1**

_____ **SUBTOTAL PARTE 2**

_____ **PONTUAÇÃO TOTAL**

Parte 2

6. 0 Eu tenho breves períodos de pensamento acerca de me matar, que passam rapidamente
1 Eu tenho períodos de pensamento acerca de me matar, que duram um tempo moderado
2 Eu tenho longos períodos de pensamento acerca de me matar

7. 0 Eu raramente ou apenas ocasionalmente penso acerca de me matar
1 Eu tenho pensamentos constantes acerca de me matar
2 Eu penso continuamente acerca de me matar

8. 0 Eu não aceito a ideia de me matar
1 Eu não aceito nem rejeito a ideia de me matar
2 Eu aceito a ideia de me matar

9. 0 Eu consigo conter-me de cometer suicídio
1 Eu estou incerto de que consigo conter-me de cometer suicídio
2Eu não consigo conter-me de cometer suicídio

10. 0 Eu não me mataria por causa da minha família, amigos, religião, danos possíveis de uma tentativa não sucedida, etc.
1 Eu estou algo preocupado acerca de me matar por causa da minha família, amigos, religião, danos possíveis de uma tentativa não sucedida, etc.
2 Eu não estou preocupado ou apenas um pouco acerca de me matar por causa da minha família, amigos, religião, danos possíveis de uma tentativa não sucedida, etc.

11. 0 As minhas razões para querer cometer suicídio são apontadas primariamente a influenciar outras pessoas, como a vingar-me de pessoas, fazer pessoas mais felizes, fazer prestarem-me atenção, etc.
1 As minhas razões para querer cometer suicídio não estão apenas apontadas a influenciar outras pessoas, mas também representam um meio de resolver os meus problemas
2 As minhas razões para querer cometer suicídio são baseadas primariamente em escapar aos meus problemas

12. 0 Eu não tenho um plano específico acerca de como me matar
1 Eu tenho considerado formas de me matar, mas não trabalhei os detalhes
2 Eu tenho um plano específico para me matar

13. 0 Eu não tenho acesso a um método ou a uma oportunidade para me matar
1 O método que eu usaria para cometer suicídio demora tempo, e eu realmente não tenho uma boa oportunidade para usar este método
2 Eu tenho acesso ou antecipo ter acesso ao método que eu escolheria para me matar e também tenho ou terei a oportunidade para o usar

14. 0 Eu não tenho a coragem ou a habilidade para cometer suicídio
1 Eu estou incerto de que tenho a coragem ou a habilidade para cometer suicídio
2 Eu tenho a coragem ou a habilidade para cometer suicídio

15. 0 Eu não espero fazer uma tentativa de suicídio
1 Eu estou incerto de que farei uma tentativa de suicídio
2 Eu estou certo de que farei uma tentativa de suicídio

16. 0 Eu não fiz quaisquer preparativos para cometer suicídio
1 Eu fiz alguns preparativos para cometer suicídio
2 Eu quase acabei ou completei os meus preparativos para cometer suicídio

17. 0 Eu não escrevi uma nota de suicídio
1 Eu pensei acerca de escrever uma nota de suicídio ou comecei a escrever uma, mas ainda não a completei
2 Eu completei uma nota de suicídio

18. 0 Eu não fiz quaisquer preparativos para o que acontecerá após eu ter cometido suicídio
1 Eu pensei acerca de fazer alguns preparativos para o que acontecerá após eu ter cometido suicídio
2 Eu fiz preparativos definitivos para o que acontecerá após eu ter cometido suicídio

19. 0 Eu não escondi o meu desejo de me matar das outras pessoas
1 Eu contive-me de contar às pessoas acerca de querer matar-me
2 Eu tentei esconder, ocultar, ou mentir acerca de querer cometer suicídio

Vá para o grupo 20.

QUESTIONÁRIO DE AUTO-RESPOSTA - PSS-10

Cohen e Williamson (1988)

Versão portuguesa preparada por
Miguel Trigo e Danilo Silva, 2003

INSTRUÇÃO

Para cada questão, pedimos que indique **com que frequência pensou ou se sentiu de determinada maneira, durante o último mês**. Apesar de algumas perguntas serem parecidas, existem diferenças entre elas e deve responder a cada uma como uma pergunta separada. **Responda de forma rápida e espontânea**. Indique, com uma cruz (X), a alternativa que melhor se ajusta à sua situação.

	Nunca	Quase nunca	Algumas vezes	Frequentemente	Muito frequente
	0	**1**	**2**	**3**	**4**
1. No último mês, com que frequência esteve preocupado(a) por causa de alguma coisa que aconteceu inesperadamente?					
2. No último mês, com que frequência se sentiu incapaz de controlar as coisas importantes da sua vida?					
3. No último mês, com que frequência se sentiu nervoso(a) e em stresse?					
4. No último mês, com que frequência sentiu confiança na sua capacidade para enfrentar os seus problemas pessoais?					
5. No último mês, com que frequência sentiu que as coisas estavam a correr à sua maneira?					
6. No último mês, com que frequência sentiu que não aguentava com as coisas todas que tinha para fazer?					
7. No último mês, com que frequência foi capaz de controlar as suas irritações?					
8. No último mês, com que frequência sentiu ter tudo sob controlo?					
9. No último mês, com que frequência se sentiu furioso(a) por coisas que ultrapassaram o seu controlo?					
10. No último mês, com que frequência sentiu que as dificuldades se estavam a acumular tanto que não as conseguia ultrapassar?					
	0	**1**	**2**	**3**	**4**

Quadro 1 – Escala Hospitalar de Ansiedade e Depressão

Este questionário ajudará o seu médico a saber como você está se sentindo. Leia todas as frases. Marque com um "X" a resposta que melhor corresponder a como você tem se sentido na ÚLTIMA SEMANA. Não é preciso ficar pensando muito em cada questão. Neste questionário as respostas espontâneas têm mais valor do que aquelas em que se pensa muito. Marque apenas uma resposta para cada pergunta.

A 1) Eu me sinto tenso ou contraído:
- 3 ( ) A maior parte do tempo
- 2 ( ) Boa parte do tempo
- 1 ( ) De vez em quando
- 0 ( ) Nunca

D 2) Eu ainda sinto gosto pelas mesmas coisas de antes:
- 0 ( ) Sim, do mesmo jeito que antes
- 1 ( ) Não tanto quanto antes
- 2 ( ) Só um pouco
- 3 ( ) Já não sinto mais prazer em nada

A 3) Eu sinto uma espécie de medo, como se alguma coisa ruim fosse acontecer:
- 3 ( ) Sim, e de um jeito muito forte
- 2 ( ) Sim, mas não tão forte
- 1 ( ) Um pouco, mas isso não me preocupa
- 0 ( ) Não sinto nada disso

D 4) Dou risada e me divirto quando vejo coisas engraçadas:
- 0 ( ) Do mesmo jeito que antes
- 1 ( ) Atualmente um pouco menos
- 2 ( ) Atualmente bem menos
- 3 ( ) Não consigo mais

A 5) Estou com a cabeça cheia de preocupações:
- 3 ( ) A maior parte do tempo
- 2 ( ) Boa parte do tempo
- 1 ( ) De vez em quando
- 0 ( ) Raramente

D 6) Eu me sinto alegre:
- 3 ( ) Nunca
- 2 ( ) Poucas vezes
- 1 ( ) Muitas vezes
- 0 ( ) A maior parte do tempo

A 7) Consigo ficar sentado à vontade e me sentir relaxado:
- 0 ( ) Sim, quase sempre
- 1 ( ) Muitas vezes
- 2 ( ) Poucas vezes
- 3 ( ) Nunca

D 8) Eu estou lento para pensar e fazer as coisas:
- 3 ( ) Quase sempre
- 2 ( ) Muitas vezes
- 1 ( ) De vez em quando
- 0 ( ) Nunca

A 9) Eu tenho uma sensação ruim de medo, como um frio na barriga ou um aperto no estômago:
- 0 ( ) Nunca
- 1 ( ) De vez em quando
- 2 ( ) Muitas vezes
- 3 ( ) Quase sempre

D 10) Eu perdi o interesse em cuidar da minha aparência:
- 3 ( ) Completamente
- 2 ( ) Não estou mais me cuidando como deveria
- 1 ( ) Talvez não tanto quanto antes
- 0 ( ) Me cuido do mesmo jeito que antes

A 11) Eu me sinto inquieto, como se eu não pudesse ficar parado em lugar nenhum:
- 3 ( ) Sim, demais
- 2 ( ) Bastante
- 1 ( ) Um pouco
- 0 ( ) Não me sinto assim

D 12) Fico esperando animado as coisas boas que estão por vir:
- 0 ( ) Do mesmo jeito que antes
- 1 ( ) Um pouco menos do que antes
- 2 ( ) Bem menos do que antes
- 3 ( ) Quase nunca

A 13) De repente, tenho a sensação de entrar em pânico:
- 3 ( ) A quase todo momento
- 2 ( ) Várias vezes
- 1 ( ) De vez em quando
- 0 ( ) Não sinto isso

D 14) Consigo sentir prazer quando assisto a um bom programa de televisão, de rádio ou quando leio alguma coisa:
- 0 ( ) Quase sempre
- 1 ( ) Várias vezes
- 2 ( ) Poucas vezes
- 3 ( ) Quase nunca