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Lack of association of indoleamine 2,3-dioxygenase polymorphisms with interferon-alpha-related depression in hepatitis C

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ABSTRACT

Background: Major depression is a frequent adverse effect of interferon-alpha (IFN-α) therapy. Although the indoleamine 2,3-dioxygenase (IDO) enzyme seems to be involved in the pathophysiology of IFN-α-induced depression, no pharmacogenetic study has investigated whether variation in the IDO gene modifies vulnerability to this adverse effect.

Methods: A cross-sectional study assessing 277 hepatitis C patients recruited in two specialized outpatient clinics of Brazil. They were interviewed with the Mini International Neuropsychiatric Interview (MINI) approximately 1 month after the end of IFN-α plus ribavirin therapy. Genomic DNA of individuals was extracted from venous blood. Three IDO single-nucleotide polymorphisms (SNPs) were genotyped (rs3824259; rs10089084 and rs35099072).

Results: MINI indicated that 21.3% of the sample met criteria for a major depressive episode during the course of IFN-α therapy. No association with the diagnosis of a major depressive episode during the course of IFN-α therapy was observed genotype or allele-wise ($p > 0.05$). Current major depression and/or current anxiety disorder was significantly associated with IFN-α-related depression ($p < 0.005$). However, gender, age, route of infection, result of the antiviral treatment, past history of substance use disorders, depression or any other psychiatric disorder showed no association with IFN-α-related depression ($p > 0.05$).

Conclusions: Our results suggest no influence of the variants in the IDO gene and the diagnosis of interferon-α-related depression in the Brazilian population. Interferon-α-related depression may impose persistent psychopathology on at least 15% of the depressed patients even 2 years after antiviral therapy termination. The cross-sectional design is a limitation of our study, predisposing memory bias. Prospective pharmacogenetic studies are warranted to continue investigation of the impact of IDO polymorphisms on the development of IFN-α-induced depression.

1. Introduction

Chronic hepatitis C affects over 170 million individuals worldwide (Capuron et al., 2002; Asnis et al., 2003; Asnis and De La Garza, 2006; Hauser et al., 2000; Keefe, 2007). The gold standard treatment for hepatitis C is interferon-alpha (IFN-α) combined with ribavirin (RBV). This treatment offers the opportunity for cure in more than 50% of hepatitis C virus (HCV)-infected patients (Asnis and De La Garza, 2006). However, IFN-α-induced major depression episodes (MDEs) are a frequent adverse effect in 30–45% of patients who receive this treatment (Capuron et al., 2002; Asnis et al., 2003). This IFN-α-related neuropsychiatric side effect may lead to severe outcomes such as suicidal behavior, therapy withdrawal, and poor virological response (Capuron et al., 2002; Raison et al., 2007; Leutscher et al., 2010).

The primary pathophysiological hypothesis for IFN-α-induced depression involves the interaction between immune and central

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nervous systems. IFN-α stimulates the synthesis and secretion of pro-inflammatory cytokines, which are important for viral clearance in the therapy of HCV, but which also mediate the "sickness behavior", characterized by loss of appetite, sleep disturbance, fatigue, malaise, lethargy, inability to concentrate, and loss of interest in the surroundings (Asnis et al., 2003; Raison et al., 2005; Quarantini et al., 2007). These features overlap with depressive symptoms, which explain why non-mental-health professionals may fail to promptly diagnose this adverse effect, thus resulting in additional damage to HCV patients, including chronic or recurrent depression (Galvão-de Almeida et al., 2010a,b).

Apart from the possible direct actions of proinflammatory cytokines in the brain, it seems they modulate the serotonergic system through the upregulation of the indoleamine 2,3-dioxygenase enzyme (IDO). IDO over-stimulation may result in lower plasma concentrations of tryptophan, and consequently in decreased availability of serotonin, one of the neurotransmitters implicated in pathophysiology of major depression, in the central nervous system (CNS) (Wichers and Maes, 2002; Bonaccorso et al., 2002; Capuron and Miller, 2004; Comai et al., 2011). This mechanism may also result in higher production of kynurenine, another tryptophan metabolite, the metabolites of which (i.e., quinolinic acid, and 3-hydroxykynurenine) have been demonstrated to be involved in such degenerative diseases as Alzheimer's and amyotrophic lateral sclerosis, as well as in depression and schizophrenia (Chen et al., 2010; Maes, 2010).

These hypotheses are additionally supported by such clinical findings as reduced acid 5-hydroxy-indoleacetic acid (5-HIAA) in the cerebrospinal fluid of patients treated with IFN-α, and by the efficacy of selective serotonin reuptake inhibitors (SSRIs) in the treatment of IFN-α-induced depression (Capuron and Miller, 2004; Vignau et al., 2005). The involvement of IDO is also corroborated by a study in which untreated depressed patients, during IFN-α use, displayed a significant increase in serum kynurenine, and more prolonged reductions in tryptophan concentrations than those not depressed during the same treatment (Capuron and Miller, 2004).

Considering that the HCV-major depression comorbidity remains under-diagnosed (Batista-Neves et al., 2008) and affects both the quality of life and the course of the somatic illnesses (Batista-Neves et al., 2009), many authors have suggested systematically treating IFN-α-induced depression prophylactically with antidepressants (Raison et al., 2007; Musselman et al., 2001; Schaefer et al., 2005; Kraus et al., 2005; Gleason et al., 2007; Morasco et al., 2007). A recent review of six clinical trials by our group did not support this strategy (Galvão-de Almeida et al., 2010a,b). Thus, risk factors for depression during IFN-α treatment in HCV individuals need to be identified.

Recent studies (Bull et al., 2009; Lotrich et al., 2009; Pierucci-Lagha et al., 2010) have suggested that genetic evaluation may be informative for screening "at-risk" HCV patients and may produce more successful individualized preventive and therapeutic approaches. Considering the significant role played by IDO in the regulation of serotonin levels during IFN-α treatment and its possible influence on IFN-α-induced depression, variation in IDO gene may influence risk of developing treatment-induced depression. To test this hypothesis, we conducted an association study with three IDO functional polymorphisms and the diagnosis of major depression during the course of IFN-α plus RBV therapy in HCV patients.

2. Methods and Materials

A cross-sectional study was performed evaluating the association of three functional polymorphisms in IDO gene and the diagnosis of IFN-α-related depression in HCV patients who had completed IFN-α plus RBV therapy.

2.1. Sample

The sample comprised HCV patients recruited between February 2008 and March 2010 from the outpatient of the Hepatology clinics of the Teaching Hospital, Federal University of Bahia (UFBA), Bahia, Brazil, and the São Paulo Hospital, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

Initially, medical charts were screened in order to select potential subjects. Sequentially, the patients that had fulfilled the inclusion and exclusion criteria were invited, personally during the regular medical appointments or by phone, to participate.

Inclusion criteria included: 1. Age between 18 and 65; 2. Diagnosis of chronic hepatitis C with anti-HCV positive by ELISA III, and confirmed by qualitative determination of HCV RNA; 3. Treatment with conventional or pegylated IFN-α plus RBV for at least 3 months (if discontinued due to lack of efficacy); 4. Therapy termination at least 1 month prior to evaluation.

Exclusion criteria were: 1. Co-infections (hepatitis B virus- HBV; human immunodeficiency virus- HIV; human T lymphotropic virus- HTLV); 2. Decompensated liver disease (Child-Pugh B or C); 3. Diagnoses of other causes of liver disease than hepatitis C; 4. Decompensated general medical conditions (e.g., patients with a recent diagnosis of hypertension/diabetes, or with unstable clinical status - i.e., high blood pressure or high glycemia despite regular use of specific therapy); 5. Neurological conditions (epilepsy, Parkinson's Disease, past history of cerebrovascular events); 6. Cancer diagnosis; 7. Previous diagnosis (before initiation of antiviral therapy) of major depression, schizophrenia, bipolar disorder, organic mental disorder, or moderate to severe mental retardation; 8. Difficulty understanding the study and its objectives.

2.2. Phenotypic assessment

After the complete antiviral therapy, the HCV patients were cross-sectionally assessed with a comprehensive interview. It included a sociodemographic and clinical characteristics questionnaire, a structured psychiatric diagnostic interview and two psychiatric symptoms severity scales.

Assessed clinical features included the probable route of infection, viral genotype, hepatic fibrosis according to the METAVIR classification (Bedossa and Poynard, 1996), and family psychiatric history. Medical charts were also consulted in order to guarantee the best available information.

Lifetime psychiatric diagnoses were evaluated by the Mini International Neuropsychiatric Interview, Brazilian version 5.0.0 (MINI Plus) (Amorim, 2000), which encompasses the main axis I disorders of DSM-IV (American Psychiatric Association, 1994), and International Classification of Diseases (World Health Organization, 1991). Beck Depression Inventory (BDI) (Beck et al., 1961), and Hospital Anxiety and Depression Scale (HADS; Brazilian version) (Botega et al., 1995) were used to assess the severity of depressive and anxiety symptoms.

The minimum time for the assessment, after the end of IFN-α plus RBV treatment, was set at 1 month but was not given a deadline.

2.3. Genotypic assessment

2.3.1. DNA Extraction

Genomic DNA of individuals was extracted from samples of 5 ml of peripheral venous blood using the "salting out" method and stored in individual tubes labeled for later analysis (Miller et al., 1988).

2.3.2. IDO Polymorphisms and Population Markers Selection and Genotyping

To comprehensively screen the IDO gene, the Tagger program (http://www.broad.mit.edu/mpg/tagger/) (de Bakker et al., 2005) from the HapMap Project database (http://www.hapmap.org/index.html.en) (The International HapMap Consortium, 2003) for the CEU population (Individuals with European ancestry) was used in the Tagger Pairwise mode. Minor allele frequency cutoff was set at 0.05 and r^2 cutoff was set at 0.7. Two tag single-nucleotide polymorphisms (SNPs) (rs3824259; rs10089084) located in the 5' region of the IDO gene, capturing a total of 5 of the 7 existing SNPs in the IDO gene, exhibiting a minor allele frequency higher than 5%, were selected. According to the database, the two selected SNPs are representative of the common genetic variation in the gene, since they work as proxy markers for the other untyped SNPs in the region, with a mean r^2 value of 0.916. The third SNP selected in this study (rs35099072) was chosen based on its potential functional properties. Arefayene et al. (2009) recently demonstrated that this polymorphism resulted in significantly reduced protein expression and enzyme activity. Genotyping was performed by Prevention Genetics (Marshfiled, MA, USA) using allele-specific PCR with universal energy transfer-labeled primers (Myakishev et al., 2001).

Additionally, a set of 35 ancestry informative markers (AIMs), which exhibit a high level of allele frequency difference among the three founder populations of the Brazilian individuals (Europeans, West-Africans and Native Americans) (Shriver et al., 2005; Guindalini et al., 2006), were selected for genetic admixture analyzes. The number of ancestral populations (K) among the sample and individual admixture proportions was estimated using the Bayesian Markov Chain–Monte Carlo (MCMC) method implemented in the STRUCTURE 2.1 program (Pritchard et al., 2000). The program was run under the admixture model, using correlated allele frequencies and no prior population information with a burn-in of 100,000 interactions and 1000,000 interactions after burn-in. Genotyping of all markers was performed using the same method described above. Only genotypes with a level of confidence $\geqslant$90% were included in the analysis.

2.4. Statistical analysis

Student's *T* tests and Fisher's exact test were used to test for differences between groups in sociodemographic and clinical features. Fisher exact test was performed for analysis of categorical variables. Continuous data were evaluated with *T* tests and presented as mean ± S.D. (standard deviation). The odds ratios and 95% confidence intervals for the genotypic analyses were derived from multivariate logistic regression models using the Statistical Package for the Social Sciences (SPSS) v15.0. Two-tailed hypotheses were used with a statistical significance level set at $p < 0.05$.

2.4.1. Ethical Aspects

The study was approved by the Medical Review Ethics Committees of UFBA and UNIFESP and performed in accordance with the ethical standards set in the 1996 Declaration of Helsinki, and with Resolution 196/96 on research involving human subjects. All patients had provided written informed consent prior to their inclusion in the study.

3. Results

During the first stage of the study, 759 medical charts were screened. Two hundred and thirty-six HCV subjects were excluded because they had never been treated, 17 were older than 65, 4 had only been treated with IFN-α (without RBV; e.g., chronic renal failure and sickle cell anemia), 5 patients had schizophrenia, 2 had bipolar disorder, 4 had already been diagnosed with depression, 20 were excluded for co-infections, 12 for neurological conditions, 7 for cancer, and 9 were classified as Child-Pugh B. Finally, 412 patients were eligible to participate in the study: 113 could not be contacted for the second screening; 4 demonstrated some intellectual deficit and were therefore unable to understand the purpose of the study; and 27 refused to participate. Two hundred and ninety-nine interviews were ultimately performed, but 22 protocols could not be completed.

3.1. Demographic and Clinical Characteristics of the Total Study Sample

The sample of 277 patients was predominantly made up of males (56.7%), presented a mean age of 51.3 years (standard deviation [SD]: 7.7), and a mean duration of chronic HCV diagnosis of 6.4 years (SD: 3.7). Thirty-four percent of the patients had been infected through blood transfusion, and of those who acquired HCV sharing syringes, 69% did so to use vitamin complex injections. Almost 75% of the sample had acquired genotype 1 HCV, and 81.5% had been treated with pegylated IFN-α. The most common co-occurring diseases were systemic arterial hypertension (32.1%), diabetes mellitus (17%), and hepatic cirrhosis (15.9%). Table 1 summarizes the characteristics of the individuals who met criteria for a major depressive episode during the course of IFN-α therapy. The level of fibrosis revealed by the hepatic biopsy was the only variable associated with the diagnosis of IFN-α-related depression ($p = 0.03$).

3.2. Psychiatric and Psychometric Characteristics of the Total Study Sample

Regarding psychiatric features, MINI indicated that 21.3% of the sample met criteria for a major depressive episode during the course of IFN-α therapy, 10.1% met criteria for lifetime major depressive episode with no relation to IFN-α exposure, and 4.7% of the patients were depressed at the time of the evaluation. The mean current scores of BDI and HADS were, respectively, 11.2 ± 10.0, and 11.4 ± 7.7. Approximately 18% of the patients referred to a current or past psychiatric treatment, 17.7% fulfilled criteria for lifetime anxiety disorder, and 35.7% for lifetime substance abuse or dependence.

Table 2 summarizes the data concerning the psychiatric disorders detected, personal and family psychiatric history, and the psychometric measures in the groups of individuals with and without IFN-α-related depression. Current major depression and/or current anxiety disorder was significantly associated with IFN-α-related depression ($p < 0.005$). However, lifetime major depression non-related to IFN-α and lifetime substance use disorders showed no association with IFN-α-related depression ($p > 0.05$). The current anxiety disorders associated with the diagnosis of IFN-α-related depression were generalized anxiety disorder (GAD) ($p = 0.03$), and specific phobia ($p = 0.003$). The only past anxiety disorder with a statistically significant correlation was panic disorder ($p = 0.04$) although only 2 patients presented with this diagnosis.

3.3. Genetic analyses

The observed genotype frequencies for the rs3824259; rs10089084 and rs35099072 SNPs were demonstrated in Hardy–Weinberg Equilibrium in our sample ($p > 0.05$). Based on the genotypic data of the 35 AIMs, for the sample as a whole, the STRUCTURE 2.1 program estimated the mean ancestry proportions of the individuals to be: 53.5 ± 19.3% European, 29.1 ± 18.8% West-African, and 17.3 ± 10.9% Native American. The ancestry

Table 1
Sociodemographic and clinical characteristics of the patients concerning their IFN-α-related depression status, according to the MINI.

Characteristics	IFN-α-related depression		*p* value
	Yes	No	
Age in years (mean ± SD)	50.9 ± 7.0	51.4 ± 7.9	0.67
Male gender% (*N*)	52.5% (31)	57.8% (126)	0.55
Marital Status% (N)			
Stable marital relationship	66.1% (39)	72.5% (158)	0.33
Education% (N)			
Complete high school degree	49.2% (29)	42.2% (92)	0.37
Occupational status% (N)			
Employed, student or housekeeper	52.5% (31)	59.2% (129)	0.37
Paid activity, source of income	71.2% (42)	82.1% (179)	0.07
Probable Route of Infection% (N)			
Transfusion	39% (23)	32.1% (70)	
Tattoo	3.4% (2)	5.0% (11)	
Sharing needles or straws for drug use	13.6% (8)	16.5% (36)	
Other (accidental, sharing blades, surgery)	44.1% (26)	46.3% (101)	0.81
Time in Years since HCV Diagnosis (mean ± SD)	6.62 ± 3.69	6.39 ± 3.74	0.67
Virus genotype% (N)			
1	72.9% (43)	75.1% (160)	
2	3.4% (2)	2.8% (6)	
3	22% (13)	22.1% (47)	
4	1.7% (1)	0% (0)	0.38
Inflammatory activity (METAVIR)% (N)			
A0	23.5% (12)	26.6% (51)	
A1	39.2% (20)	31.8% (61)	
A2	27.5% (14)	30.2% (58)	
A3	9.8% (5)	11.5% (22)	0.89
Fibrosis (METAVIR)% (N)			
F0–F1	1.9% (1)	14.1% (28)	
F2–F3	81.1% (43)	70.2% (139)	
F4	17.0% (9)	15.7% (31)	0.03
Duration of the last antiviral therapy (months)			
6	40.7% (24)	45% (98)	
12	59.3% (35)	55% (120)	0.65
Antiviral Therapy			
Pegylated IFN-α plus RBV% (N)	84.7% (50)	80.6% (175)	0.57
Number of IFN-α trials% (N)			
1	78% (46)	83.5% (182)	
2 or more	22% (13)	16.5% (36)	0.33
Successful antiviral therapy% (N)	44.8% (26)	46.5% (99)	0.88
Time in years since therapy termination (mean ± SD)	2.15 ± 1.41	2.54 ± 1.73	0.07
Presence of any other general medical condition	62.7% (37)	64.2% (140)	0.87

Abbreviations: IFN-α, interferon-alpha; SD, standard deviation; HCV, hepatitis C virus; RBV, ribavirin.

Table 2
Psychiatric and psychometric characteristics of the sample concerning its IFN-α-related depression status, according to the MINI.

Characteristics	IFN-α-related Depression		*p* value
	Yes	No	
Current major depression% (*N*)	15.3% (9)	1.8% (4)	<0.001
Lifetime major depression non-related to IFN-α% (*N*)	15.3% (9)	8.7% (19)	0.14
Current anxiety disorder% (*N*)	32.2% (19)	13.3% (29)	0.002
Lifetime substance abuse or Dependence% (*N*)	44.1% (26)	33.5% (73)	0.16
Report of current or past psychiatric treatment% (*N*)	35.6% (21)	13.8% (30)	<0.001
Lifetime suicidal attempt% (*N*)	5.1% (3)	3.7% (8)	0.7
Current suicide risk according to the MINI% (*N*)			
Low	91.5% (54)	98.2% (214)	
Moderate	1.7% (1)	0.5% (1)	
High	6.8% (4)	1.4% (3)	0.03
Family history of any psychiatric disorder% (*N*)	79.7% (47)	67.9% (148)	0.1
Family history of depression% (*N*)	15.3% (9)	10.1% (22)	0.25
Family history of suicide% (*N*)	8.5% (5)	7.3% (16)	0.78
Current BDI scores (mean ± SD)	17.7 ± 12.2	9.3 ± 8.4	<0.001
Current HADS scores (mean ± SD)	16.6 ± 7.5	10.0 ± 7.1	<0.001

Abbreviations: IFN-α, interferon-alpha; SD, standard deviation; MINI, Mini International Neuropsychiatric Interview; BDI, Beck Depression Inventory; HADS, Hospital Anxiety and Depression Scale.

proportions did not differ between groups of patients with and without IFN-α-related depression ($p > 0.05$). Genotype and allele frequencies for the three studied markers in both groups are shown in Table 3. No association with the diagnosis of major depressive episode during the course of IFN-α therapy was observed genotype or allele-wise ($p > 0.05$).

Multivariate logistic regression analyses including fibrosis, current major depression, current anxiety disorder, report of psychiatric treatment, current suicide risk, current BDI and HADS scores, as well as the genotype groups and genetic ancestry estimations, confirmed the lack of association between the rs10089084 (OR = 1.17; 95% CI = 0.56–2.45; $p = 0.676$) and the rs3824259 polymorphisms (OR = 1.10; 95% CI = 0.50–2.41; $p = 0.810$), and the diagnosis of IFN-α-related depression.

3.4. Discussion

The enzyme IDO is known to act by metabolizing tryptophan in serotonin and kynurenine. Although the role of IDO in IFN-α-induced depression is supported by many studies (Wichers and Maes, 2002; Bonaccorso et al., 2002; Capuron and Miller, 2004; Comai et al., 2011), to the best of our knowledge, this is the first study to investigate the influence of the genetic variants of this enzyme and the diagnosis of major depression during the course of IFN-α therapy. Contrary to our hypothesis, no association between the rs3824259, rs10089084, and rs35099072 polymorphisms and IFN-α-related depression was indentified.

We accounted for the potential bias related to population stratification in the Brazilian ethnically heterogeneous population using thirty-five AIMs that show large differences in allele distribution among the three main ethnic groups (European, African and Indigenous). However, the inclusion of the estimated ancestry did not affect the genetic association results. It is important to note that the high level of admixture found in our sample has a strong influence on the haplotype structure of the gene, and therefore other SNPs in and around the gene should be further evaluated before any conclusion regarding the effect of the IDO gene variation in the predisposition of IFN-α-related depression can be reached. In addition, power calculation revealed that the total sample has approximately 80% power to detect differences in genotype group frequency > 18%, assuming the frequency of 46.8% and 63.5% of the CG/GG and GT/GG genotype groups among individuals who have not developed IFN-α-related depression, for the rs10089084 and rs3824259, respectively.

Table 3
Genotype and Allele frequencies concerning the IFN-α-related depression status of the patients.

Genotypes and Alleles	IFN-α-related Depression		*p* value
	Yes	No	
rs10089084			
CC	52.5% (31)	53.2% (115)	1.0
CG/GG	47.5% (28)	46.8% (101)	
C	69.5% (82)	72% (311)	0.65
G	30.5% (36)	28% (121)	
rs3824259			
TT	41.8% (23)	36.5% (77)	0.5
GT/GG	58.2% (32)	63.5% (134)	
T	65.5% (72)	58.3% (246)	0.19
G	34.5% (38)	41.7% (176)	
rs35099072			
GG	100% (56)	99.1% (212)	1.0
GA	0	0.9% (2)	
G	100% (112)	99.5% (426)	1.0
A	0	0.5% (2)	

Abbreviation: IFN-α, interferon-alpha.

The fact that an association between these polymorphisms and the diagnosis of depression related to IFN-α therapy was not found in our HCV patients suggests that other genetic variations either influence or are influenced by IDO and its metabolites' actions. Indeed, polymorphisms of pro-inflammatory cytokines that may be associated to the overstimulation of IDO, such as IL-6 (Bull et al., 2009) and IL-28B (Lotrich et al., 2010), of the IFN-α-receptor 1 (Yoshida et al., 2005), the serotonin transporter (5-HTT) (Bull et al., 2009; Lotrich et al., 2009), and the serotonin-1A receptor gene (HTR1A) (Kraus et al., 2007) have been investigated and seem to play a role in the vulnerability to IFN-α-induced depression. Moreover, genetic variations of phospholipase A2 (PLA2) and cyclooxygenase-2 (COX2), the two key enzymes of the polyunsaturated fatty acid (PUFA) metabolism and prostaglandin E2 (PGE2) synthesis, have also increased the risk of IFN-α-induced depression in a recent study (Su et al., 2010).

Nevertheless, a number of relevant clinical findings pertaining to the Brazilian sample should be noted. Importantly, regarding the natural course of this substance-related depression, our study raises questions related to the possibility of psychic consequences to IFN-α administration lasting many years after the therapy cessation. In fact, only 4 of the 13 patients who were depressed at the evaluation did not meet criteria for IFN-α-related major depression. Usually known to be limited to the regular 6 to 12 months of treatment (Capuron et al., 2002), this adverse effect may impose persistent psychopathology on at least 15% (9 out of 59) of the depressed patients up to 2 years after antiviral therapy termination. Therefore, we have recently hypothesized that, in some vulnerable patients, IFN-α may trigger a pathophysiological pathway which may become autonomous and maintain the depressive symptoms, even in the absence of the exogenous cytokine, generating a chronic depressive episode (Galvão-de Almeida et al., 2010b).

Concerning the relevant association of this adverse effect and the diagnosis of current anxiety disorder, we speculate that since depression and anxiety have been proposed as parts of the same psychopathological spectrum (Gorman, 1996–1997; Nestadt et al., 2003), the latter may represent sequelae of IFN-α-triggered depression (Bonaccorso et al., 2001). Another explanation is that this comorbidity reveals an artifact of the current nosological classifications, and consequently of the diagnostic instrument that was applied.

The main limitation of our study is that these patients were not evaluated before the antiviral therapy. Consequently, although patients previously diagnosed with a mood disorder have been excluded, we cannot affirm that the depressive symptoms began only after cytokine initiation. In order to contemplate this limitation, we have chosen to use the term "IFN-α-related depression", rather than "IFN-α-induced depression". Moreover, it should also be noted that a placebo or a control group of IFN-α-naïve HCV patients was not included to assure that diagnosed major depression episodes were really a consequence of the cytokine exposure, and not only part of the natural course of chronic hepatitis C. In addition, it is possible that the relatively low number of patients found to be diagnosed with depression during antiviral therapy (Capuron et al., 2002; Asnis et al., 2003) may be a result of memory bias. In fact, the variable *Time since Therapy Termination* showed an association trend with the main outcome ($p = 0.07$), suggesting that the longer the time since the end of the last treatment, the less likely the patient would be to fulfill the criteria for IFN-α-related depression. Our aim was to guarantee a higher number of patients assessed; however, prospective studies are more adequate for the purpose of evaluating psychopathology induced by IFN-α. In this study, a major risk factor for this adverse effect, which is baseline subclinical affective symptoms, could not be evaluated (Hauser et al., 2002; Dieperink et al., 2003).

Our results corroborate the results of many other studies suggesting that the development of this substance-induced depression is not related to gender, age, route of infection, type of IFN used, result of the antiviral treatment, past history of substance use disorders, depression or any psychiatric disorder not related to cytokine administration, and family history of mood disorder (Horikawa et al., 2003; Schaefer et al., 2002; Capuron and Ravaud, 1999; Otsubo et al., 1997). These data are in contrast to a recent study which found that female gender independently predicted the emergence of major depression during IFN-α treatment in hepatitis C (Leutscher et al., 2010).

On the other hand, the higher severity of liver fibrosis showed a significant association with the diagnosis of IFN-α-related depression in our sample. Otsubo et al. (1997) also evaluated the effect of this variable in a prospective design study examining a sample of 85 Japanese patients and could not find evidence of such association. In addition to population-specific characteristics, and a smaller sample size, differently from our approach, the previous study used DSM-III-R criteria and Hamilton Depression Scale scores to reach major depression diagnosis. Combined, these factors may explain the discordant results. Additionally, it's reasonable to assume that severe liver dysfunctions may result in more neurovegetative symptoms, cognitive impairment, and monoamine disturbance in CNS, predisposing the fulfillment of major depression diagnosis (Quarantini et al., 2009).

Additionally, as other studies with Brazilian samples demonstrated (Parana et al., 1999; Quarantini et al., 2006), the main routes of infection of HCV in our study were blood transfusion and sharing syringes to use vitamin complexes. Therefore, it may not be possible to extend our findings to groups of patients with hepatitis C including a significant percentage of illicit intravenous drug users, the main source of HCV infection worldwide (Lavanchy, 2009).

In summary, since the role of IDO in the pathophysiology of this cytokine-triggered depression has proven relevant (Comai et al., 2011), the inability to identify any association between the selected polymorphisms and our diagnosis of major depression related to IFN-α plus RBV therapy does not completely exclude the possibility of the role of genetic variants in the modulation of IFN-α response. Although IDO polymorphisms evaluated in this study may not be directly involved in the vulnerability to IFN-α-related depression, additional variants in and around the gene, not in close disequilibrium linkage with the present ones, should be examined. Our results also suggest that investigating neuropsychiatric adverse effects that may develop or persist years after the therapy termination is as important as detecting these adverse effects during the antiviral therapy.

Finally, prospective pharmacogenetic studies are warranted to continue investigation of the impact of IDO polymorphisms on the development of IFN-α-induced depression; and the search for other candidate genes that may fill the gaps in prediction of this substance-induced affective disorder must continue.

Conflicts of interest

The authors do not have any actual or potential conflict of interest, including any financial, personal, or other relationships with other people or organizations, within three years of beginning the work submitted that could inappropriately influence, or be perceived to influence, their work.

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References

American Psychiatric Association, 1994. Diagnostical and Statistical Manual of Mental Disorders, fourth ed. American Psychiatric Press, Washington, DC.

Amorim, P., 2000. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev. Bras. Psiquiatr. 22, 106–115.

Arefayene, M., Philips, S., Cao, D., Mamidipalli, S., Desta, Z., Flockhart, D.A., Wilkes, D.S., Skaar, T.C., 2009. Identification of genetic variants in the human indoleamine 2, 3-dioxygenase (IDO1) gene, which have altered enzyme activity. Pharmacogenet. Genomics 19, 464–476.

Asnis, G.M., de La Garza II, R., 2006. Interferon-induced depression in chronic hepatitis C: a review of its prevalence, risk factors, biology, and treatment approaches. J. Clin. Gastroenterol. 40, 322–335.

Asnis, G.M., de la Garza 2nd, R., Kohn, S.R., Reinus, J.F., Henderson, M., Shah, J., 2003. IFN-induced depression: a role for NSAIDs. Psychopharmacol. Bull. 37, 29–50.

Batista-Neves, S.C., Quarantini, L.C., de Almeida, A.G., Bressan, R.A., Lacerda, A.L., de-Oliveira, I.R., Paraná, R., Miranda-Scippa, A., 2008. High frequency of unrecognized mental disorders in HCV-infected patients. Gen. Hosp. Psychiatry 30, 80–82.

Batista-Neves, S., Quarantini, L.C., Galvão-de Almeida, A., Cardeal, M., Lacerda, A.L., Paraná, R., Reis de-Oliveira, I., Bressan, R.A., Miranda-Scippa, A., 2009. Impact of psychiatric disorders on the quality of life of brazilian HCV-infected patients. Braz. J. Infect. Dis. 13, 40–43.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.

Bedossa, P., Poynard, T., 1996. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24, 289–293.

Bonaccorso, S., Marino, V., Puzella, A., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Verkerk, R., Meltzer, H., Maes, M., 2002. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J. Clin. Psychopharmocol. 22, 86–90.

Bonaccorso, S., Puzella, A., Marino, V., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Levrero, M., Egyed, B., Bosmans, E., Meltzer, H.Y., Maes, M., 2001. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res. 105, 45–55.

Botega, N.J., Bio, M.R., Zomignani, M.A., Garcia, Jr.C., Pereira, W.A.B., 1995. Transtornos de humor em enfermarias de clínica médica e validação de escala de medida (HAD) de ansiedade e depressão. Rev. Saúde. Pública. 29, 355–363.

Bull, S.J., Huezo-Diaz, P., Binder, E.B., Cubells, J.F., Ranjith, G., Maddock, C., Miyazaki, C., Alexander, N., Hotopf, M., Cleare, A.J., Norris, S., Cassidy, E., Aitchison, K.J., Miller, A.H., Pariante, C.M., 2009. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. Mol. Psychiatry 14, 1095–1104.

Capuron, L., Gumnick, J.F., Musselman, D.L., Lawson, D.H., Reemsnyder, A., Nemeroff, C.B., Miller, A.H., 2002. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology 26, 643–652.

Capuron, L., Miller, A.H., 2004. Cytokines and psychopathology: lessons from interferon-alpha. Biol. Psychiatry 56, 819–824.

Capuron, L., Ravaud, A., 1999. Prediction of the depressive effects of interferon alfa therapy by the patient's initial affective state. N. Engl. J. Med. 340, 1370.

Chen, Y., Stankovic, R., Cullen, K.M., Meininger, V., Garner, B., Coggan, S., Grant, R., Brew, B.J., Guillemin, G.J., 2010. The kynurenine pathway and inflammation in amyotrophic lateral sclerosis. Neurotox. Res. 18, 132–142.

Comai, S., Cavalletto, L., Chemello, L., Bernardinello, E., Ragazzi, E., Costa, C.V., Bertazzo, A., 2011. Effects of PEG-interferon alpha plus ribavirin on tryptophan metabolism in patients with chronic hepatitis C. Pharmacol. Res. 63, 85–92.

De Bakker, P.I., Yelensky, R., Pe'er, I., Gabriel, S.B., Daly, M.J., Altshuler, D., 2005. Efficiency and power in genetic association studies. Nat. Genet. 37, 1217–1223.

Dieperink, E., Ho, S.B., Thuras, P., Willenbring, M.L., 2003. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. Psychosomatics 44, 104–112.

Galvão-de Almeida, A., Guindalini, C., Batista-Neves, S., de Oliveira, I.R., Miranda-Scippa, A., Quarantini, L.C., 2010a. Can antidepressants prevent interferon-alpha-induced depression? A review of the literature. Gen. Hosp. Psychiatry 32, 401–405.

Galvão-de Almeida, A., Quarantini, L.C., Batista-Neves, S., Lyra, A.C., Paraná, R., de Oliveira, I.R., Miranda-Scippa, A., Guindalini, C., 2010b. Is the interferon-α-triggered depressive episode a self-limited kind of depression? Four cases of

persistent affective symptoms after antiviral treatment in HCV-infected individuals. World J. Biol. Psychiatry 11, 914–918.
Gleason, O.C., Fucci, J.C., Yates, W.R., Philipsen, M.A., 2007. Preventing relapse of major depression during interferon-alpha therapy for hepatitis C—A pilot study. Dig. Dis. Sci. 52, 2557–2563.
Gorman, J.M., 1996–1997. Comorbid depression and anxiety spectrum disorders. Depress Anxiety 4, 160–168.
Guindalini, C., Howard, M., Haddley, K., Laranjeira, R., Collier, D., Ammar, N., Craig, I., O'Gara, C., Bubb, V.J., Greenwood, T., Kelsoe, J., Asherson, P., Murray, R.M., Castelo, A., Quinn, J.P., Vallada, H., Breen, G., 2006. A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. Proc. Natl. Acad. Sci. U. S. A. 103, 4552–4557.
Hauser, P., Khosla, J., Aurora, H., Laurin, J., Kling, M.A., Hill, J., Gulati, M., Thornton, A.J., Schultz, R.L., Valentine, A.D., Meyers, C.A., Howell, C.D., 2002. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol. Psychiatry 7, 942–947.
Hauser, P., Soler, R., Reed, S., Kane, R., Gulati, M., Khosla, J., Kling, M.A., Valentine, A.D., Meyers, C.A., 2000. Prophylactic treatment of depression induced by interferon-alpha. Psychosomatics 41, 439–441.
Horikawa, N., Yamazaki, T., Izumi, N., Uchihara, M., 2003. Incidence and clinical course of major depression in patients with chronic hepatitis type C undergoing interferon-alpha therapy: a prospective study. Gen. Hosp. Psychiatry 25, 34–38.
Keefe, B., 2007. Interferon-induced depression in hepatitis C: an update. Curr. Psychiatry Rep. 9, 255–261.
Kraus, M.R., Al-Taie, O., Schäfer, A., Pfersdorff, M., Lesch, K.P., Scheurlen, M., 2007. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. Gastroenterology 132, 1279–1286.
Kraus, M.R., Schäfer, A., Al-Taie, O., Scheurlen, M., 2005. Prophylactic SSRI during interferon alpha re-therapy in patients with chronic hepatitis C and a history of interferon-induced depression. J. Viral Hepat. 12, 96–100.
Lavanchy, D., 2009. The global burden of hepatitis C. Liver Int. 29, 74–81.
Leutscher, P.D., Lagging, M., Buhl, M.R., Pedersen, C., Norkrans, G., Langeland, N., Mørch, K., Färkkilä, M., Hjerrild, S., Hellstrand, K., Bech, P.NORDynamIC Group, 2010. Evaluation of depression as a risk factor for treatment failure in chronic hepatitis C. Hepatology 52, 430–435.
Lotrich, F.E., Ferrell, R.E., Rabinovitz, M., Pollock, B.G., 2009. Risk for depression during interferon-alpha treatment is affected by the serotonin transporter polymorphism. Biol. Psychiatry 65, 344–348.
Lotrich, F.E., Loftis, J.M., Ferrell, R.E., Rabinovitz, M., Hauser, P., 2010. IL28B polymorphism is associated with both side effects and clearance of hepatitis C during interferon-alpha therapy. J. Interferon Cytokine Res.. doi:10.1089/jir.2010.0074, e-pub ahead of print 6 Dec 2010.
Maes, M., 2010. Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry. doi:10.1016/j.pnpbp.2010.06.014, e-pub ahead of print 20 Jun 2010.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Morasco, B.J., Rifai, M.A., Loftis, J.M., Indest, D.W., Moles, J.K., Hauser, P., 2007. A randomized trial of paroxetine to prevent interferon-alpha-induced depression in patients with hepatitis C. J. Affect. Disord. 103, 83–90.
Musselman, D.L., Lawson, D.H., Gumnick, J.F., Manatunga, A.K., Penna, S., Goodkin, R.S., Greiner, K., Nemeroff, C.B., Miller, A.H., 2001. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N. Engl. J. Med. 344, 961–966.
Myakishev, M.V., Khripin, Y., Hu, S., Hamer, D.H., 2001. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res. 11, 163–169.
Nestadt, G., Addington, A., Samuels, J., Liang, K.Y., Bienvenu, O.J., Riddle, M., Grados, M., Hoehn-Saric, R., Cullen, B., 2003. The identification of OCD-related subgroups based on comorbidity. Biol. Psychiatry 53, 914–920.
Otsubo, T., Miyaoka, H., Kamijima, K., Onuki, M., Ishii, M., Mitamura, K., 1997. Depression during interferon therapy in chronic hepatitis C patients. Seishin Shinkeigaku Zasshi 99, 101–127.
Parana, R., Lyra, L., Trepo, C., 1999. Intravenous vitamin complexes used in sporting activities and transmission of HCV in Brazil. Am. J. Gastroenterol. 94, 857–858.
Pierucci-Lagha, A., Covault, J., Bonkovsky, H.L., Feinn, R., Abreu, C., Sterling, R.K., Fontana, R.J., Kranzler, H.R.HALT-C Trial Group, 2010. A functional serotonin transporter gene polymorphism and depressive effects associated with interferon-alpha treatment. Psychosomatics 51, 137–148.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Quarantini, L.C., Bressan, R.A., Galvão, A., Batista-Neves, S., Paraná, R., Miranda-Scippa, A., 2007. Incidence of psychiatric side effects during pegylated interferon-alpha retreatment in nonresponder hepatitis C virus-infected patients. Liver Int. 27, 1098–1102.
Quarantini, L.C., Miranda-Scippa, A., Batista-Neves, S., Powell, V.B., Abreu, N., Abreu, K.C., Moura, I., Crane, J., Sampaio, A.S., Netto, L.R., Deoliveira, I.R., Paraná, R., Bressan, R.A., Lacerda, A.L., 2009. A neuropsychological study comparing patients infected with HCV and HBV without psychiatric comorbidities. J. Med. Virol. 81, 1184–1188.
Quarantini, L.C., Miranda-Scippa, A., Schinoni, M.I., Sampaio, A.S., Santos-Jesus, R., Bressan, R.A., Tatsch, F., de Oliveira, I., Parana, R., 2006. Effect of amantadine on depressive symptoms in chronic hepatitis C patients treated with pegylated interferon: a randomized, controlled pilot study. Clin. Neuropharmacol. 29, 138–143.
Raison, C.L., Demetrashvili, M., Capuron, L., Miller, A.H., 2005. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. CNS Drugs 19, 105–123.
Raison, C.L., Woolwine, B.J., Demetrashvili, M.F., Borisov, A.S., Weinreib, R., Staab, J.P., Zajecka, J.M., Bruno, C.J., Henderson, M.A., Reinus, J.F., Evans, D.L., Asnis, G.M., Miller, A.H., 2007. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. Aliment. Pharmacol. Ther. 25, 1163–1174.
Schaefer, M., Engelbrecht, M.A., Gut, O., Fiebich, B.L., Bauer, J., Schmidt, F., Grunze, H., Lieb, K., 2002. Interferon alpha (IFN-alpha) and psychiatric syndromes: a review. Prog. Neuropsychopharmacol. Biol. Psychiatry 26, 731–746.
Schaefer, M., Schwaiger, M., Garkisch, A.S., Pich, M., Hinzpeter, A., Uebelhack, R., Heinz, A., van Boemmel, F., Berg, T., 2005. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J. Hepatol. 42, 793–798.
Shriver, M.D., Mei, R., Parra, E.J., Sonpar, V., Halder, I., Tishkoff, S.A., Schurr, T.G., Zhadanov, S.I., Osipova, L.P., Brutsaert, T.D., Friedlaender, J., Jorde, L.B., Watkins, W.S., Bamshad, M.J., Gutierrez, G., Loi, H., Matsuzaki, H., Kittles, R.A., Argyropoulos, G., Fernandez, J.R., Akey, J.M., Jones, K.W., 2005. Large-scale SNP analysis reveals clustered and continuous patterns of human genetic variation. Hum. Genomics 2, 81–89.
Su, K.P., Huang, S.Y., Peng, C.Y., Lai, H.C., Huang, C.L., Chen, Y.C., Aitchison, K.J., Pariante, C.M., 2010. Phospholipase A2 and cyclooxygenase 2 genes influence the risk of interferon-alpha-induced depression by regulating polyunsaturated fatty acids levels. Biol. Psychiatry 67, 550–557.
The International HapMap Consortium, 2003. The International HapMap Project. Nature 426, 789–796.
Vignau, J., Karila, L., Costisella, O., Canva, V., 2005. Hepatitis C, interferon α and depression: main physiopathologic hypothesis. Encephale 31, 349–357.
Wichers, M., Maes, M., 2002. The psychoneuroimmuno-pathophysiology of cytokine induced depression in humans. Int. J. Neuropsychopharmacol. 5, 375–388.
Organization, World.Health., 1991. International Classification of Diseases (ICD-10), 10th ed. Geneva, World Health Organization.
Yoshida, K., Alagbe, O., Wang, X., Woolwine, B., Thornbury, M., Raison, C.L., Miller, A.H., 2005. Promoter polymorphisms of the interferon-alpha receptor gene and development of Interferon-induced depressive symptoms in patients with chronic hepatitis C: preliminary findings. Neuropsychobiology 52, 55–61.