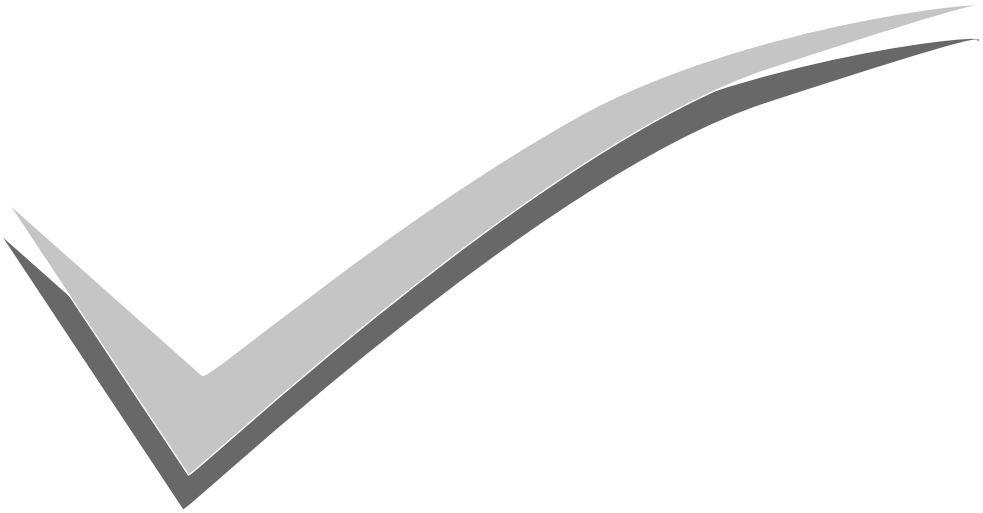
Performance Evaluations



Flaviscreen®HCV

Rapid test for detection of antibodies to HCV

Performance Evaluations

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S. No.	**Name of the Publication**	**Pg Nos**
1.	PLOS ONE | https://doi.org/10.1371/journal.pone.0210556 January 17, 2019	1-10
2.	PLOS ONE | DOI:10.1371/journal.pone.0118610 March 3, 2015	1-20
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Performance Evaluations

EXTERNAL EVALUATIONS

INDEX	
S. No.	**Name of the Evaluating Body**
5.	Virology laboratory of Saint Antoine Hospital, Paris

PLOS ONE

RESEARCH ARTICLE

Evaluation of five rapid diagnostic tests for detection of antibodies to hepatitis C virus (HCV): A step towards scale-up of HCV screening efforts in India

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Abstract

Objectives

Hepatitis C virus (HCV) infection is a major contributor to morbidity and mortality worldwide. Early detection and curative treatment of HCV can reduce the risk of liver-related mortality and serve to prevent transmission of new infections. India is estimated to have about six million HCV infected individuals, most of whom are unaware of their infection status. Rapid diagnostic test kits (RDTs) could help identify HCV infected persons more expeditiously and thus availability of high performing, quality-assured RDTs is essential to scale-up HCV screening efforts. The present study was thus undertaken to evaluate the performance characteristics of five anti-HCV RDTs.

Methods

Five anti-HCV RDTs (Alere Truline, Flaviscreen, Advanced Quality, SD Bioline and OraQuick) were evaluated using two panels of known anti-HCV positive and negative samples; one characterized from Indian patient samples (n = 360) and other obtained from the US Centers for Disease Control and Prevention (CDC), Atlanta (n = 100). Sensitivity, specificity, inter-observer agreement, test validity and operational characteristics of RDTs were assessed.

Results

The combined sensitivities across both panels for Alere Truline, Flaviscreen, Advanced Quality, SD Bioline and OraQuick RDTs were 99.4% (95%CI-96.6%-99.9%), 86.2% (95% CI-79.8%-91.1%), 96.2% (95%CI-91.9%-98.6%), 99.4% (95%CI-96.6%-99.9%) and 99.4%

necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the US Department of Health and Human Services, or the US Centers for Disease Control and Prevention.

(95%CI-96.6%-99.9%) respectively. The overall specificities across both panels for all RDTs were 99.7%. The inter-observer agreement was 100% for Alere Truline, SD Bioline and OraQuick, while it was 99.5% and 98.6% with Advanced Quality and Flavicheck respectively. Discordant results were significantly associated with human immunodeficiency virus (HIV) positivity for both Advanced Quality and Flavicheck ($p<0.001$).

Conclusion

The present evaluation demonstrated that Alere Truline, SD Bioline and OraQuick RDTs had sensitivity and specificity in accordance with the acceptance criteria of the Drug Controller General, India, the national regulatory authority, had excellent inter-observer agreement and superior operational characteristics. Our findings suggest that certain HCV RDTs perform well and can be a useful tool in screening of HCV infections expeditiously.

Introduction

Hepatitis C virus (HCV) infection is a major contributor to morbidity and mortality worldwide. It is estimated that globally 71 million people are living with chronic HCV infection, and about 400,000 succumb to this infection annually [1]. The burden of HCV infection is enormous in low and middle-income countries from the Southeast Asian region [2]. India alone is estimated to have about 6 million individuals living with chronic HCV infection, most of whom are unaware of their infection status [1, 3]. Chronic HCV infection is associated with long term complications such as liver fibrosis, cirrhosis and hepatocellular carcinoma [4]. HCV infection is primarily spread through exposure to contaminated blood, and rates of infection are particularly high among people who inject drugs (PWID) and men who have sex with men (MSM) [5, 6]. Since HCV and HIV share similar routes of transmission, co-infection rates are high and are associated with higher morbidity and mortality as well [7–9].

Early diagnosis and curative treatment of HCV infection can reduce the risk of liver-related morbidity and mortality and also serve to prevent transmission of new infections [10–13]. Furthermore, availability of efficacious, well-tolerated, relatively cheap, and easy to administer directly acting antivirals (DAAs), offer an opportunity to provide treatment and management using a public health model in India [14]. The first step to scaling up access to curative HCV treatment is to identify individuals who are chronically infected with HCV. Determination of current HCV infection requires screening for the presence of antibodies to HCV (anti-HCV) followed by confirmation of current infection either by using nucleic acid testing (NAT) for HCV RNA or an immunoassay (IA) for HCV core antigen wherever available [1]. Laboratory-based IAs, including automated platforms or manual enzyme immunoassays (EIAs), have proven accuracy in detection of HCV antibodies, however they require standard laboratory set up, skilled personnel, and have longer turn-around times (TAT), and necessitate extensive sample transport should screening be decentralized to sites without necessary infrastructure [15].

Rapid diagnostic tests (RDTs), eliminate the need for highly trained healthcare workers, sample transport, and provide fast TAT. Several commercial RDTs are available in the Indian market for the detection of HCV antibodies, however their performance characteristics have not been independently determined. Additionally, there are RDTs available globally that have not been approved for use in India by the Drug Controller General of India (DCGI).

Evaluating their performance will help in decisions pertaining to approval of RDTs by DCGI. There have been previous reports of high rates of false negative anti-HCV rapid test results in HIV-HCV co-infected individuals [16, 17]; hence, evaluation of HCV RDTs in this group is essential.

The present study was conducted to evaluate the performance of five RDTs for detection of HCV antibodies. Data from this evaluation will provide valuable information for making decisions about scale-up of HCV screening in India as the government launches the National Program for the control of viral hepatitis.

Methods

Evaluation panels

Two panels were used in this evaluation, one sourced from patients in India and the other from the Division of Viral Hepatitis Laboratory, US Centers for Disease Control and Preventions, (US-CDC) Atlanta, GA (Table 1). The Indian panel was composed of a mix of both serum and plasma and includes representative samples from different geographical areas of the country, collected during years 2015–17 and stored at -70˚C as part of sample repository at ICMR-National AIDS Research Institute (NARI). Samples from the co-infected (anti-HCV positive and HIV-positive samples) were prospectively collected in collaboration with the ART Plus Center, Amritsar, Punjab after obtaining written informed consent from donors. The CD4+ cell count and antiretroviral treatment data was obtained from the medical records.

The US-CDC, consisted of 100 anonymized well characterized plasma samples, previously tested for all HCV markers [18]. These plasma samples were collected from a US plasma donor center that were rejected due to anti-HCV-reactivity and/or HCV-RNA-positivity

Reference tests

The samples from the Indian panel were screened for anti-HCV by both Ortho HCV version 3.0 (Ortho Clinical Diagnostics, USA) and Murex anti-HCV version 4.0 (Diasorin, Italy) ELISA. All positive samples were tested by Bio Rad Western Blot to confirm antibody positivity as a supplemental test. A sample was considered positive when it was positive by both

Table 1. Description of the two specimen panels used for evaluation.

Anti-HCV				**Total**
Indian panel	**Anti-HIV**			**360**
	Positive		Negative	
Positive	N = 60 30 serum and 30 plasma		N = 60 40 serum and 20 plasma	120
Negative	N = 120 50 serum and 70 plasma		N = 120 100 serum and 20 plasma	240
US-CDC panel	**HCV RNA**			**100**
	Positive		Negative	
Positive	N = 25		N = 15	40
	18 = GT1a	2 = GT1b	NA	
	1 = GT2b	4 = GT3a		
Negative	N = 46		N = 16	60
	26 = GT1a	1 = GT1b	NA	
	10 = GT2b	9 = GT3a		

GT: genotype, NA: Not applicable

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ELISA tests and Western Blot and negative when it was negative by both ELISA tests and Western Blot. For HIV status, the samples were tested by two ELISA Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio Rad, USA) and HIVASE 1+2 ELISA (General Biologicals Corp, Taiwan) and three rapid tests, Alere Determine HIV 1/2 (Alere, Ireland), HIV Tri Dot (J Mitra Co Pvt Ltd, India) and HIV 1/2 Stat Pak Dipstick (Chembio Diagnostic Systems, USA). A sample was considered positive when it was positive by all five tests and confirmed by New LAV Blot Western blot (Bio Rad, USA), while negative when it was negative by all 5 tests. Discordant or indeterminate samples were not included in the panel. All assays were performed according to manufacturer instructions including criteria for analyte reactivity. Specimens in the US-CDC evaluation panel were tested for HCV markers as described previously, specifically using the Vitros 3600 CMIA platform (Ortho Clinical Diagnostics) for HCV serology [18]. All samples were plasma/serum; no whole blood specimens were analyzed.

Anti-HCV rapid diagnostic test kits for evaluation

Five anti-HCV RDTs were evaluated: Alere Truline rapid test kit for HCV antibodies, Alere Medical Pvt Ltd, Gurgaon, India (Product code: 11304191030), Flaviscreen Plus HCV, Qualpro Diagnostics, Goa, India (Product code: 402170050), Advanced Quality Rapid Anti-HCV Test, Intec Products, Inc, Xiamen, China (Product code: ITP01151-TC40), SD Bioline HCV, Standard Diagnostics Inc, Republic of Korea (Product code: 02FK10) and OraQuick HCV rapid antibody test, OraSure Technologies, Bethlehem, PA, USA (Product code: 0006656483). The kits were stored at room temperature as recommended by the manufacturer. All are in vitro, qualitative, immune-chromatographic, single use, disposable chamber tests that provide visual results within 20 minutes for anti-HCV detection. Alere Truline, Flaviscreen and SD Bioline use antigens from structural (core) and nonstructural (NS3, NS4, and NS5) regions, while Advanced Quality kit uses the NS2 region antigens additionally. The OraQuick uses antigens from core and NS3 and NS4 regions.

Evaluation procedures

The RDTs were performed as per the manufacturers' instruction manuals. The procedure for testing and interpretation of the results was similar for all assays. An assay was interpreted as negative if a control line was present (regardless of intensity) with no corresponding test line. The appearance of a control line and a test line indicated a positive result. A missing or broken control line indicated an invalid result, regardless of presence of test line. Each specimen was tested by one laboratory technician and read by 2 independent laboratory staff in addition to the performer. A rapid assay result was classified as positive if at least 2 of the 3 observers interpreted the assay as positive.

Statistical analysis

Data analyses were performed using SPSS 17.0 (SPSS, Chicago) software. Sensitivity and specificity with confidence intervals (CIs) for each RDT was calculated by comparing results obtained using the RDT to the reference result. Inter-observer agreement between the three technicians was measured by Fleiss' kappa statistics. Invalid rate was calculated as the proportion of total samples tested in which the result was invalid out of the total number of samples tested. Each RDT was assessed for its operational characteristics by same three technicians. Tests were scored for clarity of kit instructions, technical ease of use and ease of result interpretation. Each of these characteristics was allotted marks out of 5, giving a maximum of 15. Responses to individual questions were analyzed to assign an overall score.

Ethical considerations

The study was approved by Ethics Committee of ICMR-National AIDS Research Institute, Pune, India and the Chesapeake Institutional Review Board in the USA. Written informed consent was obtained from participants prior to sample collection.

Results

Performance of anti-HCV rapid diagnostic test kits

The sensitivity and specificity across both panels and per panel for the five HCV RDTs evaluated are shown in Table 2. The overall sensitivities of the RDTs ranged from 86.3% (95% confidence interval (CI), 79.9%-91.2%) to 99.4% (95% CI, 96.6%-99.9%). The Flaviscreen and Advanced Quality RDTs exhibited lower sensitivities. All five RDTs had specificities of 100% (95% CI, 98.8%-100%).

Using the Indian sourced panel, the sensitivity and specificity for the RDTs was evaluated according to HIV serostatus. We observed that Alere Truline, SD Bioline and OraQuick had 100% (95% CI, 94.0%-100%) sensitivities for both HIV-positive and for HIV-negative samples. The sensitivity of the Flaviscreen kit for HIV-positive samples was 76.7% (95% CI, 63.9%-86.6%) and for HIV-negative samples was 100% (95% CI, 94.0%-100%), while the sensitivity of Advanced Quality kit for HIV-positive samples was 91.7% (95% CI, 81.6%-97.2%) and for HIV-negative samples was 100% (95% CI, 94.0%-100%). There was a statistically significant difference between the sensitivities for HIV-positive and HIV-negative samples for both Flaviscreen and Advanced Quality RDTs ($p<0.001$) [Fig 1]. We did not observe any association between the discordant test results in the HIV-positive samples with CD4 cell count or antiretroviral treatment status. Using the US-CDC panel, we analyzed the influence of HCV viral load on the performance of RDTs and found no statistically significant difference between concordant (mean log HCV viral load: 5.1±1.2) and discordant (mean log HCV viral load: 4.9±3.5) RDT results, $p = 0.45$.

All five RDTs had 100% (95% CI, 96.9%-100%) specificity for both HIV-positive and for HIV-negative samples. False negative anti-HCV results were observed in 14/60 (23.3%) and

Table 2. Performance of anti-HCV rapid diagnostic test kits.

Anti-HCV rapid diagnostic test kits	Panel	Sensitivity (95% CI)	Specificity (95% CI)
Alere Truline (Product code: 11304191030)	Indian	100% (96.9%-100%)	100% (98.5%-100%)
	US-CDC	97.5% (86.5%-99.9%)	100% (94%-100%)
	Overall	99.4% (96.6%-99.9%)	100% (98.8%-100%)
Flaviscreen (Product code: 402170050)	Indian	88.3% (81.2%-93.5%)	100% (98.5%-100%)
	US-CDC	80% (64.4%-90.9%)	100% (94%-100%)
	Overall	86.3% (79.9%-91.2%)	100% (98.8%-100%)
Advanced Quality (Product code: ITP01151-TC40)	Indian	95.8% (90.5%-98.6%)	100% (98.5%-100%)
	US-CDC	97.5% (86.8%-99.9%)	100% (94%-100%)
	Overall	96.3% (91.9%-98.6%)	100% (98.8%-100%)
SD Bioline (Product code: 02FK10)	Indian	100% (96.9%-100%)	100% (98.5%-100%)
	US-CDC	97.4% (86.5%-99.9%)	100% (94%-100%)
	Overall	99.4% (96.6%-99.9%)	100% (98.8%-100%)
OraQuick (Product code: 0006656483)	Indian	100% (96.9%-100%)	100% (98.5%-100%)
	US-CDC	97.4% (86.5%-99.9%)	100% (94%-100%)
	Overall	99.4% (96.6%-99.9%)	100% (98.8%-100%)

95% CI: 95% confidence interval, US-CDC: US-Centers for Disease Control and Prevention

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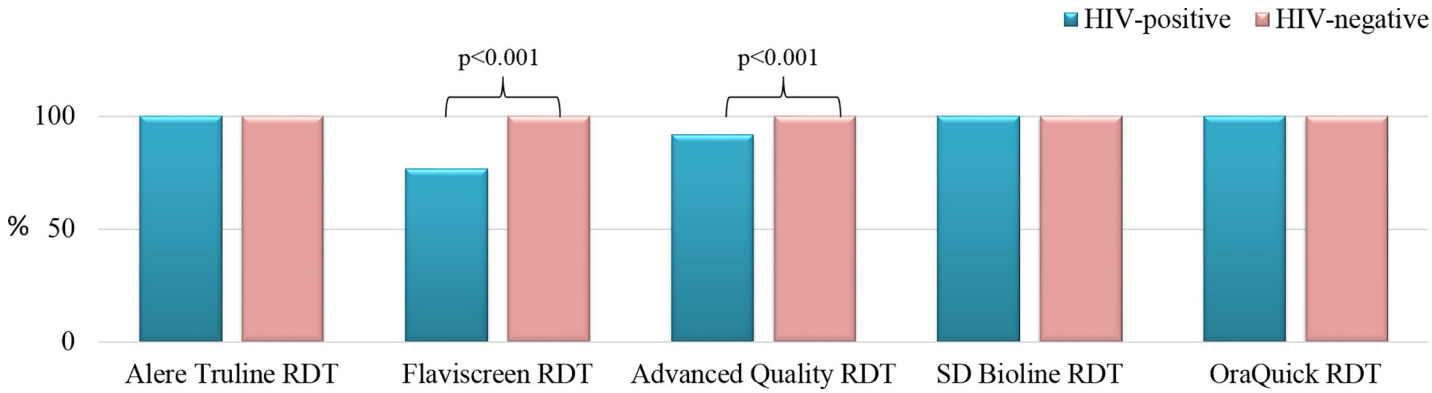

Fig 1. Sensitivities of anti-HCV rapid diagnostic test kits by HIV sero-status.

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5/60 (8.3%) HIV-HCV co-infected samples with Flaviscreen and Advanced Quality RDTs respectively from the Indian Panel. HCV genotype data was available for all HCV RNA positive CDC samples, of which the same 2 samples (GT3a and GT1a) gave discordant results with Flaviscreen and Advanced Quality RDTs. There was one HCV seropositive sample in the US panel that was missed by all RDTs, which was HCV RNA negative.

Inter-observer agreement, test validity and operational characteristics

The inter-observer agreement in the test results between three technicians was excellent for SD Bioline, Oraquick and Alere Truline HCV RDTs. The inter-observer agreement for the Advanced Quality HCV RDT was 0.993 and for Flaviscreen HCV RDT was 0.979 (Table 3).

No invalid test results were observed with any of the five RDTs evaluated. The scores for operational characteristics of the RDTs are summarized in Table 4. The OraQuick obtained the highest score (14/15) with a significant superiority on clarity of kit instruction and ease of result interpretation, while Flaviscreen scored lowest on technical ease of use and ease of result interpretation.

Discussion

This study investigated the diagnostic accuracy of five RDTs using two well-characterized serum/plasma panels, one an Indian and panel and other from US CDC. We observed that Alere Truline, SD Bioline and OraQuick had higher overall sensitivities as compared to Flaviscreen and Advanced Quality RDTs. These data are consistent with prior reports noting the good performance of the OraQuick and SD Bioline tests in other countries [15, 19–21]. To our knowledge, this is the first study to report the performance characteristics of Alere Truline,

Table 3. Inter-observer agreement for anti-HCV rapid diagnostic test kits.

Anti-HCV rapid diagnostic test kits	Agreement (%)	Kappa (95% CI)
Alere Truline	100%	1.00 (0.94–1.05)
Flaviscreen	98.6%	0.97 (0.93–1.03)
Advanced Quality	99.5%	0.99 (0.94–1.04)
SD Bioline	100%	1.00 (0.94–1.05)
OraQuick	100%	1.00 (0.94–1.05)

95% CI—95% confidence interval

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Table 4. Operational characteristics of anti-HCV rapid diagnostic test kits.

Operational characteristics	Top score	Mean scores				
		Alere Truline	Flaviscreen	Advanced Quality	SD Bioline	OraQuick
Clarity of kit instructions	5	5	4	4	4	5
Technical ease of use	4	4	2	3	4	4
Ease of result interpretation	5	4	2	4	4	5
Overall scores	14	13	8	11	12	14

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Intec Advanced Quality and Flaviscreen anti-HCV RDTs. The DCGI has laid acceptance criteria (sensitivity >99% and specificity ≥98%) for anti-HCV rapid immunodiagnostic kits in India and based on these standards the Alere Truline, SD Bioline and OraQuick RDTs met the acceptance criteria. The Advanced Quality and Flaviscreen kits had very good specificity, but exhibited relatively low sensitivity and thus do not meet the required standards. With the need to rapidly scale up screening having poor sensitivity and thus potential missing HCV-infected individuals would present a missed opportunity for the program.

It has been estimated that the prevalence of HCV in India is between 0.5 and 1.5 per cent, but the majority of individuals remain undiagnosed [14]. Though a national serosurvey has not yet been conducted, it is hypothesized that there is wide variation in state-to-state prevalence. The Indian state of Punjab, for example, is already known to have high HCV disease burden and has launched two public health programs the tackle the HCV epidemic accordingly [22–24]. Thus far, both state programs are catering to the latent demand of HCV treatment for few patients/risk groups who are already aware of their infection status, but there are plans to expand active screening and surveillance activities through community-based screening efforts in the near future. Furthermore, the Government of India has launched the National Program for Control of Viral Hepatitis and announced free treatment for hepatitis C [25]. Thus there is an urgent need to expand HCV diagnostics. RDTs could help identify the HCV infected persons more expeditiously; the rapid TAT will also assist to limit loss to follow-up and facilitate early linkage to treatment and care. As a step towards scale-up of HCV screening efforts in India, the present study was conducted to evaluate the diagnostic performance of five anti-HCV screening RDTs. To scale up access to HCV testing, integration of anti-HCV RDTs into established HIV testing services will be critical, including the use by organizations that are effective in reaching hard-to-reach or marginalized populations, such as PWID or MSM, who have not been tested for HCV, but are at higher risk of exposure. Therefore, it is critical that the performance of screening tests in the context of HIV co-infection be interrogated.

It has been reported that HIV-positive individuals may have impaired HCV antibody response [26–28]. Previous studies have reported false-negative results among HIV-positive individuals for HCV antibody detection, including with the OraQuick test [16, 17], which was not found for this test in our study. This could also reflect geographic differences in the origin of the samples. In the present study we observed false negative results in the HIV-HCV co-infected samples, using serum/plasma specimens, and consequently lower sensitivity for the Flaviscreen and Advanced Quality RDTs. No false negative results or statistically significant difference in the sensitivities with Alere Truline, SD Bioline and Oraquick RDTs were observed when samples from HIV-positive individuals were tested. These findings confirm that choice of appropriate HCV RDTs is essential, especially in the context of HIV co-infection.

Our study has a few limitations. All assays were performed in a reference laboratory by trained technicians. Hence the results may not be easily generalizable to the field setting where

environmental conditions, sample type (whole blood) and expertise of technicians may vary. Though we analyzed the effect of HIV status on kit performance, there was an overall limited sample size, likewise other covariates like age, gender and the influence of other co-infections such as hepatitis B were not determined. As well, the virological profile of the Indian panel was not known, therefore influence of genotype or amount of virus cannot be accounted for. Lastly, both panels consisted of serum/plasma samples whereas the most likely and feasible sample type that would be in use in the field would be capillary (finger-stick) blood.

In summary, the present evaluation demonstrated that Alere Truline, SD Bioline and Ora-Quick RDTs had sensitivity and specificity in accordance with the acceptance criteria for anti-HCV RDTs as per the DCGI guidelines and had excellent inter-observer agreement and operational characteristics. The Flaviscreen and Advanced Quality kits demonstrated insufficient diagnostic accuracy, especially among HIV-infected individuals, suggesting that choice of appropriate HCV RDTs may be essential for use in the context of HIV-HCV co-infection.

Author Contributions

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Funding acquisition: Arati Mane, Jilian Sacks, Sadhya Sharma, Kartik Kacholia, Ritubhan Gautam, Raman Gangakhedkar.

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RESEARCH ARTICLE

Plasma Concentrations of BDNF and IGF-1 in Abstinent Cocaine Users with High Prevalence of Substance Use Disorders: Relationship to Psychiatric Comorbidity

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Abstract

Recent studies have identified biomarkers related to the severity and pathogenesis of cocaine addiction and common comorbid psychiatric disorders. Monitoring these plasma mediators may improve the stratification of cocaine users seeking treatment. Because the neurotrophic factors are involved in neural plasticity, neurogenesis and neuronal survival, we have determined plasma concentrations of brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and IGF-1 binding protein 3 (IGFBP-3) in a cross-sectional study with abstinent cocaine users who sought outpatient treatment for cocaine (n = 100) and age/body mass matched controls (n = 85). Participants were assessed with the diagnostic interview 'Psychiatric Research Interview for Substance and Mental Disorders'. Plasma concentrations of these peptides were not different in cocaine users and controls. They were not associated with length of abstinence, duration of cocaine use or cocaine symptom severity. The pathological use of cocaine did not influence the association of IGF-1 with age observed in healthy subjects, but the correlation between IGF-1 and IGFBP-3 was not significantly detected. Correlation analyses were performed between these peptides and other molecules sensitive to addiction: BDNF concentrations were not associated with

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inflammatory mediators, lipid derivatives or IGF-1 in cocaine users, but correlated with chemokines (fractalkine/CX3CL1 and SDF-1/CXCL12) and N-acyl-ethanolamines (N-palmitoyl-, N-oleoyl-, N-arachidonoyl-, N-linoleoyl- and N-dihomo-γ-linolenoyl-ethanolamine) in controls; IGF-1 concentrations only showed association with IGFBP-3 concentrations in controls; and IGFBP-3 was only correlated with N-stearoyl-ethanolamine concentrations in cocaine users. Multiple substance use disorders and life-time comorbid psychopathologies were common in abstinent cocaine users. Interestingly, plasma BDNF concentrations were exclusively found to be decreased in users diagnosed with both primary and cocaine-induced disorders for mood and anxiety disorders. In summary, BDNF, IGF-1 and IGFBP-3 were not affected by a history of pathological use of cocaine supported by the absence of associations with other molecules sensitive to cocaine addiction. However, BDNF was affected by comorbid mood disorders. Further research is necessary to elucidate the role of BDNF and IGF-1 in the transition to cocaine addiction and associated psychiatric comorbidity.

Introduction

Chronic cocaine use induces long-lasting neurochemical, structural and behavioral adaptive changes thought to result from altered gene and protein expression within cerebral areas playing a critical role in addiction and reward [1]. Some of these changes are not fully reversed upon prolonged abstinence, and may represent an example of aberrant cocaine-induced neuroplastic changes related to cocaine dependence and an increased susceptibility to relapse to drug taking even after a long period of abstinence [2,3].

Furthermore, long-term cocaine use is commonly associated with altered executive functions, impaired emotional processing capacity and a higher incidence of comorbid mental disorders, particularly mood and anxiety disorders [4,5,6]. The diagnosis of psychiatric comorbidity is an important consideration for effective therapies to overcome cocaine addiction. However, the accurate diagnosis of comorbid psychiatric disorders in cocaine addicts has to face two major problems, the effects of cocaine can conceal symptoms of other mental disorders and the diagnosis is defined by manifestations rather than by direct biomarkers [7,8].

Focusing on this last point, the search for peripheral biomarkers for both psychiatric and substance use disorders has caused an increasing interest in addiction psychiatry research over the last few years. This growing research has generated a number of putative biomarkers, mainly involving the immune system and inflammatory responses, which still require replication in larger studies [9,10,11]. Recently, our group has studied the plasma profile of inflammatory mediators and fatty acid derivatives in abstinent cocaine users under outpatient treatment [12,13]. We found certain cytokines and chemokines that might serve as reliable biomarkers for pathological use of cocaine (i.e., binge use and/or chronic use) and symptom severity [12]. Additionally, several fatty acid derivatives such as endocannabinoids and congeners were biomarkers for cocaine use disorders and psychiatric comorbidity [13].

Neurotrophic factors are peptides that are known for their role in mediating neuronal plasticity and neuronal growth. In addition to neurons, these factors are produced by other cells and can affect and integrate neural, immune and endocrine systems. They have been reported to mediate the effects of drugs for the treatment of mental disorders but also play a relevant role in the acute and chronic responses produced by addictive drugs [14]. We have focused this

study on two trophic factors, which are found in plasma and involved in mediating neuronal plasticity, the brain-derived neurotrophic factor (BDNF) and the insulin-like growth factor 1 (IGF-1). In addition to IGF-1, we have also measured the IGF binding protein 3 (IGFBP-3) as relevant protein modulating its effects.

BDNF is a neurotrophin that participates in neuronal survival, differentiation, synaptogenesis and maintenance. Accumulating evidence suggests that alterations in the BDNF expression underlie a variety of psychiatric and neurological disorders. BDNF has been associated positively with some disorders (e.g., major depression and bipolar disorder) but there are also many non-specific or conflicting findings (schizophrenia and autism) [11,15]. Furthermore, BDNF has been also evaluated in cocaine addiction and comorbid disorders. Indeed, serum BDNF concentrations have been recently postulated as an indication of relapse risk during early recovery from cocaine dependence in a prospective study [16]. Another study performed by Corominas-Roso and colleagues showed that an increase in serum BDNF concentrations during early abstinence correlates with cocaine craving and abstinence symptoms [17], but interestingly these increased BDNF concentrations are not observed in patients displaying cocaine-induced psychosis [18].

IGF-1 is a trophic mediator regulated by growth hormone (GH) that may either be free or bound to binding proteins. IGFBP-3 is the most abundant binding protein in human blood and this complex prolongs the half-life of IGF-1. IGF-1 regulates proliferation, development and growth of neural cells (for recent reviews [19,20]). This peptide is also involved in the pathogenesis and evolution of psychiatric disorders in preclinical models [21,22] and clinical cases in old [23] and young [24] individuals. Thus, a cross-sectional study in older adults showed that the association between depressive symptoms and memory deficits is stronger with lower concentrations of circulating IGF-1 [23]. Young adults with GH deficiency exhibit anxious or depressed moods, which can be treated by GH therapy that increases IGF-1 concentrations. Furthermore, IGF-1 concentrations negatively correlate with depression, fatigue, tension and anxiety and positively with vigor and memory [24]. Regarding drugs of abuse in humans, IGF-1 concentrations have been assessed in alcohol and opiate dependence. Studies in alcohol dependents revealed a positive correlation between blood insulin level and alcohol craving, but not between alcohol craving and IGF-1 concentrations during either the active drinking phase or during abstinence [25]. In contrast to alcohol, serum IGF-1 is found to be elevated in opiate dependence [5].

The aim of the present cross-sectional study is to examine the plasma concentrations of BDNF, IGF-1 and IGFBP-3 in a cohort of abstinent cocaine users on an outpatient basis according to cocaine use history (duration of use, length of abstinence and cocaine symptom severity) and the comorbidity of other mental disorders. We found that plasma BDNF, IGF-1 and IGFBP-3 are unaltered in abstinent cocaine users but they are affected by the presence of comorbid mood and anxiety disorders.

Methods and Materials

1 Subjects and recruitment

All participants in the present cross-sectional study were white Caucasians grouped into abstinent cocaine users and healthy controls. One-hundred and ten cocaine users were enrolled from outpatient treatment programs for cocaine addiction in the province of Málaga (Spain) for a 36 month- period (2011–2013). Eighty healthy individuals were recruited in parallel from a multidisciplinary staff working at the Hospital Regional Universitario de Málaga.

Cocaine users had to meet eligibility criteria based on inclusion and exclusion criteria. Inclusion criteria were as follows: $\geq$18 years to 65 years of age, intranasal cocaine use, diagnosis of a

lifetime 'pathological use' of cocaine (chronic intoxication and/or binge), and abstinence from cocaine for at least 2 weeks before testing. The 'pathological use' of cocaine was determined through a psychiatric interview, while the abstinence of cocaine users was checked weekly by urine analysis in the outpatient treatment centers for cocaine addiction and plasma analyses [12]. Exclusion criteria were as follows: personal history of chronic diseases (e.g. cardiovascular, respiratory, renal, hepatic, neurological or endocrine diseases), personal history of cancer, infectious diseases, incapacitating cognitive alterations and/or severe schizophrenia, and pregnancy.

Controls were matched with the cocaine group for age and body mass index (BMI) and they were required to be ≥18 years to 65 years of age. In addition to the mentioned exclusion criteria for abstinent cocaine users, controls were excluded with: personal history of drug abuse and lifetime psychiatric disorders.

2 Clinical assessments

All cocaine users were evaluated according to 'Diagnostic and Statistical Manual of Mental Disorders-4th Edition-Text Revision' (DSM-IV-TR) criteria, using the Spanish version of the 'Psychiatric Research Interview for Substance and Mental Disorders' (PRISM) [7,26]. Controls were initially evaluated by PRISM (for substance screening and abuse and dependence) and subsequently by the Spanish version of the 'Composite International Diagnostic Interview' (CIDI) for the detection of psychiatric disorders [27]. All the interviews were performed by experienced psychologists who had received both PRISM and CIDI training.

2.1 Psychiatric Research Interview for Substance and Mental Diseases (PRISM). The PRISM is a semi-structured interview that has demonstrated good psychometric properties in terms of test-retest reliability [28], inter-rater reliability [29] and validity [7] to diagnose psychiatric disorders among substance users.

Diagnoses were made using two time-frames: 'current' (criteria were met within the past year) and 'past' (criteria were met before the previous 12 months). Lifetime prevalence, taking into account both current and past diagnoses, was used to present the frequency of substance use disorders, non-substance use disorders and psychiatric comorbidity. In addition to the diagnoses of substance abuse and dependence, the PRISM differentiates 'pathological use' (chronic intoxication: substance use ≥4 days a week for ≥3 weeks; and/or binge use: ≥3 consecutive days of continuous substance use) from 'occasional use' (substance use less than 4 days a week, unless substance was used in a binge pattern).

The cocaine symptom severity was assessed combining the DSM-IV-TR criteria for cocaine use disorders: 7 dependence criteria (for diagnosis of dependence three or more co-occurring symptoms in a 12-month period are required); and 4 abuse criteria (one symptom is necessary for diagnosis of abuse), which is in agreement with the unidimensionality of DSM-5 criteria [30,31]. More details regarding analysis of cocaine symptom severity have been described previously (see [13]).

3 Laboratory methods for human samples

3.1 Collection and analysis of plasma samples. Blood samples were obtained in the morning (09:00–11:00 h AM) after fasting for 8–12 h (previous to the psychiatric interviews). Venous blood was collected into 10 mL K_2-EDTA tubes (BD, Franklin Lakes, NJ, USA) and processed to obtain plasma. Blood samples were centrifuged at 2,200×g for 15 min (4°C) and individually assayed for detecting infectious diseases by 3 rapid tests for HIV (Retroscreen HIV, QualPro Diagnostics-Tulip Group Ltd, Goa, India), hepatitis B (HBsAg Test, Toyo Diagnostics-Turklab Inc., Izmir, Turkey) and hepatitis C (Flaviscreen HCV, QualPro

Diagnostics-Tulip Group Ltd, Goa, India). Samples testing positive were discarded following safety protocols.

Plasma analyses for cocaine metabolite (Benzoylecgonine Specific Direct ELISA Kit Immunalysis, Pomona, CA, USA) were performed to confirm cocaine abstinence. Four cocaine users who tested negative for drugs of abuse in urine analyses at the outpatient treatment centers for cocaine addiction were positive for benzoylecgonine in plasma, and these cocaine users were excluded from this study. Plasma samples were stored at -80°C until further analyses.

3.2 Multiplex immunoassay analysis. A Bio-Plex Suspension Array System 200 (Bio-Rad Laboratories, Hercules, CA, USA) was used to quantify the plasma concentrations of anti- and pro-inflammatory cytokines, homeostatic and pro-inflammatory chemokines and BDNF following the manufacturer´s instructions as previously reported [12]. Human protein panels were used to simultaneously detect the following analytes: Tumor necrosis factor-alpha (TNFα); interleukin-1 beta (IL-1β); interleukin-6 (IL-6); interleukin-10 (IL-10); CX3CL1 [Chemokine (C-X_3-C motif) ligand 1], commonly referred to as fractalkine; CCL2 [Chemokine (C-C motif) ligand 2], also referred to as monocyte chemotactic protein-1 (MCP-1); CXCL12 [Chemokine (C-X-C motif) ligand 12], also referred to stromal cell-derived factor-1 (SDF-1); and BDNF. Raw data (mean fluorescence intensity) were analyzed using the Bio-Plex Manager Software 4.1 (Bio-Rad Laboratories, Hercules, CA, USA). Data of plasma concentrations (pg of protein/mL) were used to perform multiple correlation studies and analyses of the means.

3.3 Radioimmunoassay analysis for IGF1 and IGFBP-3. Plasma concentrations of total IGF-1 were estimated by double antibody radioimmunoassay (RIA), after removal of serum IGFBPs by acid-ethanol extraction. To confirm the removal of IGFBPs, extracted and non-extracted plasma fractions were incubated with ^{125}I-IGF-1 at 4°C for 24 h. Dextran charcoal was used to separate the bound and free tracers. The IGF-1 antiserum (UB2–495) was a gift from Drs Underwood and Van Wisk distributed by the Hormone Distribution Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) through the National Hormone and Pituitary Program. Concentrations of IGF-1 were expressed in terms of rat IGF-1 from Gropep Bioreagents Pty Ltd (Adelaide, SA, Australia). Test sensitivity was 10 ng/mL and the intra-assay coefficient of variation was 8%. All samples were run in the same batch.

Plasma IGFBP-3 concentration was determined in duplicate by RIA using a commercially available kit (Mediagnost GmbH, Reutlingen, Germany) following the manufacturer´s protocol. The assay sensitivity was 500 pg/mL and the intra-assay coefficient of variation was 7.5%.

3.4 Quantification of acyl derivatives. The following lipid derivatives and their respective deuterated forms were used for quantification: N-stearoyl-ethanolamine (SEA), N-palmitoyl-ethanolamine (PEA) and PEA-d_4, N-oleoyl-ethanolamine (OEA) and OEA-d_4, N-palmitoleoyl-ethanolamine (POEA), N-arachidonoyl-ethanolamine (AEA) and AEA-d_4, N-linoleoyl-ethanolamine (LEA) and LEA-d_4, N-docosahexaenoyl-ethanolamine (DHEA) and DHEA-d_4, N-dihomo-γ-linolenoyl-ethanolamine (DGLEA), 2-arachidonoyl-glycerol (2-AG) and 2-AG-d_5, and 2-linoleoyl-glycerol (2-LG). PEA-d_4, OEA-d_4 and AEA-d_4 were used for quantification of POEA, SEA and DGLEA respectively, since their deuterated forms were not commercially available. All reagents were obtained from Cayman Chemical (Ann Arbor, MI, USA).

Sample extraction and the chromatographic separation were performed in a Liquid Chromatography-tandem Mass Spectrometry System (Agilent Technologies, Wilmington, DE, USA) as previously reported [13]. The tandem quadrupole mass spectrometer operated on the positive electrospray mode. The multiple reaction monitoring mode was used for the analysis with the following precursor to product ion transitions: m/z 328.1/62 for SEA, m/z 300.1/62 for PEA, m/z 304.4/66 for PEA-d_4, m/z 326.1/62 for OEA, m/z 330.4/66 for OEA-d_4, m/z 298.2/62 for POEA, m/z 348.3/62 for AEA, m/z 352.2/66 for AEA-d_4, m/z 324.5/62 for LEA, m/z 328.5/

66 for LEA-d_4, m/z 372.6/62 for DHEA, m/z 376.3/66 for DHEA-d_4, m/z 350.2/62 for DGLEA, m/z 379.2/287 for 2-AG, m/z 384.3/287 for 2-AG-d_5 and m/z 355.2/263 for 2-LG. A six-point external calibration curve prepared in the mobile phase (10:90, A:B) and spiked with 0.4–25 ng of N-acyl-ethanolamines and 0.8–50 ng of 2-acyl-glycerols was used for the quantification [32]. Data of plasma concentration (ng of acyl derivative /mL) were used to perform multiple correlation studies.

4 Ethics statement

Written informed consent was obtained from each subject after they had received a complete description of the present study and had been given the chance to discuss any questions or issues. The study and protocols for recruitment were approved by the Ethics Committee of the Hospital Regional Universitario de Málaga (07/19/2009 PND049/2009 and PI0228–2013; CEI Provincial de Málaga) and therefore were conducted in accordance with the Declaration of Helsinki (seventh revision in 2013, Fortaleza, Brazil).

5 Statistical analyses

All data for graphs and tables are expressed as number and percentage of subjects [*n* (%)] or mean and standard deviation (SD) of concentrations [mean (SD)]. The significance of differences in categorical variables was determined by using the Fisher's exact test; while continuous variables were evaluated by different statistical approaches according to the number of comparisons and the distribution of variables. For comparisons of two groups, the Student's t-test was used for normally distributed continuous variables and the Mann-Whitney U test was used as non-parametric test. For comparisons of three or more groups, one-way and analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons was used for normal distributions whereas the Kruskal—Wallis analysis with the Dunn's post test was used as non-parametric analysis. Correlation analyses were performed by using the Pearson´s correlation coefficient (r) for continuous variables with normal distribution and Spearman´s rank correlation coefficient (rho) for continuous variables without normality and discrete variables. The Holm-Bonferroni correction was employed for multiple comparisons of correlation coefficients for controlling the type I errors. The normal distribution of variables was evaluated using the D'Agostino & Pearson omnibus normality test. Thresholds of 0.05 were applied for p-values and adjusted p-values. Statistical analyses were performed using the computer program Graph-Pad Prism version 5.04 (GraphPad Software, San Diego, CA, USA).

Results

1 Sample demographics and clinical characteristics

A total of 185 subjects both sexes were selected for this study and grouped into the cocaine (n = 100) and control (n = 85) groups. The average participant was a 36–37 year-old male with a BMI of 26 (weighing 75–77 kg). A description of the sample is presented in Table 1.

Cocaine users displayed cocaine abstinence for 234.7 (436.3) days [mode: 30 days (range: 2,555)]. The percentages of cocaine users treated for substance use and psychological condition were 81.0% and 35.0% respectively. In contrast, only 17.6% of controls received psychological treatments.

Cocaine use disorders were the most prevalent lifetime substance use disorders (89%) followed by lifetime alcohol (64%), cannabis (23%), benzodiazepines (8%) and heroin (8%) use disorders (not including caffeine or nicotine).

Table 1. Baseline socio-demographic variables and lifetime psychiatric and substance use disorders.

VARIABLE		COCAINE GROUP *n* = 100	CONTROL GROUP *n* = 85	p-value
AGE (≥18) [MEAN (SD)]		35.8 (8.9)	37.3 (10.7)	0.299 [a]
SEX [*n* (%)]	Women	18 (18.0)	25 (29.4)	0.081 [b]
	Men	82 (82.0)	60 (70.6)	
BODY MASS [MEAN (SD)]	Body Mass Index	25.5 (4.5)	26.1 (4.0)	0.343 [a]
	Weight (kg)	77.1 (14.4)	75.3 (11.0)	0.337 [a]
PSYCHOLOGICAL TREATMENT (EVER) [*n* (%)]	No	67 (67.0)	80 (94.1)	**<0.001** [b]
	Yes	35 (35.0)	5 (5.9)	
SUBSTANCE USE TREATMENT (EVER) [*n* (%)]	No	21 (21.0)	85 (100)	**<0.001** [b]
	Yes	81 (81.0)	0 (0.0)	
LIFETIME SUBSTANCE USE DISORDERS [*n* (%)]	Cocaine	89 (89.0)	-	-
	Alcohol	64 (64.0)	-	
	Cannabis	23 (23.0)	-	
	Benzodiazepines	8 (8.0)	-	
	Heroin	8 (8.0)	-	
	Hallucinogens	6 (6.0)	-	
	Others	7 (7.0)	-	
LIFETIME COCAINE USE DISORDERS [*n* (%)]	Abuse or Dependence	89 (89.0)	-	-
	Abuse	78 (78.0)	-	
	Dependence	84 (84.0)	-	
LIFETIME COMMON PSYCHIATRIC DISORDERS [*n* (%)]	Mood Disorders	33 (33.0)	-	-
	Anxiety Disorders	22 (22.0)	-	
	Psychosis Disorders	13 (13.0)	-	
	Personality Disorders	31 (31.0)	-	

[a] p-value from Student's t-test.

[b] p-value from Fisher's exact test or Chi-square test.

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Regarding common psychiatric disorders assessed with the PRISM, we found high prevalences of comorbid mood (33%), anxiety (22%), psychosis (13%) and personality (31%) disorders. Therefore, a vast majority of cocaine users who participated in this study were diagnosed with substance use disorders or multiple substance use disorders and psychiatric comorbidity.

2 Plasma concentrations of BDNF, IGF-1 and IGFBP-3

Mean plasma concentrations for BDNF, IGF-1 and IGFBP-3 were similar in both groups. The plasma concentrations of each protein were as follows: 274.9±200.3 pg/mL and 269.4±242.7 pg/mL for BDNF, 212.2±63.9 ng/mL and 210.7±51.2 ng/mL for IGF-1, and 3.91±0.94 μg/mL and 3.78±0.93 μg/mL for IGFBP-3, in the cocaine and control groups respectively.

2.1 Plasma concentrations of BDNF, IGF-1 and IGFBP-3 in relation to sex. As indicated in Fig. 1, we examined whether the sex composition in the cocaine and control groups affects the plasma concentrations of BDNF, IGF-1 and IGFBP-3. A two-way ANOVA was performed taking into consideration cocaine use and sex as factors. We did not observe main effects or interaction of these factors on the concentrations of BDNF, IGF-1 and IGFBP-3 between men and women in both groups.

2.2 Plasma concentrations of BDNF, IGF-1 and IGFBP-3 in relation to age. The influence of age on growth factors has been extensively described, especially with IGF-1 [33,34,35].

Figure 1

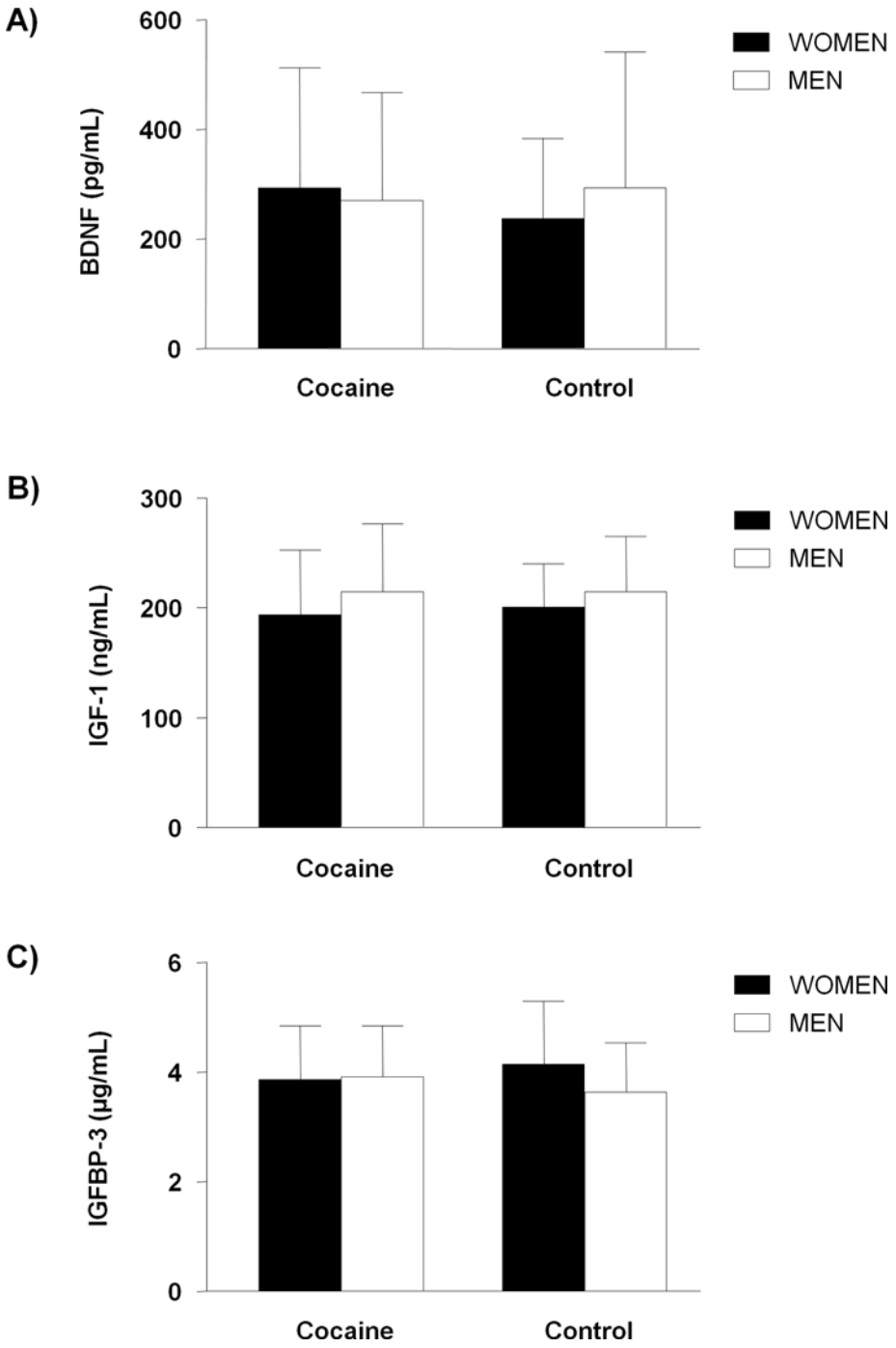

Fig 1. Plasma concentrations of BDNF, IGF-1 and IGFBP-3 according to sex in abstinent cocaine users and control subjects. A) BDNF (pg/mL); **B)** IGF-1 (ng/mL); and **C)** IGFBP-3 (µg/mL). Bars are the means and SD. Data were analyzed by two-way analyses (cocaine use [cocaine group and control group] and sex [women and men]).

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Correlation analyses were performed between plasma concentrations of BDNF, IGF-1 or IGFBP-3 and age for each study group, as shown in Fig. 2. The plasma IGF-1 concentrations were negatively correlated with age in the cocaine ($r = -0.33$, $p<0.001$) and control ($r = -0.32$, $p<0.001$) groups using the Pearson's correlation coefficient for normal distributions. These correlations were not significant for BDNF and IGFBP-3 concentrations.

Figure 2

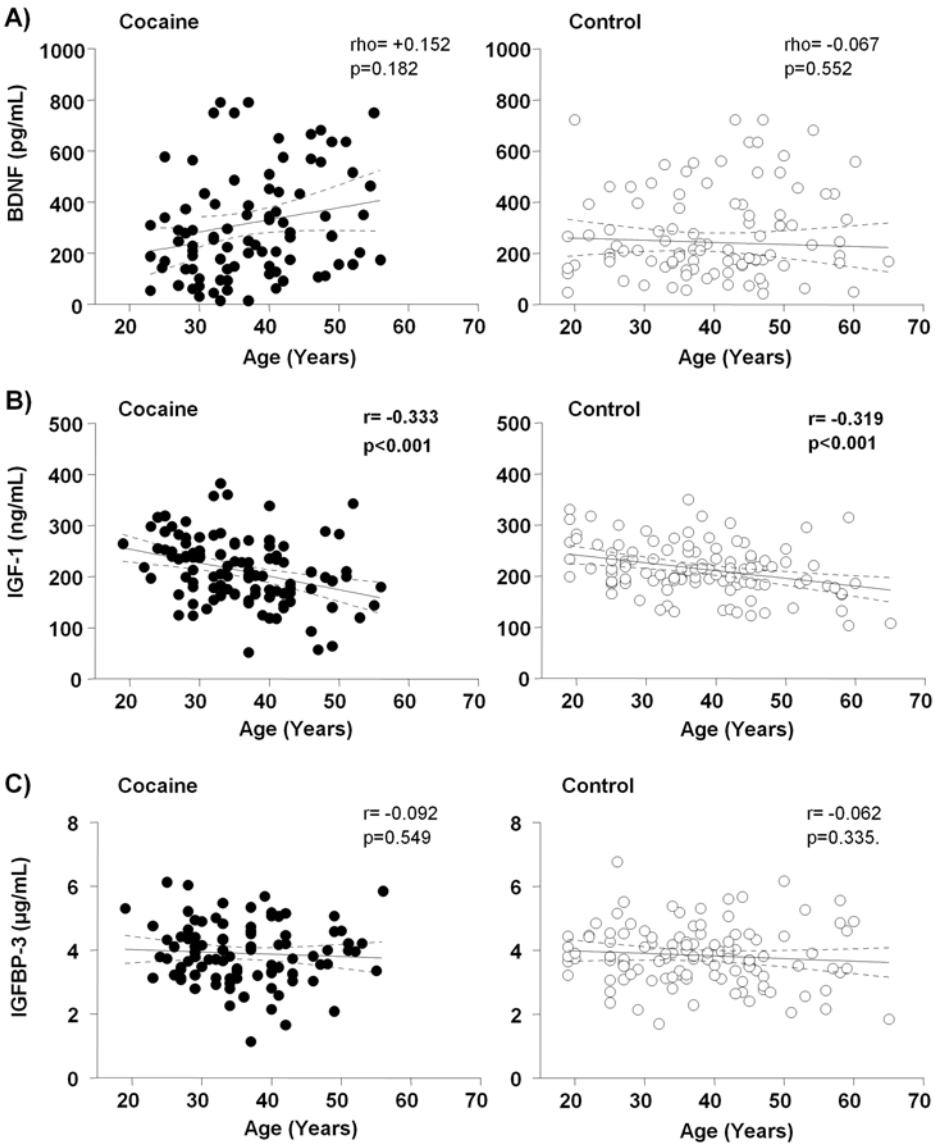

Fig 2. Correlation analyses between plasma concentrations of BDNF, IGF-1 and IGFBP-3 and age in abstinent cocaine users (black circles) and control subjects (white circles). A) BDNF (pg/mL); **B)** IGF-1 (ng/mL); and **C)** IGFBP-3 (µg/mL). Dots are individual values. (r) Pearson´s correlation coefficient; (rho) Spearman´s correlation coefficient; (p) p-value for statistical significance.

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3 Multiple correlation analyses of BDNF, IGF-1 and IGFBP-3 with other plasma molecules sensitive to cocaine addiction

We determined the degree of association among these peptides (BDNF, IGF-1 and IGFBP-3) and other plasma mediators in the cocaine and control groups. Recently, we have reported that certain circulating pro-inflammatory mediators and fatty acid derivatives are influenced by cocaine addiction [12,13]. Thus, we examined the degree of association of BDNF, IGF-1 and IGFBP-3 with these molecules and among themselves in the sample. The significances of the resulting correlation coefficients were statistically corrected using the Holm-Bonferroni method to counteract the problem of multiple comparisons for the control and cocaine groups.

3.1 BDNF. Plasma concentrations of BDNF showed a non-normal distribution in abstinent cocaine subjects and controls and the correlation coefficients calculated were rho (Table 2).

In the cocaine group, BDNF concentrations did not correlate with the concentrations of chemokines (CX_3CL1, CCL2 and CXCL12), cytokines (IL1β, TNFα, IL6 and IL10), fatty acid derivatives (N-acyl-ethanolamines and 2-acyl-glycerols), IGF-1 or IGFBP-3. In contrast, BDNF concentrations correlated positively with the concentrations of certain chemokines and N-acyl-ethanolamines in the control group. Concretely, BDNF significantly correlated with CX_3CL1 (rho = +0.69, adjusted $p<0.001$) and CXCL12 (rho = +0.78, adjusted $p<0.001$); PEA

Table 2. Multiple correlations between plasma concentrations of BDNF and other plasma molecules in the cocaine group.

	MULTIPLE CORRELATION ANALYSIS [1,2]					
VARIABLE	**BDNF**					
	COCAINE			**CONTROL**		
	rho	**p-value**	**adjusted p-value**	**rho**	**p-value**	**adjusted p-value**
CX_3CL1 (fractalkine)	-0.094	0.441	ns	**+0.693**	**<0.001**	**<0.001**
CCL2 (MCP-1)	-0.005	0.968	ns	+0.009	0.941	ns
CXCL12 (SDF-1)	-0.110	0.363	ns	**+0.784**	**<0.001**	**<0.001**
IL1β	-0.109	0.372	ns	-0.135	0.269	ns
TNFα	-0.133	0.272	ns	-0.189	0.120	ns
IL6	-0.260	0.029	ns	+0.054	0.660	ns
IL10	-0.110	0.365	ns	+0.031	0.799	ns
SEA	-0.047	0.695	ns	+0.325	**0.007**	ns
PEA	-0.058	0.629	ns	**+0.492**	**<0.001**	**0.003**
OEA	-0.133	0.270	ns	**+0.400**	**<0.001**	**0.033**
POEA	-0.037	0.760	ns	+0.331	**0.006**	ns
AEA	-0.150	0.213	ns	**+0.447**	**<0.001**	**0.005**
LEA	-0.187	0.119	ns	**+0.423**	**<0.001**	**0.014**
DGLEA	-0.170	0.156	ns	**+0.524**	**<0.001**	**0.002**
DHEA	+0.009	0.938	ns	+0.301	**0.012**	ns
2-AG	+0.074	0.538	ns	+0.054	0.661	ns
2-LG	-0.056	0.640	ns	+0.122	0.320	ns
IGF-1	-0.157	0.210	ns	-0.111	0.291	ns
IGFBP-3	-0.170	0.156	ns	-0.221	0.106	ns

[1] All variables were assessed for normality to select the adequate correlation coefficient (r; rho).

[2] Adjusted p-values were calculated using Holm-Bonferroni correction (3x18 correlations per group).

Abbreviations: ns, non-significant.

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Table 3. Multiple correlations between plasma concentrations of IGF-1 and other plasma molecules in the cocaine group.

MULTIPLE CORRELATION ANALYSIS [1,2]						
VARIABLE	**IGF-1**					
	COCAINE			**CONTROL**		
	r	**p-value**	**adjusted p-value**	**r**	**p-value**	**adjusted p-value**
CX_3CL1 (fractalkine)	-0.062	0.608	ns	-0.062	0.792	ns
CCL2 (MCP-1)	+0.087	0.472	ns	+0.087	0.137	ns
CXCL12 (SDF-1)	+0.013	0.914	ns	+0.013	0.845	ns
IL1β	+0.067	0.582	ns	+0.067	0.180	ns
TNFα	+0.048	0.693	ns	+0.048	0.638	ns
IL6	+0.078	0.519	ns	+0.078	0.520	ns
IL10	+0.089	0.462	ns	+0.089	0.799	ns
SEA	+0.086	0.436	ns	+0.086	0.187	ns
PEA	+0.054	0.624	ns	-0.281	**0.019**	ns
OEA	-0.064	0.560	ns	-0.264	**0.028**	ns
POEA	-0.142	0.195	ns	+0.006	0.964	ns
AEA	-0.085	0.437	ns	-0.186	0.125	ns
LEA	-0.095	0.387	ns	-0.133	0.275	ns
DGLEA	-0.018	0.873	ns	-0.184	0.131	ns
DHEA	+0.171	0.118	ns	-0.309	0.010	ns
2-AG	-0.120	0.275	ns	-0.097	0.430	ns
2-LG	-0.074	0.503	ns	-0.119	0.331	ns
IGFBP-3	+0.327	**0.006**	ns	**+0.463**	**<0.001**	**0.003**

[1] All variables were assessed for normality to select the adequate correlation coefficient (r; rho).

[2] Adjusted p-values were calculated using Holm-Bonferroni correction (3x18 correlations per group).

Abbreviations: ns, non-significant.

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(rho = +0.49, adjusted $p<0.01$), OEA (rho = +0.40, adjusted $p<0.05$), AEA (rho = +0.45, adjusted $p<0.01$), LEA (rho = +0.42, adjusted $p<0.05$) and DGLEA (rho = +0.52, adjusted $p<0.01$). Although BDNF was initially associated with other N-acyl-ethanolamines (SEA, POEA and DHEA), the statistical adjustment for multiple comparisons rejected them.

3.2 IGF-1. Plasma concentrations of IGF-1 were normally distributed in the cocaine and control groups and Pearson´s correlation coefficients (r) were calculated for all comparisons. As shown in Table 3, IGF-1 concentrations did not correlate with the concentrations of the plasma molecules that were assessed in the cocaine group. Only we found an initial association with its binding protein IGFBP-3 prior to apply the correction test that was rejected. However, in the control group this positive correlation between the concentrations of IGF-1 and IGFBP-3 was significant after adjusting the significance (r = +0.46, adjusted $p<0.01$). Similar to BDNF, IGF-1 showed correlations with some N-acyl-ethanolamines (PEA and OEA) that were also discarded after correcting them.

3.3 IGFBP-3. As indicated in Table 4, IGFBP-3 concentrations passed the normality test in both groups and Pearson´s correlation coefficients (r) were calculated. In the cocaine group, plasma concentrations of IGFBP-3 were not associated with the plasma mediators that were examined with the exception of SEA. Thus, plasma concentrations of IGFBP-3 correlated positively with SEA (r = +0.40; adjusted $p<0.05$). Regarding control subjects, IGFBP-3 concentrations were not correlated with the rest of molecules although some positive

Table 4. Multiple correlations between plasma concentrations of IGFBP-3 and other plasma molecules in the cocaine group.

MULTIPLE CORRELATION ANALYSIS [1,2]						
VARIABLE	**IGFBP-3**					
	COCAINE			**CONTROL**		
	r	**p-value**	**adjusted p-value**	**r**	**p-value**	**adjusted p-value**
CX_3CL1 (fractalkine)	-0.006	0.963	ns	+0.113	0.356	ns
CCL2 (MCP-1)	+0.126	0.303	ns	+0.171	0.159	ns
CXCL12 (SDF-1)	-0.113	0.357	ns	+0.141	0.250	ns
IL1β	-0.062	0.611	ns	+0.317	**0.008**	ns
TNFα	-0.047	0.699	ns	+0.233	0.054	ns
IL6	+0.067	0.587	ns	+0.321	**0.007**	ns
IL10	-0.052	0.672	ns	+0.244	**0.044**	ns
SEA	**+0.397**	**<0.001**	**0.011**	-0.091	0.459	ns
PEA	+0.216	0.058	ns	-0.048	0.696	ns
OEA	-0.226	0.056	ns	+0.115	0.348	ns
POEA	-0.121	0.272	ns	+0.046	0.710	ns
AEA	-0.079	0.473	ns	+0.125	0.307	ns
LEA	+0.038	0.732	ns	-0.003	0.979	ns
DGLEA	-0.084	0.446	ns	+0.006	0.963	ns
DHEA	+0.042	0.707	ns	+0.079	0.521	ns
2-AG	+0.010	0.926	ns	+0.145	0.235	ns
2-LG	+0.093	0.398	ns	+0.132	0.280	ns

[1] All variables were assessed for normality to select the adequate correlation coefficient (r; rho).

[2] Adjusted p-values were calculated using Holm-Bonferroni correction (3x18 correlations per group).

Abbreviations: ns, non-significant.

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associations were observed in paired-comparisons with cytokines (IL1β, IL6 and IL10) without adjustment for multiple comparisons.

Overall, while BDNF concentrations correlated positively with chemokines and N-acyl-ethanolamines in the control group, IGFBP-3 was found to be positively associated only with SEA in the cocaine group. Moreover, IGF-1 and IGFBP-3 correlated positively in the control group but not in the cocaine group.

4 Plasma concentrations of BDNF, IGF-1 and IGFBP-3 in relation to variables associated with cocaine use

In the cocaine group, correlation analyses were performed between plasma concentrations of BDNF, IGF-1 or IGFBP-3 and length of cocaine abstinence (days) and duration of cocaine use (years) (S1 Fig.). Because both length of cocaine abstinence and duration of cocaine were variables with a non-normal distribution, Spearman's rank correlation coefficients (rho) were used for determining associations. However, we observed no correlations between these mediators and variables related to abstinence and cocaine use.

Additional correlation analyses were performed between plasma concentrations of BDNF, IGF-1 or IGFBP-3 and individual scores of DSM-IV-TR criteria for cocaine abuse and dependence. The concentrations of these peptides were not associated with the cocaine symptom severity (S2 Fig.). As expected, the cocaine group displayed an increased number of criteria for cocaine use disorders (7.3±3.3 criteria) which indicates a high cocaine symptom severity.

Table 5. Plasma concentrations of BDNF, IGF-1 and IGFBP-3 in abstinent cocaine users grouped by diagnosis of common psychiatric disorders in substance users.

	VARIABLE			
PSYCHIATRIC DISORDER	***n* (%)**	**BDNF pg/mL [mean (SD)]**	**IGF-1 ng/mL [mean (SD)]**	**IGFBP-3 μg/mL [mean (SD)]**
MOOD DISORDERS[1]	33 (33.0)	240.2 (177.4)	213.1 (83.4)	3.83 (0.99)
Primary	11 (11.0)	297.0 (223.2)	246.4 (59.7)	4.31 (0.73)
Cocaine-induced	17 (18.0)	227.6 (150.4)	196.5 (91.8)	3.83 (1.07)
Primary & Cocaine-induced	5 (5.0)	**131.8 (66.7)** [a]	186.5 (38.8)	3.30 (0.67)
NO MOOD DISORDERS	67 (67.0)	294.9 (211.4)	210.8 (52.5)	3.87 (0.92)
ANXIETY DISORDERS	22 (22.0)	268.2 (171.1)	191.6 (61.9)	3.80 (0.91)
Primary	12 (12.0)	278.9 (170.0)	182.3 (67.6)	3.84 (0.87)
Cocaine-induced	7 (7.0)	293.6 (199.3)	199.9 (67.2)	4.11 (1.09)
Primary & Cocaine-induced	3 (3.0)	**137.9 (0.9)** [b,*]	209.8 (16.0)	3.16 (0.75)
NO ANXIETY DISORDERS	78 (78.0)	276.8 (208.9)	217.1 (63.7)	3.94 (0.95)
PSYCHOTIC DISORDERS	13 (13.0)	262.9 (209.9)	206.7 (59.3)	3.83 (0.84)
Primary	2 (2.0)	163.4 (168.1)	178.5 (0.0)	3.49 (0.30)
Cocaine-induced	11 (11.0)	281.0 (218.4)	211.0 (62.9)	3.89 (0.90)
Primary & Cocaine-induced	-	-	-	-
NO PSYCHOTIC DISORDERS	87 (87.0)	277.2 (199.9)	213.0 (64.8)	3.92 (0.95)
PERSONALITY DISORDERS (CLUSTER-B) [2]	31 (31.0)	232.8 (176.8)	197.0 (64.5)	3.83 (0.91)
NO PERSONALITY DISORDERS	69 (69.0)	294.5 (208.9)	218.1 (63.4)	3.94 (0.95)
CONTROL GROUP		**269.4 (242.7)**	**210.7 (51.2)**	**3.78 (0.93)**

[1] Mood disorders include major depressive disorder, dysthymic disorder, bipolar disorders (mania and hypomania) and cocaine-induced mood disorders.

[2] Cluster B personality disorders include borderline and antisocial personality disorders.

[a] $p<0.05$ denotes significant differences compared to the *no mood disorders* subgroup.

[b] $p<0.05$ denotes significant differences compared to the *no anxiety disorders* subgroup.

* $p<0.05$ denotes significant differences compared to the control group.

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5 Psychiatric comorbidity and substance use disorders

Because all outpatient cocaine users display high rates of comorbid psychiatric disorders, we evaluated plasma concentrations of BDNF, IGF-1 and IGFBP-3 in the most common psychiatric comorbidities among substance users, as shown in Table 5.

The DSM-IV-TR Axis I disorders included mood disorders, anxiety and psychosis. Considering the exclusive diagnoses, the prevalences of cocaine-induced mood and psychotic (18.0% and 11.0% respectively) disorders were higher than the primary mood and psychotic (11.0% and 2.0% respectively) disorders, unlike cocaine-induced and primary anxiety disorders (7.0% and 12.0% respectively). Also, some cocaine users were diagnosed with both primary and cocaine-induced mood ($n = 5$) and anxiety ($n = 3$) disorders. With respect to personality disorders, the prevalence reached a 31.0%.

Considering the plasma concentrations of BDNF, IGF.1 and IFGBP-3 in these outpatient users diagnosed with psychiatric comorbidities, we only observed significant changes in BDNF concentrations. Concretely, abstinent cocaine users diagnosed with both primary and cocaine-induced disorders (for mood or anxiety disorders) displayed significant decreases in plasma BDNF concentrations ($p<0.05$) compared to those users with no mood disorders or no anxiety. Additionally, the decrease in BDNF concentrations in subjects diagnosed with both primary and cocaine-induced anxiety disorders were also significant ($p<0.05$) relative to the control group.

Regarding IGF-1 and IGFBP-3, we observed no changes in their plasma concentrations by the presence of psychiatric comorbidities.

Discussion

The present exploratory and cross-sectional study found that the plasma concentrations of BDNF and IGF-1 are unaltered by a lifetime pathological use of cocaine in abstinent cocaine users under treatment. Indeed, plasma concentrations of these factors were not influenced by the length of abstinence, duration of cocaine use or cocaine symptom severity. The association of plasma IGF-1 concentrations with age was not affected by the pathological use of cocaine, although the association of IGF-1 and IGFBP-3 did not reach statistical significance after correcting it in abstinent cocaine users. Additionally, the correlations of BDNF concentrations with chemokines and N-acyl-ethanolamines in the control group were not observed in cocaine subjects. However, we detected a positive correlation between the concentrations of IGFBP-3 and SEA in the cocaine group. On the other hand, we found an elevated prevalence of comorbid psychiatric disorders in these patients with polysubstance use and changes in BDNF concentrations related to the diagnosis of mood and anxiety disorders. Thus, plasma BDNF concentrations were reduced in cocaine users diagnosed with both primary and cocaine-induced mood and anxiety disorders.

BDNF in abstinent cocaine users

Although we found no changes in BDNF concentrations, several clinical studies have reported changes in the serum BDNF concentrations of cocaine dependents during abstinence, suggesting that BDNF is a reliable biomarker for cocaine addiction. A first study in cocaine dependent individuals found that BDNF concentrations are increased during early abstinence, and the elevated BDNF is predictive of relapse risk during early recovery from cocaine dependence [16]. Related to this finding, another study reported that BDNF concentrations are positively correlated with cocaine craving and abstinence symptoms [17]. Similarly, more recent studies in crack cocaine dependent individuals have found high blood BDNF concentrations during early abstinence [36] and negative correlations with severity [37] and amount of cocaine used [38]. All these prospective studies were conducted in cocaine addicts following inpatient detoxification treatments during 2–4 weeks, unlike our study that is a cross-sectional study in cocaine users seeking treatment in outpatient programs. Consequently, we have a single measure for BDNF from each cocaine user, variable periods of abstinence and uncontrolled environmental factors (inherent in outpatient treatments) that may be interfering with the present data.

IGF-1 and IGFBP-3 in abstinent cocaine users

Numerous studies in mammals have reported that IGF-1 concentrations decline with advancing age, showing the association of IGF-1 with longevity and age-related diseases (e.g., cancer, cardiovascular disease, diabetes, osteoporosis, and neurodegenerative diseases) [39,40,41]. We observed that IGF-1 concentrations correlated significantly with the age of the participants. According to the present results, the history of a pathological use of cocaine did not influence the association of IGF-1 concentrations and aging because the significant and negative correlation between both variables was identical to the control group. However, we did not evaluated the cognitive impairment in the sample [42].

Focusing on cocaine addiction, we observed no changes in the plasma concentrations of IGF-1 and IGFBP-3 of abstinent users and concentrations did not correlated with addiction-related variables such as severity cocaine symptom, abstinence or duration of cocaine use. We did not find literature about an association between IGF-1 and cocaine but other drugs of

abuse have been investigated. Studies in rodents suggest that IGF-1 is altered in cerebral areas related to the development of addiction after chronic exposure to morphine [43,44]. In humans, a recent study in patients with opiate use dependence has demonstrated that serum IGF-1 is elevated [5]. In addition to opiates, various studies evaluating IGF-1 have been conducted in alcohol dependent subjects but the authors did not observe any interaction between alcohol addiction and this peptide in blood or brain [25,45]. However, a recent study in alcohol dependent patients has found that IGF-1 might play a role in the cognitive function in these subjects [46].

Lack of association with biomarkers of cocaine addiction

Overall, the lack of influence of cocaine addiction on plasma concentrations of BDNF and IGF-1 was confirmed through multiple analyses of correlation coefficients of these factors with other circulating molecules sensitive to cocaine addiction and/or psychiatric comorbidity in cocaine abstinent subjects from similar observational studies [12,13]. Chemokines and pro-inflammatory mediators are affected by the cocaine symptom severity, while anti-inflammatory fatty acid derivatives such as endocannabinoids and their congeners are affected by the history of pathological use of cocaine and the presence of comorbid disorders. Thus, the significant correlations between these cocaine-sensitive molecules and BDNF and IGF-1 (and IGFBP-3) in controls vanished in the cocaine group. However, we observed only one significant correlation after multiple comparisons in the cocaine group, in particular IGFBP-3 and SEA concentrations, but the SEA concentrations is not affected by cocaine use unlike other N-acyl-ethanolamines [13].

Because IGF-1 forms a ternary complex with IGFBP-3 [47], we also studied the association between both peptides. We detected a positive correlation between plasma concentrations of IGF-1 and IGFBP-3 in the control participants, but such association was weakly affected by the lifetime pathological use of cocaine.

BDNF and IGF-1 in abstinent cocaine users with psychiatric comorbidity

Similar to these previous cross-sectional studies in abstinent cocaine users recruited from outpatient programs, we have detected a high prevalence of comorbid psychiatric disorders, approximately 60% [12,13]. Although these plasma peptide concentrations were unaltered in cocaine users, we examined these concentrations according to the diagnosis of primary and cocaine-induced disorders. Substantial evidence indicates neurotrophic/growth factors such as BDNF and IGF-1 are involved in the pathogenesis of common psychiatric disorders. We have indeed observed in this study changes in circulating concentrations of these neurotrophic factors in mood and anxiety disorders, especially in BDNF.

BDNF in psychiatric comorbidity

Current literature on BDNF and mental disorders is particularly focused on depressive and bipolar disorders. Thus, circulating concentrations of BDNF are decreased in depressed patients compared to controls and that they increase significantly with antidepressant treatment [48,49,50]. The reduction of plasma BDNF concentration has also been related to suicidal behavior in major depression [51] whereas other studies in bipolar patients have found a significant association with the severity of depression [52,53], suggesting that plasma BDNF concentrations may be a marker of depression and/or bipolar disorder. Our findings are consistent with the literature because BDNF concentrations in cocaine users diagnosed with mood disorders, both primary and cocaine-induced, were found to be decreased. However, only five cocaine users were identified with this complicated diagnosis and, therefore, the statistical

significance of this reduction may be called into question. We observed no differences in the BDNF concentrations of those abstinent subjects displaying primary mood disorders or cocaine-induced mood disorders separately.

BDNF has also emerged as a potential biomarker for anxiety from preclinical studies in rodents [54]. Translational studies have reported that early stress correlates negatively with peripheral BDNF concentrations later in life [55]. A recent review of clinical studies showed that BDNF concentrations were lower in individuals with any anxiety disorder compared to those without anxiety but this is not consistent across the literature [56]. In support of this, abstinent cocaine users diagnosed with both primary and cocaine-induced anxiety disorders exhibited a significant decrease in plasma BDNF, as seen previously with mood disorders. Again, the main limitation of this observation is related to the reduced number of individuals with this dual (primary and cocaine-induced anxiety) comorbid diagnosis.

IGF-1 in psychiatric comorbidity

Several studies in old and young populations have shown the association between IGF-1 concentrations and depressive symptoms [23,24]. It has been reported that plasma IGF-1 concentrations are increased in acute depressed patients [57] and similarly, another study demonstrated that patients with bipolar disorder have elevated IGF-1 concentrations [58]. Although our data are consistent with these observations and cocaine users with primary mood disorders displayed an increase in plasma IGF-1 and IGFBP-3 concentrations, these increases did not reach statistical significance relative to the control group and abstinent users without mood disorders.

We need to perform additional studies to establish the mechanisms of action of these trophic factors (especially BDNF) and the selectivity in the influence of each factor by a specific comorbid disorder in cocaine addiction.

Limitations and future perspectives

Although our findings support the importance of monitoring BDNF and IGF-1 in the context of cocaine addiction with psychiatric comorbidity, we are aware of the limitations of the present exploratory study. Firstly, the number of cases reported in our study with comorbid disorders is small and the replication is necessary. We cannot conclude whether these changes in BDNF and IGF-1 concentrations are exclusive to cocaine addiction or not because new studies in psychiatric patients with no history of drug use will be necessary to elucidate their role in mental disorders. Additional studies to determine plasma BDNF and IGF-1 in active cocaine users are necessary to confirm the lack of effects produced by the presence of cocaine on circulating concentrations of these factors. Further, longitudinal studies during cocaine abstinence could indicate whether these concentrations are unaltered or time-dependent.

The present study was conducted on outpatient subjects and, therefore we tried to show a realistic example of individuals seeking treatment in public centers for addiction but without being isolated from their social and familiar context. Nevertheless, from our results, we believe that monitoring neurotrophic factors such as BDNF and IGF-1 in cocaine users seeking treatment remains to be investigated to further improve the stratification of these patients taking into consideration the psychiatric comorbidities.

Supporting Information

S1 Fig. Correlation analyses between plasma concentrations of BDNF, IGF-1 and IGFBP-3 and variables related to addiction: length of abstinence and amount of cocaine use in abstinent cocaine users. A) BDNF (pg/mL); **B)** IGF-1 (ng/mL); and **C)** IGFBP-3 (μg/mL). Black

dots are individual values. (r) Pearson´s correlation coefficient; (rho) Spearman´s correlation coefficient; (p) p-value for statistical significance.
(TIF)

S2 Fig. Correlation analyses between plasma concentrations of BDNF, IGF-1 and IGFBP-3 and total number of DSM-IV-TR criteria for cocaine abuse and dependence in abstinent cocaine users. A) BDNF (pg/mL); **B)** IGF-1 (ng/mL); and **C)** IGFBP-3 (μg/mL). Black dots are individual values. (rho) Spearman´s correlation coefficient; (p) p-value for statistical significance.
(TIF)

Author Contributions

Conceived and designed the experiments: FRF FJP. Performed the experiments: MP NG-M PA RC-C JJR PR-S AS JS EC-O FJP VB JAC JA AIM-V MAV FRF. Analyzed the data: LJS FRF FJP. Contributed reagents/materials/analysis tools: MT RdT LJS. Wrote the paper: FJP FRF RdT MT VB MAV. Were responsible for the study concept and design: FRF FJP. Coordinated and recruited participants from Outpatient Treatment Centers in Málaga: MP NG-M PA RC-C JJR. Contributed to the acquisition of psychiatric data by means of interviews: MP PA NG-M PR-S. Obtained and processed blood samples: AS JS EC-O FJP. Supervised the benzoylecgonine detection and supervised the quantification of acyl derivatives in plasma: RdT. Supervised and performed the quantification of cytokines, chemokines and BDNF from human plasma: VB JAC JA. Supervised and performed the quantification of IGF-1 and IGFBP-3 from human plasma: AIM-V MAV. Assisted with data analysis and interpretation of findings: LJS FRF FJP. Drafted the manuscript: FRF FJP. Provided critical revision of the manuscript for important intellectual content: MT RdT LJS VB MAV. Critically reviewed content and approved final version for publication: All authors.

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Seroprevalence of Hepatitis B and C Virus in Blood Donors at a Tertiary Care Hospital, Dhanbad, Jharkhand, India

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A B S T R A C T

Blood transfusion serves a significant role in the supportive care of healthcare system. Infectious diseases remain a major topic of interest for those involved in blood safety. The purpose of this study was to estimate the prevalence of HBV and HCV among the blood collected from the donors at a hospital based blood bank in Dhanbad, Jharkhand. A total of 7721 blood samples from donors were collected. Blood samples (3ml each) were collected aseptically in to sterile dry tube. It was allowed to stand at room temperature for clotting and retraction and then centrifuge to extract serum. Detection of Hepatitis B and C virus was done using commercially available SENSA Hep-B HBsAg kit and Flaviscreen HCV kit for screening and confirmation by respective ELISA kit. The overall prevalence of HBV and HCV was 0.28% and 0.06% respectively. The HBV Seroprevalence in male donor was 0.22% and in female donors was 0.06%. HCV seroprevalence in male and female donors was found as 0.05% and 0.01% respectively. The occurrence of hepatitis B and C among the blood donors should be monitored carefully to further reduce the rates to ensure safer and more reliable blood for transfusion.

Introduction

Donation of blood involved collection, testing, preparation, and storing of blood and blood components. Blood donors are divided into groups. A voluntary blood donor is a person who donates blood voluntarily and does not receive payment, and who donates only for an internal sense of altruism, or community responsibility. A replacement donor, either a friend or family member of the recipient, is someone who donates blood to replace the blood that is used for a transfusion, to ensure a consistent supply. Transfusion plays an important role in the supportive care of medical and surgical patients. Transfusion-transmitted infectious diseases remain a major topic of interest for those involved in blood safety. Globally, the most notable transfusion-related risks are human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) due to their high prevalence rates (Marcucci *et al.*, 2004).

The risk of transmitting hepatitis through transfusions of blood and blood products has been known since 1950 (Cerny and Chisari, 1999; Mahoney, 1999).

Hepatitis B and C are transmitted parentally mainly as a result of blood to blood contact including injury with contaminated instruments and sharing of needles or by sexual contact and also through prenatal transmission from mother to child (Robbin, 2005; Mahoney, 1999). HBV and HCV are the two established causes of post transfusion hepatitis. Prevalence of transfusion-transmitted diseases is much lower in healthy voluntary blood donors as compared to professional blood donors (Kamal, 2000).

Both infections can lead to an acute or silent course of liver disease progressing from liver impairment to liver failure, cirrhosis of the liver and to hepatocellular carcinoma. Hepatitis B virus (HBV) infection is one of the most common infectious diseases in the world with significant acute and chronic morbidity and thus has become a global public health problem. The presence of HBsAg in serum indicates active HBV infection, either acute or chronic. Usually, HBsAg is the first serologic marker in acute HBV infection and is detected 2–4 weeks before the alanine aminotransferase (ALT) level becomes abnormal and 3–5 weeks before symptoms or jaundice (Mujeeb, 2000).

The World Health Organization (WHO) has estimated that more than 2 billion people in the world have been infected with HBV at some time in their lives and about 350 million people worldwide are HBV carriers with the majority in developing world mainly in Asia and Africa (Horvat *et al.,* 2003). The worldwide carrier rate of HBV is more than 350 million; these carriers provide a huge reservoir for HBV (World Health Organization, 2015).

Hepatitis C virus (HCV) continues to be a major disease burden in the world. In 1997, WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide and 3 to 4 million new infections each year (Villano *et al.,* 1999). Among the viral hepatitis, HCV is dreadful in the aspect that its morbidity rate is high as it establishes a state of chronic infection in as many as 85% of acutely infected patients, whereas about 15% of acutely infected patients spontaneously clear the infection (Dienstag and Isselbacher, 2001; Alter *et al.*,). Considering the grave consequences of these infections and to reduce the transmission, it is extremely important to monitor these viral transfusion transmissible infections. Thus we aimed to estimate the prevalence of HBV and HCV among the blood collected from the donors at a hospital based blood bank in Dhanbad, Jharkhand. This research would help to study and identify the trend of increase or decrease of HBV and HCV.

Materials and Methods

This was a retrospective and cross-sectional study conducted at Patliputra medical college and hospital, Dhanbad District of Jharkhand, India from January 2018 to August 2018. During this period 7,721 donations were done. Three millilitres of venous blood samples were taken from each blood donor into a clean dry tube. Blood samples were allowed to stand at room temperature for clotting and retraction. Thereafter, the samples were centrifuged to give a clear serum.

Detection of HBsAg was done using commercially available SENSA Hep-B HBsAg kit (Orchid Biomedical system) for the detection of HBsAg in serum. Blood units which were shown to be HBsAg positive or at border line were retested using HBsAg confirmation kit (DIA.PRO Diagnostic, Milano, Italy), a set of reagents for the confirmation of HBsAg positivity in human sera.

Detection HCV-Ab was done using the commercially available Flaviscreen HCV kit

(Qualpro diagnostic Goa, India), an enzyme immunoassay for the detection of antibodies to hepatitis C virus in human serum. Positive and borderline HCV-Ab units were confirmed using the commercially available HCV confirmation kit (DIA.PRO), an enzyme immunoassay for the confirmation of HCV Ab positivity in human sera. Data was analyzed using a Statistical Package for Social Sciences (SPSS version 16). Ethical clearance for this study was obtained from the Institutional Ethical Committee of Patliputra Medical College and Hospital.

Results and Discussion

A total of 7,721 donors were screened and among these 97.48% (7527) were males and 2.51% (194) were females. Among them, 22 donors were found seropositive for HBV giving the seroprevalence of 0.28%. The HBV Seroprevalence in male donor was 0.22% (17) and in female donors was 0.06% (5). 5 donors were found seropositive for HCV giving a seroprevalence of 0.06%. HCV seroprevalence in male and female donors was 0.05% and 0.01% respectively. The overall prevalence of HBV and HCV was 0.28% and 0.06% respectively (Table 1).

The present study shows that the prevalence of HBV infection among native of Jharkhand in the Dhanbad district is 0.28 %. The HBsAg seropositivity results observed in this study are considerably lower than those reported by Ayoola *et al.,* who observed a prevalence of 5.4 % from Jazan Region in Saudi (Villano *et al.,* 1999). This study presumed that the most important factor that is responsible for the decline in HBV infection was the introduction of the HBV vaccination under national immunisation schedule by government of India in recent time. This decline in HBV infection could also be due to the greater awareness of HBV among blood donors. The distribution of HBV worldwide shows variations depending on geographical location. In China, 1.4% of blood donors were reported to be positive for HBV (Ayoola *et al.,* 2003). In Europe, the prevalence of HBV in blood donors ranged from 0% to 5.2%, and in the United States the prevalence ranged from 0.4% to 1.0% among blood donors (Yong-lin Yang *et al.,* 2012; Van Der Poel *et al.,* 2005).

Table.1 HBV and HCV Prevalence in Dhanbad Jharkhand, India (Jan 2018- August 2018)

Sex	N (%)	HBV Positive N (%)	HCV Positive (%)
Male	7527 (97.48)	17(0.22)	4 (0.05)
Female	194 (2.51)	5 (0.06)	1(0.01)
Total	7721	22	5

In this study, the HCV seropositivity rate among the tested blood units was 0.06%. Considering the worldwide prevalence of HCV seropositivity in blood donors from the Americas was 0.07% (Van Der Poel *et al.,* 2005), and in Europe it ranged from 0.02% to 3.03% (Yong-lin Yang *et al.,* 2012).

HBV and/or HCV infection(s) among blood donors in the study area is/are reducing. The occurrence of these infections among the blood donors should still be monitored carefully to further reduce the rates to ensure safer and more reliable blood for transfusion.

Measures such as more sensitive techniques, education, sensitization and vaccination must be carried out to ensure that people are well enlightened and protected from these infections.

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"Prevalence of Helicobacter pylori infection in chronic alcoholic liver disease patients"

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Abstract
Background*:*
Helicobacter pylori (H. pylori) is a slow-growing, microaerophilic bacteria and gram-negative rod shaped bacteria which colonises the gastric mucosa and reported to be class I carcinogenic factor and also involved in various systemic i.e. vitamin B12 deficiency, iron deficiency anaemia (IDA), idiopathic thrombocytopenic purpura type ,II diabetes mellitus (DM) and Non-alcoholic fatty liver disease (NAFLD).About half of the world's population is estimated to be infected with H. pylori with greater prevalence in developing countries like India than in developed countries. H. pylori also affects the physiology of the liver and this is observed more in those who have cirrhosis. Alcohol interferes with the symbiotic complex between gut immunity and microbe complex, thus favours the growth of H. pylori in the gut. Ingestion of alcohol is found to have a positive relationship with gastric ulcers /Peptic Ulcer Disease, ultimately leading to chronic active gastritis and gastric adenocarcinoma.Hence, we conducted this study to estimate its prevalence and significance with chronic alcoholic liver disease patients.
Methods: *This cross-sectional study was conducted in Regional Institute of Medical Sciences (RIMS), Imphal, Manipur from August 2019 to September 2021.124 cases of chronic alcoholic liver disease patients above 18 years who attended Medicine /Gastroenterology OPD or admitted in the General Medicine wards were enrolled. Helicobacter Pylori was detected by either -H. pylori specific IgG antibody detection in serum /Rapid Urease Test positivity of biopsy specimen /Histopathological examination (Gram stain & Giemsa stain) of biopsy specimen. Liver function tests, viral markers, prothrombin time, complete hemogram, USG whole abdomen and other investigations as per clinical suspicion were done.*
Results: *A total of 124 diagnosed cases of chronic alcoholic liver disease were included in the study. The mean age of the study population was 55.29 ± 10.6 with the majority in age group 49-58 years (37.1%) and majority of them were males 85.5%. Dyspepsia (97.5%) was the most common presenting symptom. In the present study, the prevalence of H.pylori was found to be 63.7%*which was the same as *H. pyloriIgG positivity. But as per the Rapid urease test and histopathological examination results, the H. pylori prevalence rate in the study was 84.8%. Thus, Rapid urease test and histopathological examination reported a higher prevalence than H. pylori IgG positivity.*
Conclusion:*The prevalence of H. pylori was found to be 63.7% and had significant association with chronic alcoholic liver disease patients with more frequent consumption of large quantity of alcohol for longer duration and those belonging to Child Turcotte Pugh Class B, thereby suggesting specific anti H. pylori therapeutic interventions in CLD patients.*
Keyword: *alcohol, alcoholic liver disease, gastric ulcer, helicobacter pylori, rapid urease test*

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I. Introduction

A symbiotic relationship exists between the human host and gastrointestinal microbiome, and both co-exist together as a complex ecosystem[1] About half of the world's population is estimated to be infected with H. pylori with greater prevalence in developing countries like India than in developed countries.[2-3]Helicobacter pylori (H. pylori) is a slow-growing, microaerophilic bacteria and gram-negative rod-shaped bacteria. H. pylori colonize in the gastric mucosawhich shields them against the acidic pH of the stomach. Adhesins like

liposaccharides and outer proteins enable H. pylori attachment to the mucosal surface. These organisms make their environment favourable by producing abundant urease enzyme that hydrolyses urea to ammonia and carbon dioxide, making environment alkaline for H. pylori.Hence, diagnostic investigations for H. pylori are also done by detecting urease either by breath analysis or Rapid Urease Test (RUT) of biopsy specimen from stomach. They also produce toxins like vacuolating toxins (VacA) which cause direct mucosal injury and highly virulent cytotoxin associated gene A (CagA), which causes actin remodelling, IL-8 induction, host cell growth and apoptosis. Few studies have reported that H. pylori bacteria can have an impact on many pathological process both in the stomach and systemically[4] H. pylori induces changes in the gut microbe or due to the release of various cytotoxic substances which activate inflammatory mediators and induce autoimmunity.[5-7] such as cytokines IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17 interferon-β, and TNF-α.[8]Hence, a positive association has been discovered between H. pylori and cardiac infarction[9] , rosacea[10]and bronchiectasis.[11] The CagA positive variants are capable of producing local and systemic, humoral and cellular inflammatory response.[12]and has a causative role in the development of chronic gastritis,[13] peptic ulcer disease,[14] gastric adenocarcinoma, low-grade B-cell lymphoma of gastric mucosa-associated lymphoid tissue (MALT-lymphoma)[15], and gastric cancer.[16] This sequalae of activation of inflammatory markers and autoimmunity can bring about inflammatory reactions which can cause atrophic gastritis, metaplasia, and dysplasia, leading to the development of gastric cancer. The World Health Organisation (WHO), has reported H. pylori as oncogenic. H. pylori has been identified as a Group I carcinogen by the International Agency for Research on Cancer and currently is considered a necessary but insufficient cause of gastric adenocarcinoma.[17-18]

H. pylori also affects the physiology of the liver and this is observed more in those who have cirrhosis.[19] It was also reported that *H.pylori* eradication therapy can increase the levels of high-density lipoprotein cholesterol in the infected patients with chronic gastritis.[20] The role of H. pylori bacteria in idiopathic thrombocytopenic purpura and iron deficiency anaemia (IDA) has been shown in all age groups.[21] Current research shows that *H. pylori* may also be associated with vitamin B12 deficiency, type II diabetes mellitus (DM) and Non-alcoholic fatty liver disease (NAFLD).

Alcohol interferes with the symbiotic complex between gut immunity and microbe complex, thus favours the growth of *H. pylori* in the gut. Ingestion of alcohol is found to have a positive relationship with gastric ulcers /Peptic Ulcer Disease. Ingestion of alcohol over a longer duration of time is also associated with chronic active gastritis. The gastric ulcers and their sequelae of inflammatory events have a crucial role in decreasing the protective function of the mucosal layer of the stomach. Alcohol is degraded in the hepatic cells majorly by an enzyme named alcohol dehydrogenase (ADH). Additionally, the microsomal ethanol oxidizing system (MEOS) and catalase are also involved in the metabolism of alcohol. Similar to alcohol dehydrogenase of the liver, the mucosal layer of the stomach also produces an isoenzyme which degrades about 10% of the consumed alcohol in the stomach itself. The *H. pylori* bacteria inhibits the alcohol dehydrogenase enzyme in the stomach ,thus enabling a higher concentration of alcohol to reach the hepatocytes.[22] Through this mechanism *H. pylori* supplements further damage to hepatocytes. *H. pylori* bacteria and alcohol together can react and produce inflammatory reaction in the stomach. In the small intestine and colon, alcohol can deplete the normal bacterial flora with its anti-inflammatory activity leading to a condition called "leaky gut" where intestines are damaged.

Since *H. pylori* is reported to be involved in various systemic diseases and is also considered as class I carcinogenic factor, a study on the prevalence of *H. pylori* infection in liver disease in this part of country is necessary for the commencement of preventive and therapeutic strategies thereby resulting in this study.

II. Materials and Methods

This cross-sectional study was conducted in Regional Institute of Medical Sciences (RIMS), Imphal, Manipur from August 2019 to September 2021.Chronic alcoholic liver disease patients who attended Medicine OPD/Liver clinic or admitted in the General Medicine wards were enrolled following the criteria.

Inclusion Criteria

Patients diagnosed as chronic alcoholic according to the working definition with evidence of underlying Chronic Liver Disease (CLD) of age more than or equal to 18 years were enrolled in the study.

Exclusion Criteria include patients with History of systemic helicobacter-pylori eradication therapy within past 6 weeks, terminally ill patients and those not giving consent.

Study procedure Independent variables: Personal details including a detailed history of presenting symptoms (Dyspepsia, Fatigue, Anorexia, Malena, Hematemesis, Yellow Eyes, Abdominal Distension), past history and personal history were recorded in proper proforma along with age, sex, socio economic status, drinking pattern and smoking. A validated World Health Organization AUDIT (Alcohol Use Disorders Inventory Test) questionnaire was used to assess alcohol consumption.A complete physical examination with emphasis on the disease activity and duration of every subject was also done. Kidney function test, complete hemogram, liver

function tests, viral markers, prothrombin time and other investigations as per clinical suspicion were done.Child Turcotte Pugh Score was calculated.*Helicobacter Pylori*was detected by either -*H. pylori* specific IgG antibody detection in serum /Rapid Urease Test positivity of biopsy specimen /Histopathological examination (Gram stain & Giemsa stain) of biopsy specimen.
Chronic liver disease was defined as the presence of cirrhosis (due to chronic alcohol intake in the absence of any other etiology). Diagnosis of cirrhosis was based on clinical findings, biochemistry (low serum albumin, AST/ALT ratio >1), imaging (heterogeneous echo texture of liver with irregular outline, altered liver size depending on etiology, Portal vein > 13, Porto systemic collateral), Upper Gastrointestinal (GI) Endoscopy (showing esophageal varices) or documentation suggestive of prior decompensation. The severity of liver disease was classified as per Child–Turcotte-Pugh criteria.

Operational definitions:
Standard Alcoholic Drink: A standard alcoholic drink contains approximately 14gms of alcohol, which is equivalent to 12 ounces of beer (~5% alcohol), 8.5 ounces of malt liquor (~9% alcohol), 5 ounces of wine (~12% alcohol), 3.5 ounces of fortified wine (e.g., sherry or port), or 1.5 ounces of liquor (distilled spirits; ~40% alcohol).

Moderate Alcohol Consumption:
Men: No more than two standard alcoholic drinks/day
Women: No more than one standard alcoholic drink/day
Heavy alcohol consumption
Men: More than 14 standard alcoholic drinks/week or more than 4 standard alcoholic drinks in a day
Women: More than 7 standard alcoholic drinks/week or more than 3 standard alcoholic drinks in a day

Study tool: Hepatitis C serology was done by Flaviscreen method, Hepatitis B serology by Viruschek rapid test, HIV I & II serology done by Retrogine HIV kit and Sono Ace X S was used For Ultrasonography. Upper GI Endoscopy was done for every patient. H-Pylori specific IgG antibody detection in serum (ELISA) was done by using a commercial ELISA kit (GAP-IgG Test). Rapid Urease Test (CLO Test kit) was used for detection of *H. pylori* from Endoscopic Biopsy specimen from antrum. Histopathological examinationwere also done.

Statistical analysis: IBM SPSS Version 21.0 for Windows, Armonk NY: IBM Corp.were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.** Categorical variables were expressed in frequencies and proportion. Pie charts and bar graphs were used to depict the results. Chi square test and Fisher's exact test were used appropriately to test the significance between the *H. pylori* infection and clinico-demographic variables. A p-value of <0.05 was considered statistically significant.

Approval of Research Ethics Board and Informed consent: The study was approved by Research Ethics Board Regional Institute of Medical Sciences, Imphal.(Reference No- A/206/REB-Comm (SP)/RIMS/2015/555/33/2019).

III. Result

A total of 124 diagnosed cases of chronic alcoholic liver disease were included in the study. Baseline characteristics of the study subjects were given in table 1. The mean age of the study population was 55.29 ± 10.6 with the majority in age group 49-58 years (37.1%) and majority of them were males 85.5%.39.5% were current smokers. Dyspepsia (97.5%) was the most common presenting symptom. Majority of study participants belonged to Child Turcotte Pugh Class B (35.5%) testing positive for *H.pylori*infection than the others in this study (p=0.005) . Anemia was present in 50.8% and thrombocytopenia was detected in 75% of the study participants(p=0.002). In the present study, the prevalence of *H.pylori* was found to be 63.7%which was the same as *H. pylori* IgG positivity. But as per the Rapid urease test and histopathological examination results, the *H. pylori* prevalence rate in the study was 84.8%. Thus, Rapid urease test and histopathological examination reported a higher prevalence than *H. pylori* IgG positivity. Association of *H.pylori*infection with demographic variables were given in table 2 and with symptoms were given in table 3.Child Turcotte Pugh Score class association with *H.pylori* infection were given in table no 4. Consumption of large quantity of alcohol (10-20 drinks per week and more than 20 drinks per week), more frequently (4 or more times week), and for longer duration (16-20 years, more than 20 years) and those belonging to Child Turcotte Pugh Class B had significant association with *H.pylori* infection. However, there was no significant association between age, religion, education, occupation, marital status, socioeconomic status, place of residence, smoking status with the *H.pylori* infection.

Table1. Baseline characteristics of the study subjects.

Demographic Characteristics	Percent (%)
Age (in years)	
18-28	1.6
29-38	8.9
39-48	14.5
49-58	37.1
59-68	29.0
69-78	8.9
Gender	
Male	85.5
Female	14.5
Smoking pattern	
Current smoker	39.5
Former smoker	30.6
Non-smoker	29.8
Drinking pattern	
> 20 /week	43.5
10-20 drinks/week	35.5
<10 drinks/week	21
Duration of drinking alcohol (in years)	
5-10	
11-15	3.2
16-20	15.3
>20	18.5
	63
Types of alcohol	
Locally brewed	23.4
Beer	5.6
Hard liquor	11.2
Locally brewed + hard liquor	58
Wine	1.6
Clinical presentations	
Dyspepsia	94.4
Anorexia	68.5
Jaundice	61.3
Fatique	51.6
Abdominal distension	47.6
Hematemesis	30.6
Malena	48.4
Anemia	50.8
Thrombocytopenia	75

Table 2: Association between demographic variables and *H.pylori*infection (N=124)

Parameters	*H. pylori* Negative n (%)	*H. pylori* Positive n (%)	p-value
Age group (in years)			
18-28	2 (100%)	0 (0.0%)	
29-38	8 (72.7%)	3 (27.3%)	
39-48	5 (27.8%)	13 (72.2%)	0.099
49-58	11 (23.9%)	35 (76.1%)	
59-68	15 (41.7%)	21 (58.3%)	
69-78	4 (36.4%)	7 (63.6%)	
Gender			
Male	34 (32.1%)	72 (67.9%)	0.031
Female	11 (61.1%)	7 (38.9%)	
Smoking pattern			
Current smokers	16 (32.7%)	33 (67.3%)	
Former smokers	17 (44.7%)	21 (55.3%)	0.433
Non - smokers	12 (32.4%)	25 (67.6%)	
Drinking pattern			
2-4 times a month	11 (84.6%)	2 (15.4%)	
4 or more times /week	17 (27.9%)	44 (72.1%)	0.001
More than 20 years	23 (29.5%)	55 (70.5%)	0.003
Standard drinks/week of Alcohol			
<10 drinks/week	22 (84.6%)	4 (15.4%)	
10-20 drinks /week	8 (18.2%)	36 (81.8%)	<0.001

>20 drinks /week	15 (27.8%)	39 (72.2%)	

Table 3: Association between various symptoms and *H. pylori* infection(N=124)

Symptoms		***H. pylori* Negative n (%)**	***H. pylori* Positive n (%)**	**p-value**
Dyspepsia	Yes	40 (34.2%)	77 (65.8%)	0.033
	No	5 (71.4%)	2 (28.6%)	
Fatigue	Yes	15 (23.4%)	49 (76.6%)	0.002
	No	30 (50.0%)	30 (50.0%)	
Anorexia	Yes	25 (29.4%)	60 (70.6%)	0.019
	No	20 (51.3%)	19 (48.7%)	
Malena	Yes	19 (31.7%)	41 (68.3%)	0.300
	No	26 (40.6%)	38 (59.4%)	
Hematemesis	Yes	17 (44.7%)	21 (55.3%)	0.194
	No	28 (32.6%)	58 (67.4%)	
Yellow Eyes	Yes	25 (32.9%)	51 (67.1%)	0.322
	No	20 (41.7%)	28 (58.3%)	
Abdominal Distension	Yes	23 (39.0%)	36 (61.0%)	0.552
	No	22 (33.8%)	43 (66.2%)	
Anemia	Yes	23 (36.5%)	40 (63.5%)	0.959
	No	22 (36.1%)	39 (63.9%)	
Thrombocytopenia	Yes	37 (39.8%)	56 (60.2%)	0.161
	No	8 (25.8%)	23 (74.2%)	

Table 4: Child Turcotte Pugh Score class and *H.pylori* infection (N=124)

Child Turcotte Pugh Score	***H. pylori* Negative n (%)**	***H. pylori* Positive n (%)**	**p-value**
Class A	17 (41.5%)	24 (58.5%)	0.005
Class B	8 (18.2%)	36 (81.8%)	
Class C	20 (51.3%)	19 (48.7%)	

IV. Discussion

Excessive alcohol consumption is a global healthcare problem with enormous social, economic, clinical consequences and damages nearly every organ in the body. However, the liver sustains the earliest and the greatest degree of tissue injury from excessive drinking because it is the primary site of ethanol metabolism.[23]

In the present study, the prevalence of *H.pylori* was found to be 63.7%, which was almost similar to the finding of Kim DJ et al[24](62.4%) ,Kirchner GI et al[25](61%)and Barbuti RC et al[26](60-70%). Most of the other studies reported a lower prevalence of *H. pylori* than the present study.Feng H et al[27]conducted the study on liver cirrhosis patients and reported a *H.pylori* prevalence of 52.26% and Gopinath JM et al[28]53.6%. Among our patients with gastroduodenal erosions, the prevalence of *H.pylori* was lower at around 47% in accordance with the study conducted by Kirchner GI et al[25]. Other studies conducted by Brenner H, et al[29] also showed that the prevalence of *H. pylori* infection was 39.2%. But Manes G et al[30]reported 38% *H.pylori*prevalence among patients with chronic pancreatitis and 28% among asymptomatic patients. Zhang L et al[31]reported *H.pylori*prevalence 27.3% in those with functional dyspepsia and Kim DJ et al[24]73.7% among patients with peptic ulcer disease. The wide variation in prevalence rates between the present study and the abovementioned studies maybe because, patient profiles chosen for each of these studies were suffering from different diseases.

As per the seropositivity, the *H.pylori* prevalence rate in the present study was 63.7%, same as that of overall prevalence. But Schulz C et al[32]and Pogorzelska J et al[33]reported a prevalence of *H.pylori* of only 21 % and 46.9%, respectively by serological tests. As per the Rapid Urease Test results, the *H.pylori* prevalence rate in the present study was 84.8% , which was higher than that by the antibody detection methodand was similar to study done by Saikumar C[34] (82.6%).In the study by Nardone G et al[35]*H. pylori* infection was diagnosed by a positive concordance of both quick urease test and histology, and it was seen that the prevalence was 41.85%.Schmulson MJ et al[36] reported that 50% of alcoholics and 42.9% of non-alcoholic cirrhotics were *H. pylori* positive by the Rapid Urease Test method.

As per the Histopathological examination results, the *H.pylori* prevalence rate in the present study was 84.8%, identical to that of Rapid Urease Test which were similarly shown by Saikumar C[34](82.6%). But Farinati F et al[37]stated that hypertensive gastropathy might not represent a favourable environment for growth of *Helicobacter pylori*, thus making the biopsy's diagnostic sensitivity lower than expected. In accordance with other studies that used the Histopathological examination method for *H.pylori*prevalence conducted by Elsebaey MA et al[38] and Auroux J et al[39]reported much lower values of 59.2% and 58% respectively. Saikumar C[34]registered a much lower *H. pylori* prevalence of 23.9% on culture.

The present study did not show any association between age and *H.pylori* infection which is in accordance withZhang L et al[31] and Schmulson MJ et al[36]studies.A significantly higher proportion of the males tested positive for *H.pylori* infection than the females in the present study (p=0.031), similar to study by Saikumar C[34]. This difference might have been due to the difference in demographic profile and alcohol

consumption patterns in the study population. Gender disparity in *H. pylori* infection is an intriguing topic, as gastric adenocarcinoma (the most serious complication of *H. pylori* infection) shows significant male predominance.

There was no association between education or occupation ,socioeconomic status and marital status with the *H.pylori*infection in the present study which is consistent with study bySyam AF et al[40]. But Lawlor DA et al[41]found that*H. pylori* infection is associated with childhood poverty. Similarly, Mitchell H et al[42]also found that higher prevalence was observed in people of lower socioeconomic status. The difference could be due to the study setting and various cultural practices followed in different geographical areas.

Regarding the clinical presentation, majority reported dyspepsia followed by thrombocytopenia, anorexia, fatigue and anaemia.But in the report of Tongtawee T et al[43]abdominal pain, followed by iron deficiency anemia, was the most common clinical presentation. Ortiz M et al[21]also showed thatthere was a significant association between *H. pylori* infection and anaemia. The present study showed that, among *H.pylori* positive patients, significantly higher proportion presented with dyspepsia (p=0.033), fatigue (p=0.002), anorexia (p=0.019) and moderate thrombocytopenia (p=0.002).

In the present study, a significantly higher proportion of the patients who consumed alcohol frequently for a duration of 4 or more times a week (p<0.001) , larger quantities i.e 10-20 standard drinks per week (p<0.001) ,more alcohol content (locally brewed alcohol and a combination of locally brewed & hard liquor) (p=0.005 & p 0.016 respectively) and those with drinking pattern of 2-3 times per week (p=0.001) and those who used for more than 20 years (p=0.002) had a higher prevalence of *H.pylori* infection, which is consistent with studies conducted byZhang L et al**[31]**,Lieber CS et al[44]and Ogihara A et al[45].These finding strengthen the hypothesis that alcohol exerts damaging effect on gastric mucosal barrier and decrease the local immunity which favours the growth of H.pylori , thus making those individuals at risk for infection. But in contrast to the findings of the present study Brenner H et al[29]andSaikumar C[34]showed findings to support the hypothesis that moderate alcohol consumption may facilitate the spontaneous elimination of *H. pylori* infection and the prevalence of *H. pylori* infection was higher in the non-alcoholics. There was no association between smoking and the *H.pylori* infection in the present study which is similar to Zhang L et al[31]study.But OgiharaA et al[45]in his study found thatsmoking was negatively associated with *H. pylori* infection and that the risk of *H. pylori* seropositivity decreased linearly with cigarette consumption per day. This was attributed to the increased acidity in the stomach through smoking.

In the present study,majority belonged toclass B of Child-Turcotte Pugh score, followed by class A and class C which were statistically significant (p = 0.005). Though in the study by Schmulson MJ et al[36] majority had class A,Kim DJ et al[24]had class C, they weresignificantly associated with *H. pylori* infection.

In the present study a statistically significant association for *H.pylori*infection was found with gender, frequency, duration, quantity and type of alcohol consumption.

V. Conclusion

The study concluded that the overall *H.pylori* prevalence rate in the study population was 63.7%. as compared to 84.8% by rapid urease test and histopathological examination. *H.pylori* had significant association with chronic alcoholic liver diseasewith more frequent consumption of large quantity of alcohol for longer duration and those belonging to Child Turcotte Pugh Class B.These findings necessitate preventive and therapeutic strategies *for H. pylori* eradication in CLD patients.

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EXTERNAL EVALUATIONS

Flaviscreen®HCV

Rapid test for detection of antibodies to HCV

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New or more precise requirements in Common Technical Specifications (CTS) for In Vitro Diagnostic Medical Devices (2009/886/EC)

Genotype 1-4: > 20 samples per genotype (including non-a subtypes of genotype 4);
5: > 5 samples;
6: if available

From the report of May 30, 2007 following genotypes were tested.

Genotype	Number of Specimen
1	224
2	43
3	85
4	53
5	13

Performance evaluation of screening assays shall include 25 positive "same day" fresh serum and/or plasma samples

The evaluation done at Virology laboratory of Saint Antoine Hospital, Paris show that all 25 fresh positive serum samples showed positive result.

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