Int J Pharma Res Health Sci. 2017; 5 (5): 1841-45
DOI:10.21276/ijprhs.2017.05.04
Deepak NG *et al*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

A Correlative Study of Interleukin-12 and Cortisol in HIV Patients with Depression

Deepak Nilsoge Guruswamy [1, *], Rajeshwari Kenchappanavar [2], SN Mothi [3]

[1]Research scholar , Karnatak University, Dharwad, India.
[2]Head of department,department of psychology Karnataka Arts College, Karnataka University, Dharwad, India.
[3]Chairman, Asha Kiran Hospital, Mysore, India.

ARTICLE INFO

Received: 31 Aug 2017
Accepted: 02 Oct 2017

ABSTRACT

Recent studies on psychological stress have extensively discussed the interaction of the psychological sequel with the human immune system. Prolonged exposure to stress may hamper the accessibility to coping resources, subsequently leading to depression in people. Depression, a major affective disorder, affects the quality of life of patients with HIV and HIV progression; the primary effector of stress response is the hypothalamic–pituitary–adrenal axis, which has been assessed on the basis of cortisol levels. Cortisol reduced levels of interleukin (IL)-12, a major proinflammatory cytokine and promoter of cell-mediated immunity, in rats. Elucidating the correlation between cortisol and IL-12 cytokine in HIV patients with and without depression may facilitate the understanding of the association of psychological depression with the immune system of patients with HIV. So we enrolled 160 patients; among these, 80 patients each comprised the pre-antiretroviral therapy and Antiretroviral therapy. After obtaining patient consent, the patients were administered the Becks depression scale; based on the scoring of this scale, categorized into the non depressed and depressed group. Furthermore, 5 ml of blood was drawn from all patients for measuring their cortisol and IL-12 levels by using spectrophotometric analysis. Statistical analysis revealed that HIV patients with depression have decreased human plasma IL-12 with increased cortisol levels compared with those without depression.

Key words: Depression, cortisol, IL-12.

1. INTRODUCTION

Interleukin (IL)-12 is an important pro-inflammatory cytokine and promoter of cell-mediated immunity. IL-12 promotes type 1 T-helper (TH1) cell differentiation by stimulating natural killer (NK) and CD4 T cells to produce interferon (IFN)-γ, by stimulating the proliferation of undifferentiated T cells (Kubin et al., 1994; Trinchieri, 2003) [1, 2], and by supporting the promotion and activation of preactivated TH1 cells (Perussia et al., 1992) [3]. Moreover,

Corresponding author *
Deepak Nilsoge Guruswamy
Research Scholar ,Karnatak University, Dharwad
Email ID: deepakng24@gmail.com

IL-12 coordinates between innate and adaptive immunities by directly stimulating leukocytes, including NK, T, and B cells, in both systems and by stimulating NK cells in innate immunity (Trinchieri, 2003; Yoo et al., 2002) [4, 5] to enhance the cytotoxicity and proliferation of cytotoxic T lymphocytes (CTLs) and B cells (Curtsinger et al., 2003; Metzger et al., 1997) [6, 7]. Thus, IL-12 contributes to a low viral load in acute HIV-1 infection (Roberts et al., 2010) [8], which is a global pandemic affecting 36.7 million persons. Until 2015, 2.1 million new cases of HIV were reported, and 1.1 million persons died of AIDS-related illness (UN AIDS Fact Sheet). HIV population reported many common mental health problems; among which, depressionconsiderably affected the quality of life; and associated with HIV disease progression and mortality (Starace et al., 1999) [9]. Depression can occur because of the lack of coping resources owing to prolonged exposure to stressors, which may downregulate the immune system and alter the hypothalamic–pituitary–adrenal axis. These factors are hypothesised mechanisms underlying the immune changes in depression (Miranda, 1999) [10]. The HPA axis responds to physical and mental challenges to maintain homeostasis in part by controlling the body's cortisol levels. Meta- analytic and enumerative reviews have shown that chronic stress can lead to fewer circulating B and T cells and large granular lymphocytes, decreased proliferative responses of lymphocytes to several mitogens, and decreased NK cell activity (NKCA) (Herbert & Cohen, 1993: Kiecolt-Glaser et al., 1992, 1995) [11, 12]. Glucocorticoids suppress the synthesis of proinflammatory cytokines (Agarwal &Marshall, 1998) [13] may be because cortisol appears to reduce IL-12 production in rats (Shaashua et al., 2012) [14]. The present study mainly determined the correlation between cortisol and IL-12 in HIV patients with depression. The preliminary finding of this study may facilitate evaluating other immunological factors affected by cortisol in the aforementioned population.

2. METHODS

In this randomised, single-arm, cross-sectional study, approval was obtained from the Human Ethics Committee of the Asha Kiran Hospital, Mysore, Karnataka. Before enrolment, consent was obtained from seropositive patients; they were subsequently administered the Becks depression scale. On the basis of the scores on this scale, the patients were categorised into the depress and nondepressed groups. Furthermore, from all patients, 5 mL of blood was drawn into an ethylenediamine tetraacetic acid (EDTA) tube, which was further subjected to centrifugation and plasma separation. By using the spectrophotometric enzyme-linked immunosorbent assay (ELISA) method, cortisol and human IL-12 levels were quantified. The study procedure was explained to the patients before obtaining their consent. We analysed the statisticalcorrelation between IL-12, plasma cortisol, in depressed and non depressed patients with HIV.

2.1 Becks depression scale

This is a self-administered scale with 21 items. After test completion, each question can be scored according to the number on the right of the question. A highest score of 63 can be obtained in this test, indicating that number three was marked for all questions. The lowest score in this test can be zero, indicating that zero was marked for all questions.

2.2 Spectrophotometric ELISA

Five millilitres of blood was drawn in an EDTA tube between 4 to 4:30 pm and immediately centrifuged at 3,800 rpm for 10 min. Plasma was stored at −20°C until thawing for assay. The plasma levels of IL-12 and cortisol were measured using the ELISA commercial kit, Calbiotech for cortisol (10461 Austin Dr, spring valley, CA, 91978) and Diaclone Human IL-12 p70 (25020 besancon cedex, France). The sensitivity of the plasma cortisol and IL-12 kits are 1.5 and 2.2 pg/mL, respectively, according to the manufacturers' instructions. The optical density of each well was measured using a microplate reader (Varioskan Flash Multimode Reader Version 4.00.53) set at 450 nm. Duplicate readings were averaged for each standard and sample and normalised by subtracting the average zero standard optical density. For the standard curve, the data points were linearised by plotting the log of the IL-12 level versus that of the optical density; the most desirable fit line was determined using regression analysis. The IL-12 and cortisol levels of each sample were determined by reading the level from the standard curve.

3. RESULTS

The patients in the pre-antiretroviral therapy (ART) had a mean age of 36.25 years, and 47.5% and 52.5% of the patients were women and men, respectively. According to Becks classification, 66.25% of the patients had depression. Linear correlation analysis revealed negative correlations between cortisol and IL-12 levels in the depressed (r^2= −0.46, p= *.000*) and nondepressed (r^2 = −0.09)groups. This observation revealed that increasedcortisol had more negative effects on IL-12 levels in HIV patients with depression than in those without depression. For the pre-ART group, the *t* testrevealed a significance of correlation of 3.699 (observed value) and 1.673(critical value). These values prove the hypothesis of a significant negative correlation between IL-12 cytokine and cortisol levels in patients with depression. To understand the association of depression with IL-12 and cortisol levels, the *t* test analysis was conducted, which revealed an observed *t* value of 5.092 and a critical *t* value of 1.991 for the cortisol with depressed (Mean= 204.04) and non depressed (Mean = 118.75) group of pre-ART patients with HIV. These findings indicate that depression is a major variable in cortisol level variation. The IL-12 levels of the aforementioned patient groups (depressed, M=5.46, nondepressed, M=5.79)yielded *t* values of −0.372 (observed value) and 1.991 (critical value), indicating that depression doesnot have a direct significance

with IL-12 levelvariation. Thus, it can be hypothesised that depression plays a major role in cortisol level variation in Pre ART patients with HIV.

Patients who were are on Antiretroviraltherapyhad a mean age of 37.91 years; 55% and 45% of these patients were men and women, respectively. Linear correlation analysis revealed a negative correlation between cortisol and IL-12 levels in the depressed (r^2 = −0.458,p=.*001*)and nondepressed (r^2= −0.015,p=.*392*) groups, indicating that increased cortisol had negative effects on IL-12 levels in HIV patients with depression than in those without depression. The *t* testrevealeda significance of correlation of 3.45 (observed value) and 1.673 (critical value).This finding proved the hypothesis that the depressed group has a significant negative correlation between IL-12 and cortisol levels. To understand the association of depression with IL-12 and cortisol levels, *t* test analysis was conducted for the ART group patients with and without depression. The test yielded *t*values of 5.445 (observed value) and 1.991 (critical value),between depressed (Mean = 209.95) and nondepressed group(Mean=120.94) of ART patients with HIVindicatingthat depression is a major variable in cortisol level variation.Moreover, IL-12 levels of the aforementioned patient groups (depressed, M= 4.67 and nondepressed, M= 7.70)yielded *t* valuesof−4.270 (observed value) and 1.991 (critical value). No statistical significance of IL-12 levels was observedin the pre-ART group; however, the statistical significance in the ART group may be because of the highly active ART(HAART)administered to this group.Only a cause and effect relation research can prove this hypothesis. Thus, it can be hypothesised that depression plays a major role in cortisol and IL-12 level variations, and HAART appears to improve IL-12 levels in the ART group.

4. DISCUSSION

This study is the first to report the association between cortisol and IL-12 levels in HIV patients with depression. In particular, the data reveals a negative correlation of plasma cortisol with IL-12 in the ART and pre-ART patients of HIV with depression. Depression is a comorbidityin the HIV population (Strance, 1999); [15] therefore, the present study facilitates further understanding of the association of IL-12 with cortisol in HIV patients with depression. A study on women with HIV measured 30 cytokines. Among those,the concentrations of five plasma cytokines, IL-12 p70, IL-12 p40, IFN-γ,IL-7, and IL-15, in womenwith acute infection as predicted 66% (adjusted R^2 = 0.6577) of the variation in viral load set point of median 415cells/µl after 12 months of post infection. IL-12 p70 and p40 as well as IFN-γ were associated with a low viral load,and IL-12 p40 and granulocyte-macrophage colony-stimulating factor (GM–CSF) were associated with a prolonged maintenance of CD4 counts exceeding 350 cells/uL (Roberts et al., 2010).The production of this above cytokines is partly regulated by a positive feedback loop, with IFN-γ and GM-CSF promoting IL-12 p70 production,which subsequently stimulates IFN-γand GM-CSF secretion (Flesch et al., 1995; Gazzinelli et al., 1993; Kubin,1994; Tripp et al., 1993).[16-19] These studies have reported the association of IL-12 p70 with HIV prognosis. In concordance with our study,an animal study analysed different types of stressful behaviours in ratsthat significantly reduced IL-12 levels. All stress paradigms and hormones used in this study resulted in reduced IL-12 levels, namely wet-cage paradigms; social confrontation; surgery;and administration of prostaglandin E2, epinephrine, and corticosterone. These findings suggest the effects of stress on this crucial TH1 cytokine (Shaashua et al., 2012) [20]. Our study is the first to report the relationship of cortisol with IL-12 p70 in HIV patients with depressionand to demonstratethe association of immune factor in thispopulation. However, an invivo studyreported the failed production of IL-12p 70 in response to CD40 ligand stimulation in dendritic cells of patients with HIV (Anna et al., 2004). [21] A depression study showed the association of depression with Natural killer lymphocytes, and this study suggeststhat depression reduces NKCA in women with HIV (Dwight et al., 2002). [22] A meta-analytic review and enumerative study reported an association between reduced NKCA and depression (Bauer et al., 1995) [23]. IL-12 plays a vital role in the activities of NK cells and T lymphocytes and mediates the enhancement of the cytotoxic activity of NK cells and CD8 CTLs (https://en.wikipedia.org/wiki/Interleukin_12). Thus, present study showsa probable correlation, suggesting negative effects of cortisol on IL-12, mainly in the depressed group compared with the nondepressed group of patients with HIV. However, studies on the cause and effect relationship will further substantiate the association of cortisol with the pathophysiology of depression in the decreased production of IL12 in patients with HIV, supporting our study on animal modules that chronic stress has detrimental effects on the immune system, mainly on the gene expression of IL-12 p40 (Saul et al., 2005) [24].

Table 1: Cortisol and IL-12 levels in pre-ART and ART patients classified into different groupsaccording to the Becks depression scale

DEPRESSION	Pre ART						ART					
	n	%	Cortisol		IL12		n	%	cortisol		IL12	
			Mean	SD	Mean	SD			Mean	SD	Mean	SD
Normal	19	23.75 %	129.5	40.52	4.94	3.35	24	30%	120.06	42.3	7.22	4.36
Mild mood disturbance	8	10%	125.7	21.3	4.23	2.06	7	8.75%	123.93	15.12	9.36	3.65
Borderline clinical Depression	8	10%	156.85	41.84	4.77	4.21	12	15%	168.34	57.19	5.54	1.32
Moderate Depression	10	12.5%	190	39.86	3.81	4.26	15	18.75 %	197	61.99	4.51	1.80

Severe Depression	24	30%	190.79	83.82	6.07	3.54	22	27.5%	240.82	102.5	4.35	2.43
Extreme Depression	11	13.75 %	280.05	101.02	3.08	2.25						

Total Score_____Levels of Depression
1-10_____Normal
11-16_____ Mild mood disturbance
17-20_____Borderline clinical depression
21-30_____Moderate depression
31-40_____Severe depression
over 40_____Extreme depression

Table 2: *t*Test analysis of cortisol/IL12 levels in ART and Pre ART patients with and without depression

t test analysis	Pre ART		ART	
	Cortisol	IL12	Cortisol	IL12
Differences	85.297	−0.329	89.010	−3.032
t(observed value)	5.092	−0.372	5.445	−4.270
|t| (Critical Value)	1.991	1.991	1.991	1.991
DF	78	78	78	78
p Value (Two-Tailed)	< 0.0001	0.711	<0.0001	<0.0001
Alpha	0.05	0.05	0.05	0.05

Table 3: Pearson correlationmatrix of IL-12 and cortisol in pre-ART patients

Variables	IL12	Cortisol
IL12	1	−0.465
Cortisol	−0.465	1

Table 4: Pearson correlation matrix of IL-12 and cortisol in ART patients

Variables	IL12	Cortisol
IL12	1	−0.458
Cortisol	−0.458	1

5. ACKNOWLEDGEMENTS

The authors would acknowledge the help of the Mr Sunil, Mr Swamy from the GASYM organization, Dr Swamy, Dr srirama, Dr Sudheer, from the Asha Kiran Hospital Mysore for the data collection. DR Hilda R, Lobo from the St Mary's Hospital HD Kote, Mysore for the moral support.

6. REFERENCES

1. Agarwal SK, Marshall GD Jr. Glucocorticoid-induced type 1/type 2 cytokine alterations in humans: a model for stress- related immune dysfunction. J Interferon Cyto
2. kine Res 1998; 18: 1059–68.
3. Anna Smed-Sörensen, Karin Loré, Lilian Walther-Jallow, Jan Andersson and Anna-Lena Spetz HIV-1-infected dendritic cells up-regulate cell surface markers but fail to produce IL-12 p70 in response to CD40 ligand stimulation BLOOD 2004 104: 2810-2817;
4. Bauer, M.E., Gauer, G.J., Luz, C., Silveira, R.O., Nardi, N.B., von Muhlen, C.A., 1995. Evaluation of immune parameters in depressed patients. Life Sciences 57, 6655674.
5. Curtsinger JM, Lins DC, Mescher MF. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. J Exp Med. 2003; 197(9):1141–1151.
6. Dwight L. Evans, M.D,Thomas R. Ten Have, Ph.D., M.P.H,Steven D. Douglas, M.D,David R,Gettes, B.S,Mary Morrison, M.D,Margaret S. Chiappini, R.N., M.N., C.C.R.C,Priscilla Brinker-Spence, B.S,Carla Job, M.S.N., C.R.N.P,Delinda E. Mercer, Ph.D,Yan Lin Wang, M.S,Dean Cruess, Ph.D,Benoit Dube, M.D,Erik A. Dalen, B.A,Tiffany Brown, B.A,Russell Bauer, Ph.D,John M. Petitto, M.D, Association of Depression With Viral Load, CD8 T Lymphocytes, and Natural Killer Cells in Women With HIV Infection ,Am J Psychiatry 2002; 159:1752–1759;
7. Flesch IE, Hess JH, Huang S, Aguet M, Rothe J, Bluethmann H, et al. Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon γ and tumor necrosis factor α. J Exp Med 1995;181:1615–1621.
8. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A. Interleukin-12 is required for the Tlymphocyte independent induction of interferon-γ by an intracellular parasite and induces resistance in T-deficient hosts. Proc Natl Acad Sci USA 1993;90:6115–6119.
9. Herbert, T.B., Cohen, S., 1993. Stress and immunity in hu- mans: a meta-analytic review. Psychological Bulletin 55, 364-379.
10. Kiecolt-Glaser, J.K., Glaser, R., 1995. Psychoneuroim-munology and health consequences: data and shared mech- anisms. Psychosomatic Medicine 57, 269-274.
11. Kiecolt-Glaser, J.K., Cacioppo, K.T., Malarkey, W.B., Glaser, R., 1992. Acute psychological stressors and short-term im- mune changes: what, why, for whom, and to what extent? (Editorial comment). Psychosomatic Medicine 54, 680-685.
12. Kubin M, Kamoun M, Trinchieri G. Interleukin 12 synergizes with B7/CD28 interaction in inducing efficient proliferation and cytokine production of human T cells. J Exp Med. 1994; 180(1):211– 222.
13. Metzger DW, McNutt RM, Collins JT, Buchanan JM, Van Cleave VH, Dunnick WA. Interleukin-12 acts as an adjuvant for humoral immunity through interferon-gamma-dependent and -independent mechanisms. Eur J Immunol. 1997; 27(8):1958–1965.
14. Miranda Olff "Stress, depression and immunity: the role of defense and coping styles"Psychiatry Research Volume 85, Issue 1, 18 January 1999, Pages 7–15
15. Perussia B, Chan SH, D'Andrea A, Tsuji K, Santoli D, Pospisil M, Young D, Wolf SF, TrinchieriG. Natural killer (NK) cell stimulatory factor or IL-12 has

differential effects on the proliferation of TCR-alpha beta+, TCR-gamma delta+ T lymphocytes, and NK cells. J Immunol. 1992;149(11): 3495–3502.
16. ROBERTS lindi , Jo-Ann S. PASSMORE, Carolyn WILLIAMSON, Francesca LITTLE, Lisa M. BEBELL, Koleka MLISANA, Wendy A. BURGERS, Francois VAN LOGGERENBERG, Gerhard WALZL, Joel F. DJOBA SIAWAYA, Quarraisha ABDOOL KARIM, and Salim S. ABDOOL KARIM Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression,AIDS . 2010 March 27; 24(6): 819–831.
17. Saul AN, Oberyszyn TM, Daugherty C, Kusewitt D, Jones S, Jewell S, Malarkey WB, Lehman A, Lemeshow S, Dhabhar FS. Chronic stress and susceptibility to skin cancer. J Natl Cancer Inst.2005; 97(23):1760–1767.
18. L. Shaashua, L. Sominsky, B. Levi1, L. Sorski, M. Reznick, G. Page, and S. Ben-Eliyahu1 In vivo suppression of plasma IL-12 levels by acute and chronic stress paradigms: potential mediating mechanisms and sex differences Brain Behav Immun . 2012; 26(6): 996–1005.
19. Starace F, Ammassari A, Trotta MP, Murri R, De Longis P, Izzo C, Scalzini A, d'Arminio Monforte A, Wu AW, Antinori A; AdICoNA Study Group. NeuroICoNA Study Group Depression is a risk factor for suboptimal adherence to highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2002 Dec 15;31 Suppl 3:S136-9.
20. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol. 2003; 3(2):133–146.
21. Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 1993;90:3725–3729.
22. Yoo JK, Cho JH, Lee SW, Sung YC. IL-12 provides proliferation and survival signals to murine CD4+ T cells through phosphatidylinositol 3-kinase/Akt signaling pathway. J Immunol. 2002; 169(7): 3637–3643.
23. http://www.unaids.org/en/resources/fact-sheet
24. https://en.wikipedia.org/wiki/Interleukin_12

Conflict of Interest: None

Source of Funding: Nil