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Research Article

A COMPARATIVE STUDY ON THE ASSESSMENT OF RISKY LIPID PROFILES AMONG TWO RETROVIRAL THERAPEUTIC STRATEGIES

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ABSTRACT

Presently there are a number of different anti-retroviral therapeutic strategies and regimens available, which are classified, based on the retroviral life-cycle stage the drug impacts on. Two among them for the present interest are Non-Nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors. The chosen drugs of the above said category are nevirapine and lopinavir/ritonavir. Present study is designed to assess the risk of hosting the ailment related to abnormal lipid profiles associated with the use of protease inhibitor regimen lopinavir/ritonavir and non-nucleoside reverse-transcriptase inhibitor nevirapine. Our results, particularly in terms of an increased risk of abnormal lipid profiles in patients receiving lopinavir/ritonavir and a reduced risk in patients receiving NNRTIs, particularly nevirapine, therefore, may give the NNRTI regimens an advantage over current PI-containing regimens. The outcomes of the present work conclude the point that treatment methods of HIV infected patients should be rationalized to reduce side effects particularly in the aspect of abnormal lipid profiles during treatment to reduce the risk of dyslipidemia and associated disorders. The work particularly says that nevirapine is safe to use in comparison with lopinavir/ritonavir combination.

Keywords: Retrovirus, HIV, Lopinavir, Ritonavir, Nevirapine and AIDS.

INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes the acquired immunodeficiency syndrome (AIDS). Dr. Suniti Solmon in 1986 diagnosed first HIV case amongst female sex workers in Chennai. It became more than 135 cases by 1987 and among these number of cases progressed to AIDS was 14. By 1990 prevalence in high-risk groups reached above 5%. In the period of 2008-2009 the number of HIV case in India raised by 0.125 million as per UNDP's 2010 report. Adult prevalence also rose from 0.29% in 2008 to 0.31% in 2009. NACO reported that India is the third largest HIV epidemic in the world. As per the UNAIDS reports 2013 had the death toll of 130,000 people, who died from AIDS related illness and HIV prevalence in India was estimated as 0.3 percent. The four states with the highest numbers of people living with HIV are Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu and account for 53 percent of all HIV infections.

Presently there are a number of different anti-retroviral therapeutic strategies and regimens available, which are classified, based on the retroviral life-cycle stage the drug impacts on. They are briefly Entry inhibitors or fusion inhibitors, Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI), Non-Nucleoside reverse transcriptase inhibitors (NNRTI), Integrase inhibitors and Protease inhibitors.

Regulation of proteolytic enzyme activity is crucial for major physiological processes and the disturbance of the equilibrium between an enzyme and its substrates would lead to dire consequences. In this prospective, the discovery of small-molecule ligands, like protease inhibitors, that can modulate catalytic activities would have promising therapeutic effect[1]. Hence, therapeutic strategy that would intervene HIV protease activity has been one of the most important approaches in HIV infection[2] and their development is in fact as major success in the field of structure-based drug design[3]. By virtue of their high effectiveness against HIV[4] they have been a key component of anti-retroviral therapies for HIV/AIDS since the 1990s[5].

Lopinavir is one among the protease inhibitors and was marketed in 2000[6]. It was originally designed to diminish the interactions between inhibitor and HIV-1 protease's Val82, which is frequently mutated in the drug resistant strains of the virus[7]. It is a peptidomimetic HIV protease inhibition[8] and its core is identical to that of ritonavir. In the place of 5-thiazolyl end group in ritonavir, lopinavir has phenoxyacetyl group. The other difference is that 2-isopropylthiazolyl group in ritonavir was replaced by a modified valine in which the amino terminal had a six-membered cyclic urea attached[7].

Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) are other kind of antiretroviral drugs, which inhibits reverse transcriptase enzyme that controls the replication of the genetic material of HIV and used in the treatment of human immunodeficiency virus (HIV). Their discovery and development of NNRTIs began in the late 1980s[9] and in the end of 2009 four NNRTI had been approved by regulatory authorities out of which nevirapine is one. NNRTIs are highly efficient in inhibition of reverse transcriptase (RT). RT is one of the most popular targets in the field of antiretroviral drug development[10].

Drug resistance develops quickly if NNRTIs are administered as monotherapy and therefore NNRTIs are always given as part of combination therapy, the highly active antiretroviral therapy (HAART)[11].

The reason for coronary heart disease (CHD) is multifaceted[12]. Among these factors, levels of high-density lipoprotein cholesterol (HDL-c) and high levels of low-density lipoprotein cholesterol (LDL-c)[13] have been identified as risk factors for coronary heart disease in the general population. Research reports have announced that decreased HDL-c and LDL-c levels are experienced by HIV-infected patients followed by an increase in plasma triglyceride (TG) levels, before the infection develops in to AIDS[14]. The treatment of HIV infection with protease inhibitors (PIs) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs) is also associated with several metabolic disorders[15], including dyslipidemia, which may result in an increased risk of CHD[16]. Increased TG, total cholesterol (TC), and LDL-c levels are associated with PI-containing combination antiretroviral therapy (CART)[17]. These associated metabolic disturbances differ according to different drugs within the PI class itself[18]. In contrast to what is observed with PI-containing CART, regimens including NNRTI increase HDL-c levels. However they also induce increases in TC and LDL-c levels[19]. More recently, some studies have suggested that nucleoside reverse-transcriptase inhibitors (NRTIs) may also contribute to the development of dyslipidemia[20].

In view of this, present study aimed at assessing the risk of hosting the ailment related to abnormal lipid profiles associated with the use of protease inhibitor regimen lopinavir/ritonavir and non-nucleoside reverse-transcriptase inhibitor nevirapine. The objectives of this work include comparing the levels of plasma HDL, LDL and triglycerides in patients undergoing antiretroviral therapy with the lopinavir/ritonavir and nevirapine in a local ART center and assessing their priority.

MATERIALS AND METHODS

Patient groups

A total of 200 HIV-infected individuals visiting ART center in Govt. general hospital, Vijayawada were informed about the study where the sample size of each drug group was set as 100 (n =100). Each of the test groups were treated with each drug i.e. lopinavir/ritonavir and nevirapine at doses recommended by the manufacturers. All the subjects of lopinavir/ritonavir and nevirapine groups were diagnosed with advanced and progressive disease (CD4 cell count M<350 cells/mm^3). A written consent was received from all the participants. None were on lipid-lowering therapy and each subject was questioned for cardiovascular risk factors as well as personal and family history of diabetes, lipid disorders, or cardiovascular diseases, alcohol and drug consumption and confirmed their fitness for the present study. After a limited physical examination venous blood was obtained after a 10-hour fast.

As all the subjects are patients undergoing treatment in the ART center their plasma lipid profiles are recorded with the ART center before they started treatment. These plasma samples had been stored at −80°C for an average of 531±22 days (mean ± SEM) and had never been thawed before.

Laboratory Methods

Triglyceride analysis

We used ENSURE biotech triglyceride detection kit (TRIGLYCERIDES LS) and its principle is that triglycerides present in the serum are catabolized in to glycerol and free fatty acids by the action of lipoprotein lipase. Liberated glycerol is converted in to glycerol-3-phosphate in the presence of glycerol kinase and ATP. Glycerol-3-phosphate is acted upon by Glycerol-3-phosphate oxidase to form hydrogen peroxide. This along with phenolic compound gives pink colour, which can be measured at 505nm.

Low density lipoprotein analysis

We used ENSURE biotech LDL detection kit (LDL CHOLESTEROL) and its principle is that the LDL cholesterol upon serial enzymatic reactions form a quinone dye, which can be detected at 585nm.

High density lipoprotein analysis

We used ENSURE biotech HDL detection kit (HDL CHOLESTEROL) and its principle is that the HDL cholesterol upon serial enzymatic reactions involving the enzyme cholesterol esterase form a quinone dye, which can be detected at 585nm.

Statistical Analysis

Normal values of triglycerides, LDL and HDL are <180 mgs/dl, 100-190 mgs/dl and 30-60 mgs/dl respectively. As HIV infected patients are generally burdened with abnormal lipid profile, where triglyceride levels increase and LDL and HDL levels decrease. The present statistical analysis was designed to compare the mean lipid profile values by independent t-test for each of triglycerides, HLD and LDL. Values are expressed as mean $\pm$ standard deviation (SD). The confidence interval was set at 95%. P-value < 0.05 was considered to indicate statistical significance. Statistical analysis was performed using Minitab15 for windows 8.0; hence all the results presented would be in Minitab15 output format.

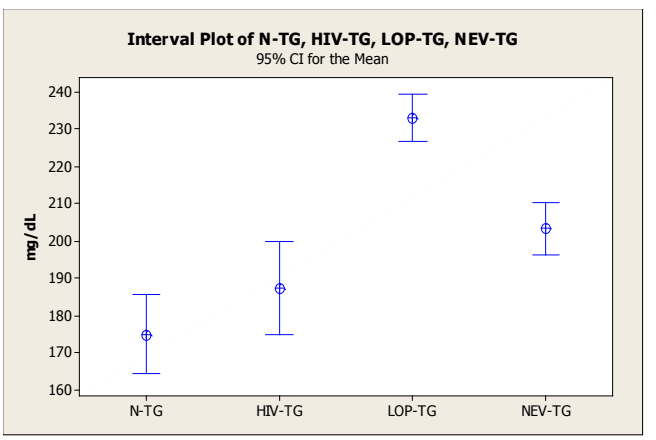

Figure 1: Interval plot of levels of triglycerides in control (N-TG), positive control (HIV-TG), lopinavir/ritonavir treated HIV patients (LOP-TG) and nevirapine treated HIV patients (NEV-TG).

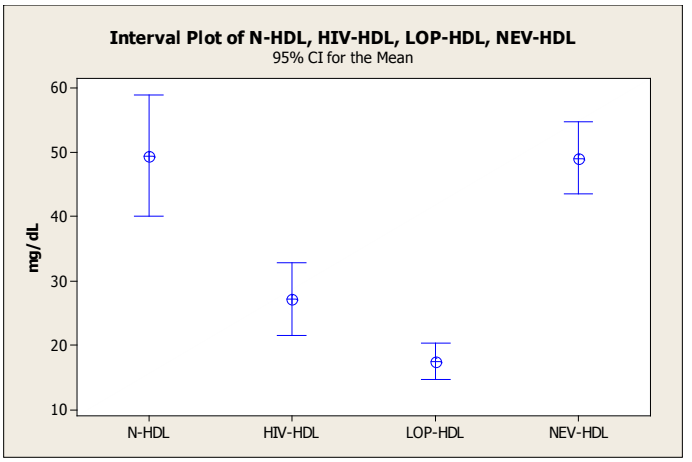

Figure 2: Interval plot of levels of HDL in control (N-HDL), positive control (HIV- HDL), lopinavir/ritonavir treated HIV patients (LOP- HDL) and nevirapine treated HIV patients (NEV- HDL).

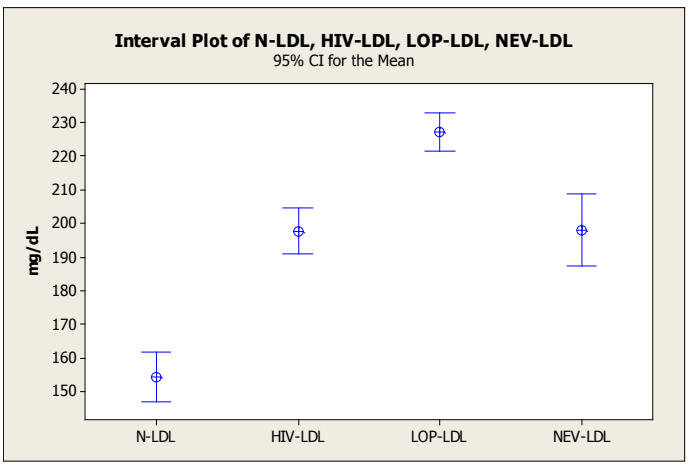

Figure 3: Interval plot of levels of LDL in control (N- LDL), positive control (HIV- LDL), lopinavir/ritonavir treated HIV patients (LOP- LDL) and nevirapine treated HIV patients (NEV- LDL).

RESULTS

Figure 1 infers that the level of plasma triglycerides (TG) had a correlation with the HIV infection and the treatment regimen. There is a little or insignificant variation in TG levels between HIV negative and HIV positive individuals. Comparatively high triglyceride levels recorded with the use of lopinavir/ritonavir based treatment and among the two treatment regimens nevirapine use manifests the healthy TG levels.

Figure 2 infers that the level of plasma HDL had a correlation with HIV infection and the treatment regimen. There is a significant variation in HDL levels between HIV negative and HIV positive individuals and the lowest HDL levels correlated with the use of lopinavir/ritonavir. Comparatively healthy HDL levels recorded with HIV negative patients and the patients who used nevirapine based treatment. Among the two treatment regimens nevirapine use manifests the healthy HDL levels.

Figure 3 infers that the level of plasma LDL had a correlation with HIV infection and the treatment regimen. There is a significant variation in LDL levels between HIV negative and HIV positive individuals. Comparatively high LDL levels recorded with the use of lopinavir/ritonavir based treatment and nevirapine had negligible impact on LDL levels. Hence among the two treatment regimens nevirapine use manifests the healthy TG levels.

Two-Sample T-Test and CI: NEV-HDL, LOP-HDL

	N	Mean	StDev	SE Mean
NEV-HDL	100	49.00	4.47	2.0
LOP-HDL	100	17.60	2.30	1.0

Difference = mu (NEV-HDL) - mu (LOP-HDL)
Estimate for difference: 31.40
95% lower bound for difference: 26.87
T-Test of difference = 0 (vs >): T-Value = 13.96 P-Value = 0.000017 DF = 5

Two-Sample T-Test and CI: NEV-LDL, LOP-LDL

	N	Mean	StDev	SE Mean
NEV-LDL	100	198.20	8.64	3.9
LOP-LDL	100	227.40	4.56	2.0

Difference = mu (NEV-LDL) - mu (LOP-LDL)
Estimate for difference: -29.20
95% lower bound for difference: -37.69

T-Test of difference = 0 (vs >): T-Value = -6.68 P-Value = 0.000273 DF = 6

Two-Sample T-Test and CI: NEV-TG, LOP-TG

	N	Mean	StDev	SE Mean
NEV-TG	100	203.40	5.59	2.5
LOP-TG	100	233.00	5.00	2.2

Difference = mu (NEV-TG) - mu (LOP-TG)
Estimate for difference: -29.60
95% lower bound for difference: -35.96
T-Test of difference = 0 (vs >): T-Value = -8.82 P-Value = 0.999 DF = 7

The above shown Minitab produced independent t-test results show that there is a significant difference in the impact of two treatment regimens, on the levels of plasma HDL and LDL as their p-values< 0.05 indicate the significant difference. The difference in the levels of triglycerides is insignificant between the two treatment regimens as the p-value is > 0.05.

Table 1: Physical parameters of the 100 HIV positive, 100 HIV negative (control), lopinavir/ritonavir treated HIV patient (LOP/RIT) and nevirapine treated HIV patient (NEVIR) groups. The values are represented in the form of Mean ± SD (Standard deviation)

Parameter	HIV Positive	HIV Negetive	LOP/RIT Treated	Never Treated
Triglycerides (TG)	187.40 ± 0.09	175.00 ± 8.60	233.00 ± 5.00	203.40 ± 5.59
HDL	27.20 ± 4.60	49.40 ± 7.54	17.60 ± 2.30	49.00 ± 4.47
LDL	197.80 ± 5.50	154.20 ± 5.93	198.20 ± 8.64	198.20 ± 8.64

DISCUSSION

Lipid profiling as its name says is the group of methods to profile the status of the lipid metabolism. Lipid metabolism is importantly concerned in the health perspective as abnormalities in this warns us about the Micro-vascular and macro-vascular complications and many other ailments. Lipid profile tends to be abnormal in disease and infection conditions. Patients with HIV infection were reported to have hypercholesterolemia with or without hypertriglyceridemia however the mechanism of decrease in cholesterol levels is not known. Rasheed et al. present the first direct evidence that HIV replication alone in human T-cells, without any influence of antiviral drugs or other factors, can stimulate the production of novel cellular enzymes and proteins that enhance fatty acid synthesis, increase the quantity of low density lipoproteins, secrete triglycerides, alter the lipid transport and metabolism, and oxidize lipids[21].

In the present study our aim to research the abnormal lipid profiles in using two different HIV treatment regimens, specifically lopinavir/ritonavir combination, which are protease inhibitors (PI) and nevirapine, which is a non-nucleoside reverse transcriptase inhibitor (NNRTI). Our specific aim is to find which among these two is safe. We also checked the abnormal lipid profiles associated with the HIV infection alone and our present results are in accordance with the reports of Mondy K et al who observed low HDL and elevated TG in their study population in the US[22]. Similar other works were also performed where van der Valk et al. compared patients randomly assigned to a first line regimen of stavudine and didanosine, together with IDV, NVP, or lamivudine (3TC)[19]. After 24 weeks of treatment, patients receiving NVP had significantly higher increases in HDL-c levels, compared with other patients. Although the TC and LDL-c levels also increased, the TC: HDL-c ratio was significantly reduced and was lowest in patients receiving NVP. Virgili et al. reported similar differential lipid changes in antiretroviral-naive patients receiving a combination of zidovudine plus 3TC, together with either NLF or NVP[23].

Antiretroviral treatments are associated with widely described abnormal changes in the lipid profile in people with HIV infection[24, 25]. Although more frequent during treatment with protease inhibitors (PI)[26, 27] these changes are also observed during treatment with stavudine and to a lesser extent with non-nucleoside reverse-transcriptase inhibitors (NNRTI)[28].

The most lipid-friendly NNRTI drugs are abacavir, tenofovir, nevirapine, atazanavir, and most recently raltegravir and maraviroc. Unfortunately abacavir and maraviroc are currently charged with some suspicion of cardiac toxicity[17]. Although regimens including an NNRTI may induce increases in TC and LDL-c levels, they may also induce a concurrent increase in HDL-c levels, in contrast to what is observed with PI-containing CART[19].

Our results, particularly in terms of an increased risk of abnormal lipid profiles in patients receiving lopinavir/ritonavir and a reduced risk in patients receiving NNRTIs, particularly nevirapine, therefore, may give the NNRTI regimens an advantage over current PI-containing regimens, particularly in patients with preexisting known risk factors for CHD. However the present results need to be confirmed with further refined studies.

CONCLUSION

The outcomes of the present work conclude the point that treatment methods of HIV infected patients should be rationalized to reduce side effects particularly in the aspect of abnormal lipid profiles during treatment to reduce the risk of dyslipidemia and associated disorders. The work particularly says that nevirapine is safe to use in comparison with lopinavir/ritonavir combination.

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