



Journal of
AIDS and HIV Research

Volume 6 Number 5 June, 2014

ISSN 2141-2359



*Academic
Journals*

ABOUT JAHR

The Journal of AIDS and HIV Research (JAHR) is published monthly (one volume per year) by Academic Journals.

Journal of AIDS and HIV Research (JAHR) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject like the implications for gender-based HIV and AIDS prevention interventions, Sputum cellularity in pulmonary tuberculosis, Comparative tolerability and efficacy of stavudine 30 mg versus stavudine 40 mg in patients on combination antiretroviral therapy, HIV and sexual risk behaviours amongst intravenous drug users etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JAHR are peerreviewed

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jahr@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of AIDS and HIV Research will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Prof. Bechan Sharma,
*Department of Biochemistry,
University of Allahabad,
Allahabad,
India.*

Dr. John E. Lewis,
*University of Miami,
Miller School of Medicine,
1120 NW 14th Street
Suite #1474 (D21)
Miami, FL 33136
USA.*

Prof. Ruta Dubakiene,
*Vilnius University,
Lithuania.*

Prof. William Nuhu Ogala,
*Ahmadu Bello University Teaching Hospital,
Zaria, Nigeria.*

Editorial Board

Dr. Arun Kumar,
*Manipal College of Medical Sciences,
India.*

Dr. Manal Fouad Ismail,
*Faculty of Pharmacy,
Cairo University,
Egypt.*

Dr. Eshrat Gharaei Gathabad,
*Mazandaran University of Medical Sciences, Sari
Faculty of Pharmacy,
Iran.*

Dr. P. Aparanji,
*Department of Biochemistry,
Andhra University Visakhapatnam,
India.*

Dr. Amzad Hossain,
*Atomic Energy Centre,
GPO Box 164, Ramna,
Dhaka-1000,
Bangladesh.*

Prof. Irvin Mpofo,
*University of Namibia,
Namibia.*

Dr. Rajiv Nehra,
*Muzaffarnagar Medical College,
India.*

Dr. Marion W. Mutugi,
*Jomo Kenyatta University of Agriculture and Technology,
Kenya.*

Dr. Emmanuel Nwabueze Aguwa,
*Department of Community Medicine,
College of Medicine,
University of Nigeria,
Enugu Campus,
Nigeria.*

Dr. William A. Zule,
*RTI International,
USA.*

Dr. M. Abhilash,
*The Oxford College Of Engineering,
Bommanahalli, Hosur Road, Bangalore 560068,
India.*

Dr. Fukai Bao,
*Kunming Medical University,
China.*

Dr. Baligh Ramzi Yehia,
*University of Pennsylvania School of Medicine,
Philadelphia, PA,
USA.*

Dr. Khandokar Mohammad Istiak,
*University of Dhaka,
Dhaka-1000,
Bangladesh.*

Dr. Aamir Shahzad,
*Max F. Perutz Laboratories,
University of Vienna,
Vienna Bio center, A-1030 Vienna,
Austria.*

Dr. Subarna Ganguli,
*Pharmacy college in Kolkata ,
West Bengal,
India.*

Dr. Mehmet Kale,
*Dept. of Virology,
Mehmet Akif Ersoy University,
Faculty of Veterinary Medicine,
Turkey.*

Mr. Shakeel Ahmed Ibne Mahmood
*Bangladesh AIDS Prevention Society, BAPS, Bangladesh
Youth Wing, National AIDS Committee,
Bangladesh.*

Dr. Adewumi, Moses Olubusuyi,
*Department of Virology,
College of Medicine,
University College Hospital,
University of Ibadan,
Ibadan,
Nigeria.*

Dr. Theodoros Eleftheriadis,
*General Hospital of Serres,
Serres,
Greece.*

Dr. Keertan Dheda,
*University of Cape Town,
South Africa.*

ARTICLES

Research Articles

Risk for developing tuberculosis among intravenous drug users with human immunodeficiency virus (HIV) infection 104

Nimesh Poudyal, Narayan Gyawali, Hari Prasad Nepal, Rajendra Gurung, Sabitri Pandey, Ritu Amatya, Suraj Timilsina and Shyamal K. Bhattacharya

Evaluation of urine specimen as an alternative to blood for malaria diagnosis among HIV positive individuals attending HIV clinics in Abakaliki, Ebonyi State, South-Eastern Nigeria 109

Nwuzo, A. C., Iroha I. R., Okonkwo, E. C., Ilang, D. C., Agah, V. M., Afiukwa, F. N., Uhuo, A. C. and Ogbu, O.

Full Length Research paper

Risk for developing tuberculosis among intravenous drug users with human immunodeficiency virus (HIV) infection

Nimesh Poudyal¹, Narayan Gyawali^{1,2*}, Hari Prasad Nepal^{1,3}, Rajendra Gurung¹, Sabitri Pandey⁴, Ritu Amatya^{1,5}, Suraj Timilsina⁵ and Shyamal K. Bhattacharya¹

¹Department of Microbiology, BP Koirala Institute of Health Sciences, Dharan, Nepal.

²School of Medical & Applied Sciences, Central Queensland University, Rockhampton, Australia.

³Department of Microbiology, Chitwan Medical College Teaching Hospital, Bharatpur, Nepal.

⁴Department of Operation Theatre, Grande International Hospital, Kathmandu, Nepal.

⁵Department of Microbiology, Nepal Medical College, Kathmandu, Nepal.

Received 3 August 2012; Accepted 16 June 2014

Approximately one third of people living with human immunodeficiency virus (HIV) infection are co-infected with tuberculosis (TB), and TB accounts for up to a third of deaths from AIDS worldwide. Injectable drug use is an important factor in epidemiology of both TB and HIV. This study was carried out to assess the risk of intravenous drug use for developing TB in HIV infected patients. A cross-sectional study was conducted from January, 2009 to December, 2011. Equal numbers of HIV seropositive patients with and without history of intravenous drug use (IVDU) attending Department of Microbiology, BP Koirala Institute of Health Sciences for CD4 cells counting were enrolled to study the prevalence of pulmonary tuberculosis. Three early morning sputum specimens on consecutive days were collected from all participants and processed for microscopy (Auramine-O and Ziehl-Neelsen staining) and culture on Lowenstein-Jensen (L-J) media. Observation was noted. Of a total of 336 subjects enrolled in our study, group I and group II comprised HIV patients with IVDU (n = 168) and without IVDU (n = 168), respectively. In group I, *Mycobacterium tuberculosis* was detected in 40 (23.8%) patients. There was no statistical association between duration of intravenous drug use and finding of *M. tuberculosis* positivity (p > 0.05). In group II, *M. tuberculosis* was detected in 32 (19%) patients. Univariate analysis revealed that TB in HIV seropositive patients appeared with higher frequency in intravenous drug users (odds ratio (ORa) = 1.3; CI 95%, 0.786 to 2.242) than the non-users. Average CD4 cells count among HIV positive patients in group I and II was 248.6 and 292.7, respectively; with 47 patients (27.9%) in group I and 40 patients (23.8%) in group II having counts below 200. Rate of TB is high in intravenous drug user populations infected with HIV.

Key words: HIV seropositive, intravenous drug users, pulmonary tuberculosis.

INTRODUCTION

Tuberculosis (TB) and human immunodeficiency virus (HIV) infection both remain a very serious public health

problem. Despite the fact that TB is a preventable, treatable and curable disease, it is a major infectious disease

with high prevalence. One of the reasons behind the failure to control TB burden is the impact of HIV on TB. World Health Organization (WHO) has estimated 9.27 million incident cases of TB where 1.37 million (14.8%) were HIV- seropositive (Guelar et al., 1993). Persons co-infected with HIV and *Mycobacterium tuberculosis* are at much greater risk of developing active TB than HIV-seronegative individuals (Guelar et al., 1993). Synergistic action of HIV and TB is defined in such a way that HIV infection weakens the immune system of a person and tubercle bacilli can grow more easily resulting in active TB disease in persons with recently acquired or latent TB infection (WHO Regional Strategic Plan on HIV/TB SEA/TB/261. SEA/AIDS/140, 2003). The life time risk of developing TB in HIV negative is 5.0 to 10.0% but the risk may increase up to 60% in HIV positives (Dhungana et al., 2008).

A metaphor is apt: this is a time when oil (HIV) is poured into the fire (TB-infected persons) in Asia (Vermund and Yamamoto, 2007). The synergy of HIV and TB takes several forms: *M. tuberculosis* infection increases viral load; immunosuppression from advancing HIV disease increases the risk of tuberculosis, new as well as re-infection (quiescent *M. tuberculosis* will reactivate) (Selwyn et al., 1989). Preventive therapy with isoniazid (INH) can be initiated if co-infected individuals are identified as soon as possible.

Although Center for Disease Control and Prevention (CDC) has reported high rates of TB among HIV-seropositive intravenous drug users (IVDUs) (Centers for Disease Control and Prevention, 2005), clear differentiation has not been defined among IVDUs and non-IVDUs with HIV seropositivity. Therefore, we aimed to determine the prevalence of people infected with tuberculosis (PTB) among HIV seropositive IVDUs attending BP Koirala Institute of Health Sciences, Dharan, Nepal.

MATERIALS AND METHODS

A cross-sectional study was conducted during three year period (January, 2009 to December, 2011). Equal numbers of HIV seropositive patients with and without history of IVU attending Department of Microbiology, BPKIHS for CD4 cells counting were enrolled to study the prevalence of PTB. The nature and purpose of the study were explained to all the participants and they were assured of their confidentiality and anonymity. Written consent was obtained from all the participants. The participants were provided standard sputum containers and instructed to collect early morning sputum specimens on three consecutive days. The specimens were transported to laboratory and processed for microscopy [using Auramine-O fluorescent staining for screening of acid fast bacilli,

and Ziehl-Neelsen (Z-N) staining for their confirmation] and culture (World Health Organization, 1998). At least 100 oil immersion fields were on Lowenstein-Jensen (L-J) media. Acid fast bacilli (AFB) detected on the smear were graded according to WHO guideline (World Health Organization, 1998). About 300 OIF were examined to declare a slide negative. Growth on L-J media was identified.

Research ethics

Ethical clearance for conducting the study was obtained from the Institutional Review Committee of BP Koirala of Institute of Health Sciences, Dharan, Nepal.

Statistical analysis

The data obtained from questionnaire and CD4 cells counting were entered in Microsoft Excel and analyzed using SPSS version 16.0 software. Frequency of demographic variables was calculated. Association of CD4 cells count and *M. tuberculosis* positivity was determined using chi-square test.

RESULTS

Of a total of 336 HIV positive patients divided into two groups in our study, group I comprised of patients with IVU (n = 168) and group II without IVU (n = 168). Gender wise, group I included 159 males and 9 females while there were 121 males and 47 females in group II. Mean age of the subjects in these groups were 29.1 and 39.3 years, respectively. Frequency of demographic variables is presented in Table 1. In Group I, *M. tuberculosis* was detected in 40 patients (23.8%). Acid fast staining demonstrated AFB positivity in the smear from only 37 patients, while culture revealed positivity even in additional 3 patients.

There was no statistical association between duration of intravenous drug use and finding of *M. tuberculosis* positivity ($p > 0.05$) as shown in Table 2. Group II exhibited positivity of *M. tuberculosis* in 32 patients. Both acid fast staining and culture revealed the presence of the bacilli with equal sensitivity. Univariate analysis revealed that tuberculosis in HIV seropositive patients appeared with higher frequency in IVDUs (ORa = 1.3; CI 95%, 0.786 to 2.242) than non-IVDUs. Average CD4 cells count among HIV positive patients in Group I and II was 248.6 and 292.7, respectively; with 47 patients (27.9%) in group I and 40 patients (23.8%) in group II having counts below 200. Table 3 shows positivity of *M. tuberculosis* among HIV patients with (group I) and without (group II) intravenous drug use and their CD4⁺ cell counts.

*Corresponding author. E-mail: n.gyawali@cqu.edu.au. Tel: 61749306966.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Table 1. Demographic variables.

Categories	Variables	Group I	Group II
Age	20-30	67	41
	30-40	82	65
	>40	19	62
Occupation	Unemployed	74	84
	Social worker	25	18
	Business	23	37
	Employed	44	29
Education	Illiterate	-	-
	Literate	5	12
	Primary	41	29
	Secondary	93	71
	Higher secondary	29	56

Table 2. Duration of intravenous drug use and finding of *M. tuberculosis* positivity.

Duration (years)	<i>M. tuberculosis</i>		P value
	Positive	Negative	
< 5	13	39	0.932
5-10	19	60	
>10	8	29	

DISCUSSION

Early diagnosis of TB in HIV seropositive people is itself an important achievement, since active TB in HIV people is AIDS defining disease and should receive antiretroviral (ARV) therapy independent of CD4 cells counts. A number of epidemiological factors associate with development and progression of TB among HIV infected people. Moreover, drug users present a challenge among these infected people. In this study, we compared the prevalence of PTB among drug-user and non-user HIV positive people. We found 23.8 and 19% of PTB prevalence among HIV seropositive IVDUs and non-IVDUs, respectively. Evidence suggests that about 30% of HIV infected people are estimated worldwide to have simultaneous infection with *M. tuberculosis* (Getahun et al., 2010). However, a range of figure (14 to 46%) can be seen in different studies conducted in different settings (Corbett et al., 2003; World Health Organization, 2009). The findings of this study is in contrast with the lower rates reported in earlier publications from Nepal (Dhungana et al., 2008; (Sharma et al., 2010; Ghimire et al., 2004). The prevalence seemed to be fairly high (23%)

in the capital's (Kathmandu, Nepal) tertiary care center (Dhungana et al., 2008), whereas low prevalence (10.8%) had been reported from the cities with low population outside the capital (Ghimire et al., 2004). High prevalence in our study corresponds to our selection of sample population. Most of the participants were from HIV/AIDS living care homes which may provide the suitable environment for household contact of TB. High occurrence of TB in a setting of HIV prevalence has been reported from various parts of the world: South Africa (17.3%) (Lawn et al., 2011), Cambodia, Thailand, and Vietnam (15%) (Cain et al., 2010) and China (22.9%) (Yu et al., 2009). A Meta analysis (Gao et al., 2010) from china has shown a range of prevalence of TB: 0.5% (Cao et al., 2006) to 35% (Wang et al., 2007) among HIV positive people.

Our finding strongly indicated higher prevalence of PTB among HIV positive IVDUs as compared to HIV positive non-IVDUs. Effects of drug use in human physiology and varieties of risk factors (such as cluster of vulnerable people to acquire infection; smoking and alcohol use; and poor nutrition) may be responsible for high prevalence of TB among drug users. Significant relation of alcohol and smoking with the prevalence of TB has already been reported from the same place where this study was conducted (Gyawali et al., 2013). Deleterious effect of drug use on the immune system has been shown by *in vitro* studies (Friedman et al., 2003; Wei et al., 2003).

In our study, majority of participants in both groups were young (20 to 40 years), unemployed and have completed only secondary level of education. Drug use and intensity of HIV and TB were not observed to go parallel with higher education and employment status. We observed no significant relation ($P > 0.05$) between duration of drug use and finding of TB. However, few studies have reported their positive relations (Brassard et al., 2004; Grimes et al., 2007; Glynn JR., 1998). With the already discussed results, it can be summarized that HIV-induced immune-suppression along with the physiological effect of drug use and the associated risk factors play important role for the high prevalence of TB among IVDUs rather than the single effect of drug use.

Many studies have highlighted that HIV infection increases the risk of tuberculosis by approximately 7-folds, though this may vary with the stage of the HIV epidemic, the prevalence of TB, and the age groups considered (Glynn JR., 1998). And, dually infected individuals develop TB at a rate of 5 to 10% per year (Deiss et al., 2009). These conditions may be due to HIV-TB co-infection with degraded humoral and cell mediated immune responses.

Although higher proportion of TB was observed in group I patients than the group II patients with respect to all three categories of CD4+ cell counts (< 200, 200 to 400 and > 400), significant association was not proved (P

Table 3. Positivity of *M. tuberculosis* among HIV patients with (group I) and without (group II) intravenous drug use and their CD4⁺ cell counts.

CD4 cell count/ μ l of blood	Group	<i>M. tuberculosis</i>		P value
		Positive	Negative	
<200	Group I	16	31	NS
	Group II	11	29	
200-400	Group I	18	43	NS
	Group II	16	47	
>400	Group I	06	54	NS
	Group II	05	60	

> 0.05). However, the higher prevalence of TB in group I than group II patients in the present study alarms the risk of drug use for development of TB among HIV people. Hence, it demands longitudinal studies to be conducted in the future covering large population setting. Furthermore, drug abuse may increase the chances of cross-infection and it may also become important treatment barrier because of the escaping behaviours of the abusers from social network. Hence the barriers, including poor adherence and limited access to care, pose unique challenges for their treatment. It is a proved fact that treatment failure is the primary risk factor for the development of drug resistance. Thus increased attention should be paid to such group while making policies to control TB and HIV infection.

Conclusion

Drug abuse remains a risk for HIV people to develop TB. Adults of 20 to 40 years age are at risk for both illicit drug use and HIV infection among them. Rate of tuberculosis is high in such drug users infected with HIV. So, to strengthen the health policy to control HIV infection and TB, IDVUs should be screened for both of these diseases.

Conflict of interest

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the laboratory staff of the Department of Microbiology, BP Koirala Institute of Health Sciences, Dharan, Nepal for provision of technical service.

REFERENCES

- Brassard P, Bruneau J, Schwartzman K, Senecal M, Menzies D (2004). Yield of tuberculin screening among injection drug users. *Int. J. Tuberc. Lung Dis.* 8:988-993.
- Cain KP, McCarthy KD, Heilig CM, Monkongdee P, Tasaneeyapan T, Kanara N, Kimerling ME, Chheng P, Thai S, Sar B, Phanuphak N, Nguyen HD, Hoang TQ, Le HT, Varma JK (2010). An algorithm for tuberculosis screening and diagnosis in people with HIV. *N Engl J Med.* 362(8):707-716.
- Cao G, Zhi Y (2006). An analysis of the screening results of tuberculosis in patients with HIV/AIDS. *Disease Surveillance* 21:400-402.
- Centers for Disease Control and Prevention (2005). Screening HIV infected persons for tuberculosis-Cambodia, January 2004–February 2005. *MMWR* 54:1177-1180
- Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C (2003). The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* 163(9):1009-1021.
- Deiss RG, Rodwell TC, Garfein RS (2009). Tuberculosis and Illicit Drug Use: Review and Update. *Clin. Infect. Dis.* 48(1):72-82.
- Dhungana GP, Ghimire P, Sharma S, Rijal BP (2008). Tuberculosis co-infection in HIV infected persons of Kathmandu. *Nepal Med. Coll. J.* 10(2):96-9.
- Friedman H, Newton C, Klein TW (2003). Microbial infections, immunomodulation, and drugs of abuse. *Clin. Microbiol. Rev.* 16(2):209-19.
- Gao L, Zhou F, Li X, Jin Q (2010). HIV/TB Co-Infection in Mainland China: A Meta-Analysis. *PLoS One* 5(5):e10736.
- Getahun H, Gunneberg C, Granich R, Nunn P (2010). HIV infection-associated tuberculosis: the epidemiology and the response. *Clin. Infect. Dis.* 50(Suppl 3):201-207.
- Ghimire P, Dhungana GR, Bam DS, Rijal BP (2004). Tuberculosis and HIV co-infection status in United Mission Hospital, Tansen, Western Nepal. *SAARC J. Tuberc. Lung Dis. HIV/AIDS.* 1:32-37.
- Glynn JR (1998). Resurgence of tuberculosis and the impact of HIV infection. *Br. Med. Bull.* 54(3):579-93.
- Grimes CZ, Hwang LY, Williams ML, Austin CM, Graviss EA (2007). Tuberculosis infection in drug users: interferon-gamma release assay performance. *Int. J. Tuberc. Lung Dis.* 11(11):1183-9.
- Guelar A, Gatell JM, Verdejo J, Podzamczar D, Lozano L, Anzar E, Miro JM, mallolas J, Zamora L, Gonzalez J (1993). A prospective study of the risk of tuberculosis among HIV infected patients. *AIDS* 7(10):1345-1349.
- Gyawali N, Gurung R, Poudyal N, Amatya R, Shrestha R, Khanal LK, Timilsina S, Bhattacharya SK (2013). Tobacco and alcohol: the relation to pulmonary tuberculosis in household contacts. *Nepal Med.*

- Coll. J. 15(2):125-128.
- Lawn SD, Brooks SV, Kranzer K, Nicol MP, Whitelaw A, Vogt M, Bekker LG, Wood R (2011). Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the expert MTB/RIF Assay: A prospective Study. *PLoS Med.* 8(7):e1001067
- Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, Walker AT, Friedland GH (1989). A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N. Engl. J. Med.* 320(9):545-50.
- Sharma S, Dhungana GP, Pokhrel BM, Rijal BP (2010). Opportunistic infections in relation to CD4 level among HIV seropositive patients from central Nepal. *Nepal Med. Coll. J.* 12(1):1-4.
- Vermund SH, Yamamoto N (2007). Co-infection with human immunodeficiency virus and tuberculosis in Asia. *Tuberculosis (Edinb)* (87 Suppl)1:18-25.
- Wang X, Liu F, Dong B (2007). Study on the detection rate of double infection among patients with AIDS and TB and its influencing factors. *Mod. Prev. Med.* 34:4457-4459.
- Wei G, Moss J, Yuan CS (2003). Opioid-induced immunosuppression: is it centrally mediated or peripherally mediated? *Biochem Pharmacol.* 65(11):1761-1766.
- WHO Regional Strategic Plan on HIV/TB SEA/TB/261. SEA/AIDS/140 (2003). WHO regional office for South East Asia, October 2003.
- World Health Organization (WHO) (2009). Global tuberculosis control: surveillance, planning, financing. WHO report 2009, Geneva.
- World Health Organization (WHO) (1998). Laboratory services in tuberculosis control. WHO/TB98.258. Geneva.
- Yu L, Wang L, Tang Z, Huang S, Meng Z (2009). The diagnosis and investigation of active tuberculosis HIV/AIDS patients. *Chin. J. Lab. Med.* 32:377-382.

Full Length Research Paper

Evaluation of urine specimen as an alternative to blood for malaria diagnosis among HIV positive individuals attending HIV clinics in Abakaliki, Ebonyi State, South-Eastern Nigeria

Nwuzo, A. C.¹, Iroha I. R.¹, Okonkwo, E. C.¹, Ilang, D. C.², Agah, V. M.¹, Afiukwa, F. N.¹, Uhwo, A. C.^{3*} and Ogbu, O.¹

¹Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, PMB 053 Abakaliki, Ebonyi State, Nigeria.

²Department of Biological Sciences, Faculty of Science and Technology, Federal University Ndufu Aliko Ikwo, Ebonyi State, Nigeria.

³Department of Applied Biology, Ebonyi State University, Abakaliki Ebonyi State, Nigeria.

Received 5 April, 2014; Accepted 16 June, 2014

A total of 150 blood and urine samples each were collected from human immunodeficiency virus (HIV) positive patients who visited selected hospitals in Ebonyi State. The subjects were made up of 57 males and 93 female patients. The blood samples were screened for the presence of four human malaria parasites using parasitological examination of blood stained films and polymerase chain reaction (PCR). Out of the 150 urine samples from the HIV positive individuals, 88 urine specimens were identified to harbor *Plasmodium* species. 75 (50%) urine specimens were identified to harbor *Plasmodium falciparum* while 10 (6.67%) and 3 (2%) were recorded against *Plasmodium malariae* and dual infection of *P. falciparum* and *P. malariae*, respectively. The result of the comparison of the specimens used showed the same result. None of the isolates that were negative by PCR test using DNA primers (template) from blood gave positive results by urine samples. Furthermore, when the primers (rOVA₁ and rOVA₂; rV₁V₁ and rV₁V₂) specific for *Plasmodium ovale* and *Plasmodium vivax*, respectively were used, none of the two species mentioned were detected in both urine and blood samples, signifying that these species may be absent in our environment. Our study demonstrated highly, the presence of *P. falciparum* and *P. malariae*, especially when specific *Plasmodium* species DNA markers were used for the analysis.

Key words: Urine, *Plasmodium* species, human immunodeficiency virus (HIV), polymerase chain reaction (PCR), microscopy.

INTRODUCTION

Malaria is caused by protozoan parasites of the genus *Plasmodium* (phylum Apicomplexa). In humans, malaria is caused by mosquito born *Plasmodium falciparum*,

Plasmodium malariae, *Plasmodium ovale* and *Plasmodium vivax*. However, *P. falciparum* is the most important cause of the disease and is responsible for about

80% of the reported cases and 90% of deaths (Mendis et al., 2001). Malaria causes about 400 to 900 million cases of fever and approximately one to three million deaths annually (Bremam, 2001). A vast majority of cases occur in children under the age of 5 years (Greenwood et al., 2005) and more so, pregnant women are particularly vulnerable.

Malaria is a major public health problem in Nigeria where it accounts for more cases and death than any other country in the World. Malaria is a risk for 97% of Nigerian population. The remaining 3% of the population live in the malaria free highlands. There are estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215, 000 deaths per year in Nigeria from human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDs). Malaria contributes an estimated 11% of maternal mortality.

Malaria accounts for 60% outpatient visit and 30% of hospitalization among children under five years of age in Nigeria. Malaria has the greatest prevalence, close to 50% in children age 6 to 59 months in the South-West, North-Central and North-West region. Malaria has the least prevalence 27.67% in children age 6 to 59 months in the South-East region.

Malaria prevalence and mosquito vector abundance in Uli town in Ihiala Local Government Area of Anambra State, Nigeria was studied between April and July, 2010. Oral interviews were used to get personal data of participants. 178 participants made up of 111 (62.45%) males and 67 (37.6%) females from the ten villages of the town were involved in the study.

Participants were aged between 0 to 70 years and subdivided into 7 age group of ten years intervals each; in occupational and education groups of the 178 participants examined, 126 (70.8%) were positive with malaria parasite. Species detected include *P. falciparum* (80.2%), *P. malariae* (13.6%), *P. ovale* (4.4%) and mixed infection of *P. falciparum* and *P. malariae* (1.6%). The age group 31 to 40 years had the highest malaria prevalence 42 (85.71%), while 0 to 10 year has the least 6 (42.86%). The malaria prevalence in relation to age was significant (15.100 df = 5; $p < 0.05$). The males 82 (73.9%) were slightly more affected than the females 45 (65.7%) but not statistically significant (0.683 m df – 5; $p > 0.05$). Malaria prevalence was significantly higher among those without formal education 7 (77.7%) and least among those with tertiary education 5 (35.7%, $p < 0.05$). Malaria prevalence among different through farmers were slightly more infected than others ($p > 0.5$) (Umeanaeto, 2011).

HIV/AIDs and malaria are among the most prevalent infectious diseases in sub-Saharan Africa and the leading cause of morbidity and mortality in the region (World Health Organization (WHO), 2004). HIV/AIDs and malaria together cause more than 4 million deaths annually, significantly affecting those in poverty and impeding sustainable development. The dual infections of malaria and HIV increase the risk of morbidity and mortality for all individuals infected. The complexity of co-infection has an impact on public health approaches and effective management of this interaction will require integration and strengthening of current public health care delivery system (WHO, 2004). HIV is a lentivirus (classified into retroviridae family) and is the major etiological agent of AIDs, a condition in humans in which the immune system begins to deplete, leading to life-threatening opportunistic infections (Weiss, 1993).

HIV is transmitted through direct contact of a mucous membrane or the blood stream with a contaminated body fluid such as blood, semen, vaginal fluid, seminal fluid and breast milk (Centre for Disease Control (CDC), 2003). The transmission can be through anal, vaginal or oral sex with infected individual, blood transfusion, infected contaminated blood, use of contaminated hypodermic needles, vertical route from mother to her baby during pregnancy or childbirth among other routes.

Malaria and HIV often co-exist in patients in most parts of the world due to overlap of these two diseases (Hewitt et al., 2006). An estimated 28 million individuals are infected with HIV in sub-Saharan Africa with almost 3 million deaths annually (Idemiyor, 2007). These diseases demonstrate a great deal of an overlapping distribution. Co-infection with HIV and malaria is very common in sub-Saharan Africa, and an understanding of how the two infections interact is important for the control of both diseases. The mainstay of malaria diagnosis has been the microscopic examination of blood, utilizing blood films (Krafts et al., 2011). Although blood is the sample most frequently used to make a diagnosis, both saliva and urine have been investigated as alternative, less invasive specimens (Sutherland and Hallett, 2009). More recently, modern techniques utilizing antigen tests or polymerase chain reaction have been discovered, though these are not widely implemented in malaria endemic regions (Ling et al., 1986; Mens et al., 2006). Areas that cannot afford laboratory diagnostic tests often use only a history of subjective fever as the indication to treat malaria.

The most economic, preferred and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics.

*Corresponding author. E-mail: chidiebube2013@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis (Warhurst and Williams, 1996).

From the thick film, an experienced microscopist can detect parasite levels (or parasitemia) as few as 5 parasites/ μ l blood (Richard et al., 2006). Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look identical and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.

Plasmodium malariae and *P. knowlesi* (which is the most common cause of malaria in South-east Asia) look very similar under the microscope. However, *P. knowlesi* parasitemia increases very fast and causes more severe disease than *P. malariae*, so it is important to identify and treat infections quickly. Therefore modern methods such as PCR (see "Molecular methods") or monoclonal antibody panels that can distinguish between the two should be used in this part of the world (McCutchan et al., 2008). Malaria transmission and mortality rates remain unchanged in endemic countries lacking adequate health care and malaria control programme despite the use of preventive measures and treatments against the disease (Bell et al., 2006). A major obstacle for effective malaria control is the lack of affordable and accurate malaria diagnostics and prompt treatment. This has led to misuse and abuse of anti-malarial drugs of which the consequence is the development of drug resistance in strain.

Microscopic examination of blood smears as the conventional method for *Plasmodium* species detection, is currently being replaced gradually with polymerase chain reaction (PCR) based rapid diagnostic tests (RDTs) for blood, because of inaccurate microscopic evaluations of blood smears resulting in misdiagnoses and misclassification of malaria severity (Makler and Hinrich, 1993). Failure to detect cryptic *P. falciparum* infections could lead to the risk of developing severe or fatal outcomes while missed *P. vivax* malaria may result in recurrent debilitating infections and economic loss (Mayxay et al., 2004).

Nevertheless, repeated examination of blood samples from malaria patients during post-treatment follow-up may at times result in poor compliance, especially among

infants and young children. Therefore, an alternative means for a noninvasive malaria diagnosis is required hence this study was designed to detect *Plasmodium* species DNA in urine sample of HIV positive individuals using PCR amplification method.

MATERIALS AND METHODS

The study area includes, General Hospital Onuigboji Ikwo LGA, Federal Teaching Hospital, Abakaliki and Onueke General Hospital, Ezza South LGA, Abakaliki, Ebonyi State. The investigation was carried out in Applied Microbiology Laboratory Complex, Eboyin State University (EBSU) while the PCR was done at Veterinary Medicine Molecular Laboratory, University of Ibadan, Oyo State. The study population includes 57 males and 93 females that were HIV positive. One hundred and fifty (150) blood and urine samples each were collected from one hundred and fifty out-patients for this study. An easy-to-read and friendly questionnaire was provided for the collection of demographic and clinical data. A physical examination with a clinical note of any reported sign or symptom was done by a physician. Ethical approval was obtained from the mentioned health Institution.

Parasitological identification of *Plasmodium* Species

Each blood sample was subjected to parasitological examination using thick and thin blood film microscopy according to microbiological standards (Cheesbrough, 2002).

Plasmodium Species DNA extraction of using whole urine samples

Exactly 150 μ l of whole urine was pipette into micro-centrifuge tube. 95 μ l of 2x digestion buffer and 5 μ l of proteinase k were added to the microcentrifuge simultaneously. The content in the tubes were mixed well and then incubated at 55°C for 20 min. Seven hundred (700 μ l) of genomic lysis buffer were added to each tube and thoroughly mixed by vortexing. The mixture were carefully transferred to a Zymo-Spin™ IIC column in a collection tube and centrifuged at 13,900 rpm for 2 min. Two hundred (200 μ l) of DNA pre-wash buffer were added to the spin column in a new collection tube and allowed to centrifuge at 13,900 rpm for 2 min. Four hundred (400 μ l) of g-DNA wash buffer was measured and introduced in the spin column. The content was centrifuged at 13,900 rpm for 2 min. The spin column was carefully transferred into a clean micro-centrifuge tube. Forty (40 μ l) of DNA elution buffer was added to the spin column and incubated at 2 to 5 min at room temperature. The final mixture was centrifuged at 8,000 rpm for 2 min. The eluted DNA was immediately used for molecular based applications or stored at -20°C for future use. The resultant supernatant, containing DNA, was carefully transferred into a pre-labeled 1.5 ml microcentrifuge tube, excluding chelex, for immediate PCR analysis or stored at -20°C (Johnston et al., 2006).

Whole blood DNA extractions

The aforementioned procedure for whole urine DNA extraction was adopted for whole blood DNA extraction.

Table 1. Sex distribution of HIV Co-infected with malaria parasite (*P. falciparum*) among the population studied.

Sex	Number of sample examined	Number positive for HIV (%)	Percentage positive for malaria (%)
Male	57	57 (38.00)	47 (82.46)
Female	93	93 (62.00)	28 (30.12)
Total	150	-	75 (50.00)

Genomic DNA electrophoresis

The extracted genomic DNA were run on a 1.5% agarose gel in ethidium bromide (TBE) buffer (pH 8.0) at 100 mV and 500 mA for 3 h after which the gel was viewed under UV light for DNA bands. This confirms the presence and quality of the extracted genomic DNA.

PCR based detection

Plasmodium species detection was carried out using nested PCR in the thermal cycler. Two (2 µl) of the genomic DNA was added to a total volume of 23 µl amplification reaction mixture with *Plasmodium* genus-specific outer primers [rPLU5:5'-CCTGTTGTTGCCTTAACTTC-3' and rPLU6: 5'-TTAAAATTGTTGCAGTTAAAACG-3'] for first step PCR. Thirty-five cycles (94°C for 1 min, annealing at 60°C for 2 min and extension at 72°C for 2 min) were carried out. Two (2 µl) of the PCR product from the first step PCR reaction was measured and used as a DNA template for the nested PCR reaction in which amplification for *P. falciparum*, *P. vivax* and *P. malariae* was done in separate reaction tubes. The amplification reaction and thermal cycling of the first step PCR reaction was the same with the second nested PCR reaction except that the annealing temperature was reduced to 55°C for 2 min and species-specific primers for *P. falciparum* [rFAL1: 5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3' and rFAL2:5'-ACACAATGAACTCAATCATGACTACCCGTC-3'; *P. vivax*, rVIV1:5'-CGCTTCTAGCTTAATCCACATAACTGATAC-3' and rVIV2:5' ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3'; *P. malariae*, rMAL1:5'-ATAACATAGTTGTACGTTAAGAATAACCGC-3' and rMAL2:5'-AAAATTCCCATGCATAAAAAATTATACAAA-3'] while 45 thermal cycling was used for *P. ovale* with species-specific primer (rOVA1: 5'-ATCTCTTTTGCTATTTTTAGTATTGGAGA-3' and rOVA2: 5'-GGAAAAGGACACATTAATTGTATCCTAGTG-3') at denaturation temperature at 94°C for 30 s, annealing temperature at 45°C for 30 s and extension temperature at 72°C for 1 min 30 s. The aforementioned method is according to Johnston et al. (2006), with little modifications.

Electrophoresis of the PCR products

The PCR products were separated in 1.5% agarose gel for both first and second nested PCR analyses. It was thereafter stained with 1 µl ethidium bromide (TBE) and allowed to run at 100 mV and 500 mA for 3 h after which the gel was visualized under a UV light for DNA bands. The PCR amplified fragments of *P. falciparum* and *P. malariae* genes were 205 and 144 bp, respectively.

RESULT

A total of 300 samples comprising 150 each of both blood

and urine were collected from HIV positive patients who visited some selected hospitals in Ebonyi State. The subjects were made up of 57 males and 93 female patients. The samples were screened for the presence of HIV and four human malaria parasites using enzyme linked immunosorbent assay (ELISA) and parasitological examination of blood stained films. The HIV positive samples were further subjected to confirmatory test using two rapid tests. Both blood and urine samples were further analyzed using PCR.

The sex distribution of HIV patients co-infected with malaria parasite revealed that female patients had the highest occurrence of 93 (62%) and 28 (30.12%) positive cases, respectively. The least prevalence was found among male patients presenting 57 (38%) positive cases for HIV and 47 (82.46) positive results for malaria infection (Table 1). The distribution of *Plasmodium* species in blood and urine specimens using PCR identification is shown in Table 2. Out of the 150 urine samples from the HIV positive individuals 88 urine specimens were identified to harbor *Plasmodium* species. 75 (50%) urine specimens were identified to harbor *P. falciparum* while 10 (6.67%) and 3 (2%) were recorded against *P. malariae* and dual infection of *P. falciparum* and *P. malariae*, respectively. The result of the comparison of the specimens used showed the same result. None of the isolates that were negative by PCR test using DNA primers (Template) from blood gave positive results by urine samples.

Furthermore, when the primers (rOVA₁ and rOVA₂; rV₁V₁ and rV₁V₂) specific for *P. ovale* and *P. vivax*, respectively were used none of the two species mentioned were detected in both urine and blood samples, signifying that these species may be absent in our environment (Figures 4 and 5). Figures 2 and 3 highly demonstrated the presence of *P. falciparum* and *P. malariae*, respectively especially when specific *Plasmodium* species DNA markers were used for the analysis while Figure 1 revealed the presence of *Plasmodium* species with different binding patterns.

DISCUSSION

Our investigation revealed the prevalence of 100 and 50% for HIV and malaria infections, respectively. The sex distribution of HIV patients co-infected with malaria

Table 2. Distribution of *Plasmodium* species in blood and urine specimens collected from HIV positive individuals using PCR identification method.

Type of specimen	Number of sample examined	Plasmodium species isolated	Number of isolates (%)
Blood	150	<i>Plasmodium falciparum</i> (Pf)	75 (50.00)
		<i>Plasmodium malariae</i> (Pm)	10 (6.67)
		Pf + Pm	3 (2.00)
Urine	150	<i>Plasmodium falciparum</i> (Pf)	75 (50.00)
		<i>Plasmodium malariae</i> (Pm)	10 (6.67)
		Pf + Pm	3 (2.00)
Total	300		176 (58.67)

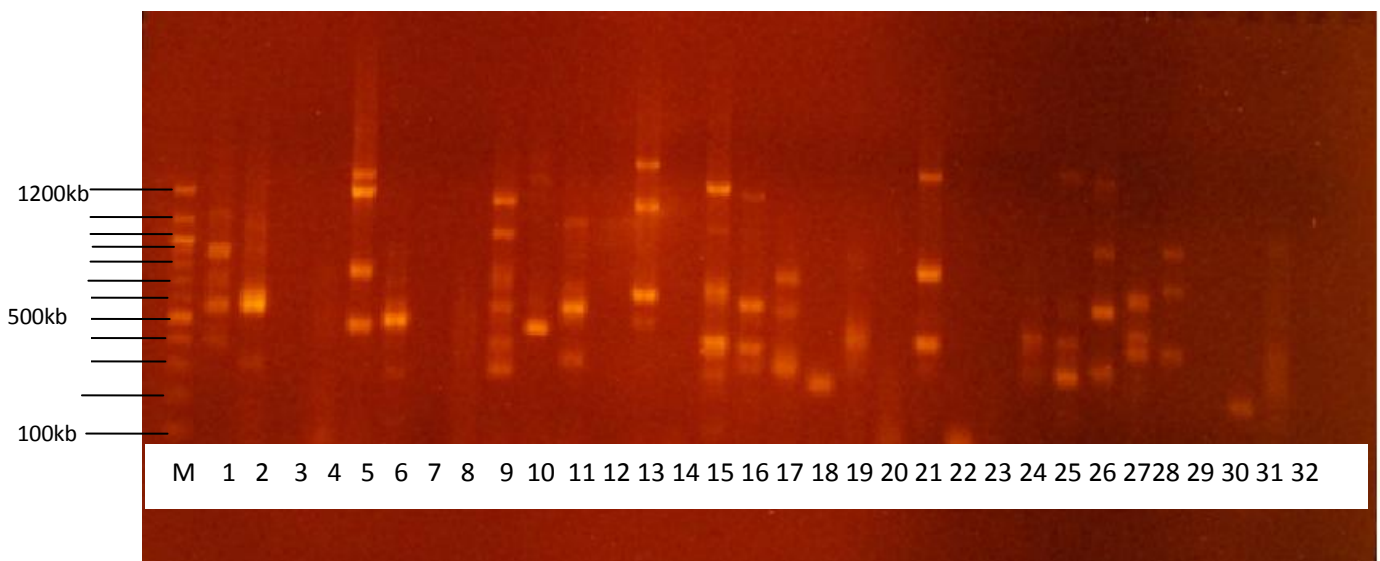


Figure 1. PCR products with primer rPLU5 and rPLU6 for *Plasmodium* species.

infection revealed that female patients gave high HIV occurrence of 62% and low malaria prevalence 30.12% while low HIV prevalence of 57(38%) was observed among the males with high malaria prevalence of 47(82.46%). Our findings here as it affect prevalence among HIV positive individuals is in agreement with UNAIDS and WHO (2008) and WHO (2004) where they reported high percentage prevalence among female subjects than male subjects. These were also supported by National Agency for the Control of AIDS (NACA) (2012) where they recorded 4.0% prevalence among HIV female patients and males 3.2%. They further observed a higher prevalence of 4.5% among female police than their male colleagues at 2.0% (Federal Ministry of Health (FMH), 2008).

In another development, population based survey observed that gender inequality is an important factor for

epidemic prevalence higher in females than males (FMH, 2011). Generally, women are more vulnerable to HIV infection than men because during sexual intercourse the receptive partner (the person who is penetrated) is more at risk than the incentive partners. Unprotected sexual intercourses among other factors were believed to be contributory factors especially among sexually active persons (UNAIDS and WHO, 2008).

High malaria profile recorded in this study against the male patients and low prevalence among female patients is in line with the report by NACA (2012) in which male patients recorded 73.9% and the female patients had 65.7%. The high percentage prevalence of malaria infection recorded in this study against the male patients could be attributed to high- risk exposure to the vector that transmits malaria. Malaria and HIV/AIDs co-exist in patients in most parts of the world due to overlap of the

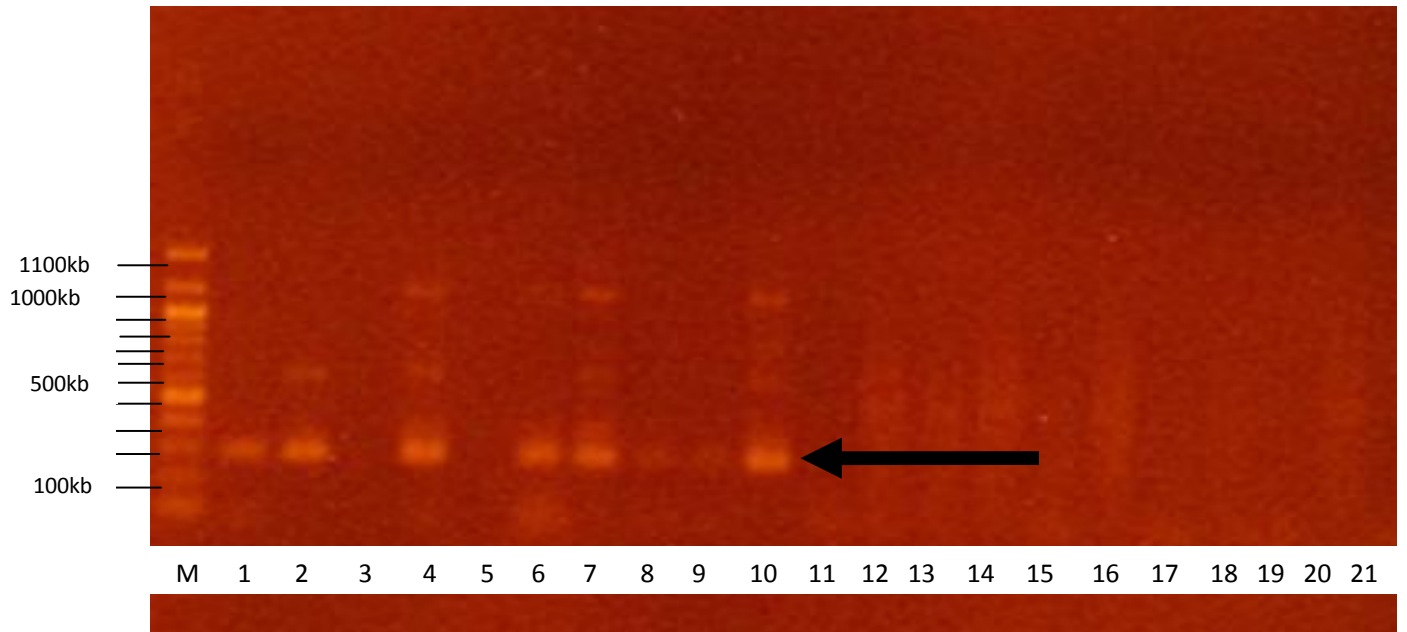


Figure 2. PCR products with primer rFAL1 and rFAL2 for *Plasmodium falciparum*.

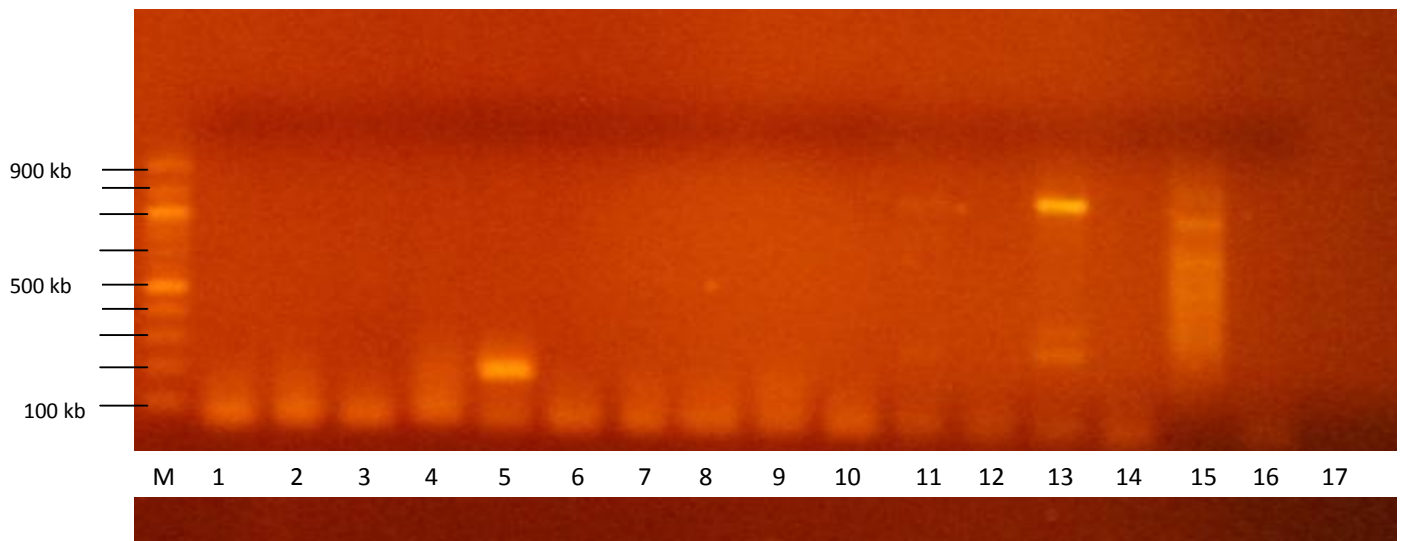


Figure 3. PCR products with primer rMAL1 and rMAL2 for *Plasmodium malariae*.

two (Hewitt et al., 2006). The Figure 2 band patterns of the presence of *Plasmodium* species isolated from the malaria and HIV individuals in this study revealed that the gene fragments are approximately 205 bp that depicts the presence of *P. falciparum*. When the results of microscopy were compared with the gel band pattern, it was observed that those blood films that were identified to be positive for *P. falciparum* gave the same band

pattern (Figure 2). The gel band pattern observed in the Figure 3 corresponds to the DNA fragment approximately 144 bp and this relates to the *P. malariae*. The result here correlates with the results of blood film microscopy. In Figure 1, the isolates in the group 2 lanes 5, 10, 13, 16, 17, 22, 28 and 29 contain multiple band patterns and they are suspected to harbor more than one *Plasmodium* species. The results also coincide with the results of blood

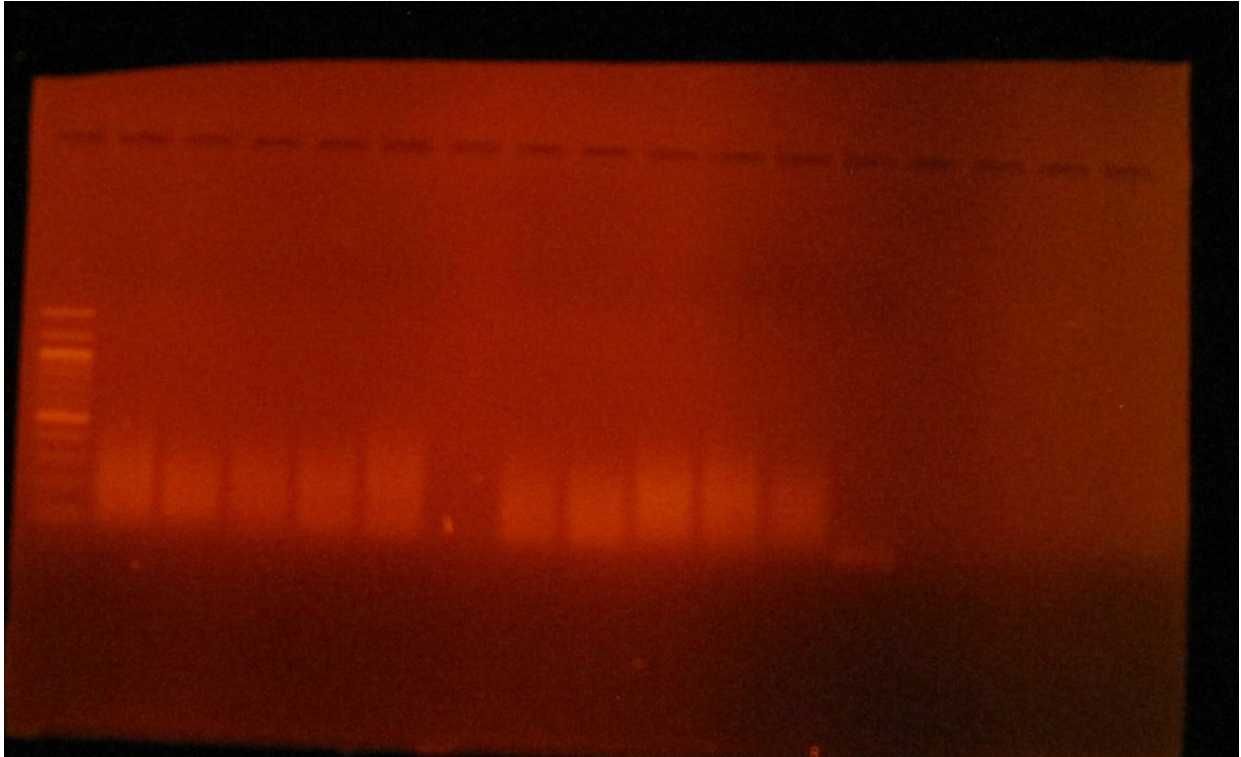


Figure 4. PCR products with primer rV1V1 and rVIV2 for *Plasmodium vivax*.

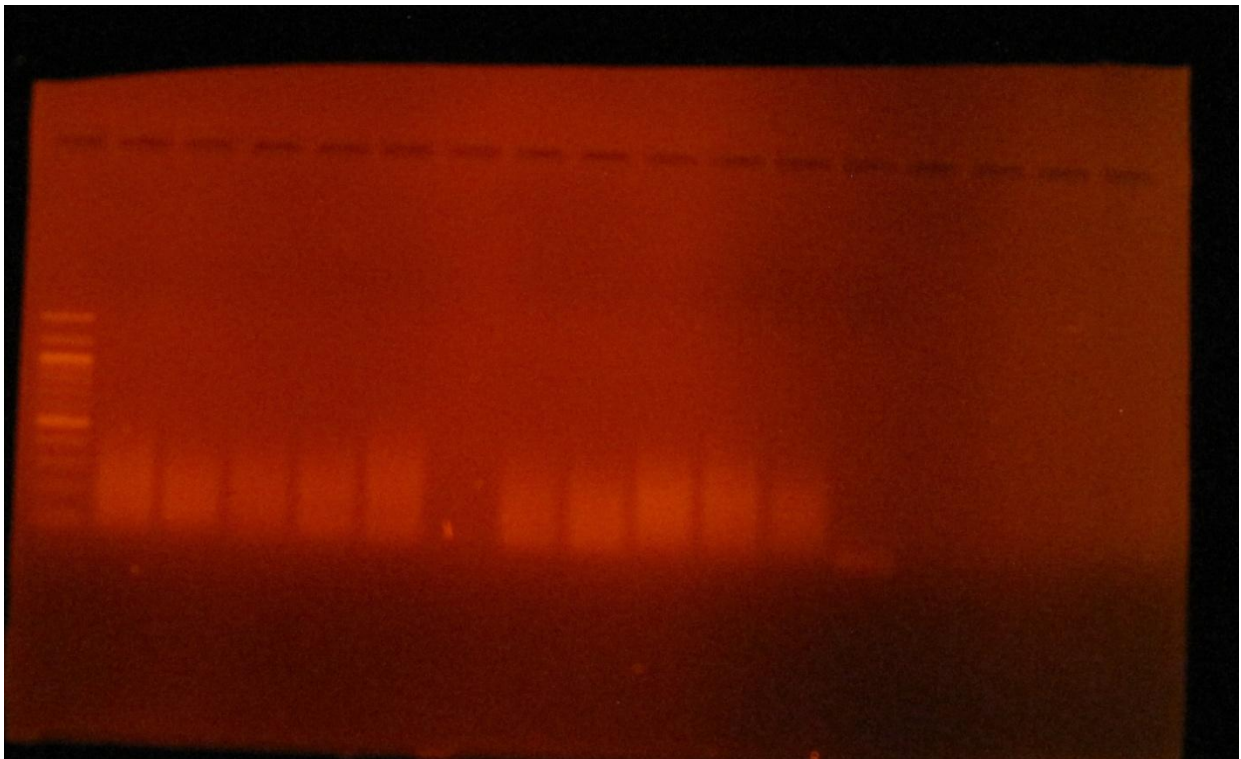


Figure 5. PCR products with primer rOVA1 and rOVA2 for *Plasmodium ovale*.

blood film microscopy. The lanes where no bands are found were suspected to be devoid of *Plasmodium* species and of course the results correspond to blood films microscopy that was negative to *Plasmodium* parasites.

The verification on the authenticity of using urine as a clinical specimen for the diagnosis of malaria in our environment was also investigated using polymerase chain reaction (PCR). Out of the 150 urine samples collected from the HIV positive individuals, 88 urine specimens were identified to harbor *Plasmodium* species. 75 (50%) was identified to be *P. falciparum* while 10 (6.67%) and 3 (2%) were recorded against *P. malariae* and dual infection of *P. falciparum* and *P. malariae*, respectively. A subsequent study in Gambia has shown that detection of the small subunit ribosomal RNA gene (SSU rRNA) of *P. falciparum* in urine has high specificity comparable to that obtained from blood samples (Nwakanma et al., 2009).

Despite a lower sensitivity of PCR detection for *P. falciparum* from urine sample than that obtained from blood-derived DNA template, repeated noninvasive sample collection during drug trials or monitoring vaccine efficacy may be warranted (Sutherland and Hallett, 2009). The findings correlate with the results gotten from blood sample PCR analysis collected from same patients. A study conducted by Buppan et al. (2010) reported 100% specificity of urine specimen for the identification of *P. falciparum* and *P. vivax* when compared with nested PCR results from blood. Results from our study also validates that *Plasmodium* species DNA could be identified in urine samples of an infected individual thereby paving way for an alternative source of non-invasive clinical specimens for potential diagnosis of malaria in our environment.

Conflict of Interests

The author(s) have not declared any conflict of interest.

REFERENCES

- Bell D, Wongsrichanalai C, Barnwell JW (2006). Ensuring Quality and Access for Malaria Diagnosis: How can it be Achieved? *Nat. Rev. Microbiol.* 4:682-695.
- Breman JC (2001). The Ears of the Hippopotamus: Manifestations Determinants and Estimates of the Malaria Burden. *Am. J. Trop. Med. Hyg.* 64(1-2 Suppl):1-11.
- Buppan P, Potaporntip C, Pattanawong U, Seethamchai S, Jonqwitiwes S (2010). Comparative Detection of *P. vivax* and *P. falciparum* DNA in Saliva and Urine Samples from Symptomatic Patients in a low endemic area. *Malar. J.* 9:72.
- Centers for Disease Control (CDC) (2003). Incorporating HIV Prevention into the Medical Care of Persons Living with HIV. Recommendation of CDC, the Health Resource and Services Administration, the National Institute of Health and the HIV Medicine Association of the Infectious Diseases Society of America. *Morbidity and Mortality Weekly Report, Recommendation Report.* 52:1-24.
- Cheesbrough M (2002). *District Laboratory Practice in Tropical Countries.* Part 2 Edition. Cambridge University Press, New York. pp. 320-329.
- Greenwood B, Bojang K, Whitty AJ, Target GA (2005). Malaria. *Lancet* 365:1487-1498.
- Hewitt K, Steketee R, Mwapasa V, Whitworth J, French N (2006). Interactions between HIV and Malaria in non-pregnant adults: Evidence and implications. *AIDS* 20(16):1993-2004.
- Idemyor V (2007). Human Immunodeficiency Virus (HIV) and Malaria Interaction in Sub-Saharan Africa: The Collision of two Tiran. *HIV Clin. Trials.* 8(4):246-253.
- Johnston SP, Pieniazek NJ, Xayavong MV, Slemenda SB, Wilkins PP, da-Silva AJ (2006). PCR as a Confirmatory Technique for Laboratory Diagnosis of Malaria. *J. Clin. Microbiol.* 44(3):1087-9.
- Krafts K, Hempelmann E, Oleksyn B (2011). The color purple: from royalty to laboratory, with apologies to Malachowski. *Biotech. Histochem.* 86(1):7-35.
- Ling IT, Cooksley S, Bates PA, Hempelmann E, Wilson RJM (1986). Antibodies to the glutamate dehydrogenase of *Plasmodium falciparum*. *Parasitology* 92(2):313-324.
- Makler MT, Hinrichs DJ (1993). Measurement of the Lactate Dehydrogenase Activity of *Plasmodium falciparum* as an Assessment of Parasitemia. *Am. J. Trop. Med. Hyg.* 48:205-210.
- Mayxay M, Pukrittayakamee S, Newton PN, White NJ (2004). Mixed-Species Malaria Infections in Humans. *Trends Parasitol.* 20(5):233-240.
- McCutchan TF, Piper RC, Makler MT (2008). Use of malaria rapid diagnostic test to identify *Plasmodium knowlesi* infection. *Emerg. Infect. Dis.* 14(11):1750-2.
- Mendis K, Sina B, Marchesini P, Carter R (2001). The Neglected Burden of *Plasmodium vivax* Malaria. *Am. J. Trop. Med. Hyg.* 64:97-106.
- Mens PF, Schoone GJ, Kager PA, Schallig HDFH (2006). Detection and identification of human *Plasmodium* species with real-time quantitative nucleic acid sequence-based amplification. *Malar. J.* 5:80.
- National Agency for the Control of Aids (NACA) (2012). National HIV/AIDS Strategic Plan 2010-2015. Abuja Nigeria: Nigeria National Agency for the Control of Aids 2010. Available at: <http://nigeria.unfpa.org/pdf/nsp.pdf>
- Nwakanma DC, Gomez-Escobar N, Walther M, Crozier S, Dubovsky F, Malkin E, Locke E, Conway DJ (2009). Quantitative detection of *Plasmodium falciparum* DNA in saliva, blood and urine. *J. Infect. Dis.* 199:1567-1574.
- Sutherland CJ, Hallett R (2009). Detecting malaria parasites outside the blood. *J. Infect. Dis.* 199(11):1561-1563.
- Umeanaeto PU (2011). Malaria Prevalence and Mosquito Vector Abundance in Uli Town, Ihiala Local Government Area, Anambra State, Nigeria. *Afr. J. Biomed. Res.* 14(3)
- UNAIDS and World Health Organization (WHO) (2008). Epidemic update. Available at: http://www.amarc.org/documents/manuals/JC1510_2008GlobalReport_en.pdf
- Warhurst DC, Williams JE (1996). ACP Broadsheet No. 148. Laboratory Diagnosis of Malaria. *J. Clin. Pathol.* 49:533-538.

The background of the entire page is a laboratory scene. A test tube containing a red liquid is tilted, with a small amount of the red liquid dripping into a beaker below. The beaker contains a green liquid. The background has a soft, blurred gradient from light green at the top to a darker green at the bottom.

Journal of AIDS and HIV Research

Related Journals Published by Academic Journals

- *Clinical Reviews and Opinions*
- *Journal of Cell Biology and Genetics*
- *Journal of Clinical Medicine and Research*
- *Journal of Diabetes and Endocrinology*
- *Journal of Medical Genetics and Genomics*
- *Medical Case Studies*

academicJournals