EPIDEMIOLOGY OF HEPATITIS B AND C VIRUS INFECTIONS AMONGST HIV PATIENTS ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY IN SOUTHEASTERN NIGERIA.

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING, FACULTY OF BIOSCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN MICROBIOLOGY (MEDICAL MICROBIOLOGY) OF NNAMDI AZIKIWE UNIVERSITY, AWKA.

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SEPTEMBER, 2019.

CERTIFICATION

This is to certify that this research work titled "EPIDEMIOLOGY OF HEPATITIS B AND C VIRUS INFECTIONS AMONGST HIV PATIENTS ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY IN SOUTHEASTERN NIGERIA" was carried out by Okoli, Chukwudum S, with Registration number NAU/PG/Ph.D/2013487011F, under the supervision of Prof. R.A.U. Nwobu, this work is original and has not been submitted in part or full for any other university.

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APPROVAL

This dissertation titled "EPIDEMIOLOGY OF HEPATITIS B AND C VIRUS INFECTIONS AMONGST HIV PATIENTS ON HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY IN SOUTHEASTERN NIGERIA" by Okoli Chukwudum Somadina with registration number NAU/PG/Ph.D/2013487011F has been approved for the award of Doctor of Philosophy (Ph.D) in Medical Microbiology, in the Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University Awka.

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DEDICATION

This work is dedicated to my parents Surv. and Mrs. Afam Nwadinobi Okoli.

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ABSTRACT

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) co-infection is the leading cause of morbidity and mortality in HIV infected patients especially in developing countries. These have been shown to decrease the life expectancy in the HIV infected patients. Hence this study was designed to study the epidemiology of HBV, HCV and HBV/HCV co-infections in HIV patients attending Government Tertiary Hospitals in Southeast Nigeria. A cross-sectional study was conducted among 2,500 HIV patients on highly active antiretroviral therapy (HAART) attending Government Tertiary Hospitals in Ebonyi, Enugu, Abia, Imo and Anambra. The hospitals are Federal Teaching Hospital Abakaliki, Enugu State University Teaching Hospital, Federal Medical Centre Umuahia, Federal Medical Centre Owerri and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka respectively from February 2016 to November 2017. Socio-demographic and risk factors were collected using a structured questionnaire. Hepatitis B and C sero markers were detected using an Enzyme Linked Immunosorbent Assay (ELISA) while HBV and HCV genotypes were detected by Polymerase Chain Reaction (PCR). Reitman-Frankel colorimetric method was used in determining Alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) while Phenolphthalein monophosphate method was used in the determination of Alkaline Phosphatase (ALP). Haemocue Haemoglobin meter 301 was used to test for Haemoglobin and the cluster of differentiation T- lymphocytes class-4 ($CD4^+$) levels were also determined using CD4 test count kit with Cyflow Counter. DNA and RNA were extracted using roche high pure viral nucleic acid kits and amplified by AB3130 Applied Biosystem Thermocycler. The results were analyzed using percentage frequency (%) and Statistical Package for Social Sciences (SPSS) software, version 20.0 and statistical significance was considered at $P<0.05$. Out of the 2,500 HIV patients sampled, 256 (10.2%), 175 (7.0 %) and 25 (1.0%) were positive for HBV infection, HCV infection and HBV/HCV co-infection, respectively. Enugu State showed the highest seroprevalence rate to hepatitis B virus surface antigen (HBsAg) with 62 (12.4%) and the least by Ebonyi State with seroprevalence rate of 42 (8.4%). In this study, 175(7.0%) were positive for Anti-HCV, the highest seroprevalence rate was recorded in Anambra and Enugu States with (10.0%) each and Imo State recorded the least seroprevalence (4.0%). The distribution of hepatitis B virus surface antigen showed that the age range of 31-40 years had the highest prevalence of 70 (17.5%) and the least prevalence was >70 years 6 (2.4%). Also, distribution of hepatitis C virus by age, 31 -40 years showed the highest prevalence of 70 (17.5%) and 51 -60 years showed the least prevalence of 15 (3.7%), but <10 years recorded 0.0%. Out of 1400 (56%) males and 1100 (44.0%) females 11.8% and 8.2% were sero positive to hepatitis B virus surface antigen (HBsAg) respectively, while 85 (6.1%) males and 90 (8.2%) females were positive to hepatitis C virus antibody. Associated risk factors and sociodemographic parameters of HIV patients positive to HBV, HCV and HBV/HCV co-infections showed statistical significance ($P<0.05$), hence are significant risk factors. This study showed that $P>0.05$ is not statistically significant with regards to haematological and biochemical parameters except for $CD4^+$ in HBV/HIV patients ($P=0.000$), also $CD4^+$ and AST in HCV/HIV patients ($P =0.000$ and 0.006) and ALT in HBV/HCV/HIV patients ($P=0.026$) which showed statistical significance ($P<0.05$). DNA and RNA genotyping respectively showed hepatitis B with genotype E and genotype 2 for hepatitis C virus. Homology with consensus sequence of HBV genotype E were 99% and that of HCV genotype 2 were 96-98%. The findings show the importance of screening for hepatitis B and viruses in HIV infected individuals. HBV and HCV being a co-morbid infection and a threat were recorded in Eastern Nigeria; hence there is a need to give health education concerning transmission, risk factors and prevention of these viral infections. Government should create centers for the vaccination of populace.

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LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AIDS	Acquired Immune Deficiency Syndrome
Anti-HCV	Hepatitis C Virus Antibodies
CAP/CTM	CobasAmpliprep/CobasTaqman
CDC	Center for Disease Control
ELISA	Enzyme Linked Immunosorbent Assay
cDNA	Complimentary DNA
ddNTP	Dideoxynuclelotide triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
HAART	Highly Active Anti-Retroviral Therapy
HCC	Hepatocellular Carcinoma
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
IU	International Units
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
UTR	Untranslated Region
UV	Ultra Violet Light
WHO	World Health Organisation