International Journal of Medical Science and Pharmaceutical Research

ISSN(e): Applied
ISSN (p): Applied

Vol. 01(01): 01-06, June 2024

Home Page: https://ijmspr.org/index.php/ijmspr

Prevalence of HCV among Gender and Age Groups by Gene Drive Molecular Platform

Onyekachi DE[1], Nwoke BEB[2], Ozims SJ[3], Chinedu-Elonu PO[3], Eberendu IF[3]

[1]Gem Mediclal Diagnostic Centre Anambra

[2]Department of Animal and Environmental Biology, Faculty of Sciences Imo State University, Owerri

[3]Department of Public Health Faculty of Health Science Imo State University, Owerri

KEYWORDS:

Prevalence, HCV, gender, age, Gene Drive molecular platform

Corresponding Author:
Onyekachi DE

ABSTRACT

With years of government and stakeholder lethargy, chronic Hepatitis C virus (HCV) infection might be seen as a quiet and unexamined murderer. The occurrence Hepatitis C virus diagnosis among blood donors in Anambra State was assessed using the Genedrive Molecular Platform and Acumen Antibody Strip. Four hundred participants made up the study's subjects; these were prospective blood donors who came to the laboratory to donate blood from Anambra State University Teaching Hospital, Awka. 60.70% of the participants were female, with 255 being female, while 39.50% (165) of the subjects were male. The participants' ages ranged from 18 to 50 years old, with a mean age of 34. 55 out of 420 people tested positive for HCV, making the research population's total prevalence of the virus 13.10%. In terms of gender stratification, the prevalence for men was 12.72% (12 out of 165), whilst the prevalence for women was marginally higher at 13.33% (34 out of 255). The age range of 31 to 35 years showed the highest prevalence of positive cases, making up 34.43% (21 out of 61) of all positive cases within that age range. With a frequency of 38.18% (21 out of 55), this age group likewise showed the highest overall prevalence among the positive cases. *This results present the current prevalence of the HCV genome 15 years after its discovery.*

INTRODUCTION

Following the discovery of an RNA viral genome in a random-prime DNA library obtained from a human plasma sample carrying the suspected non-A, non-B hepatitis agent, the term hepatitis C virus (HCV) was initially used in 1989 [1]. Epidemiological research indicated that non-A, non-B hepatitis could spread through two different pathways. As a result, enteric, parenteral, and post-transfusion versions were identified. Presently recognized as the predominant parenteral type is the hepatitis C virus (HCV). But more recently, other similar agents, such as the hepatitis G virus, have been discovered [2].

HCV is mostly transmitted parenterally through blood transfusion and contact with blood derivatives; recipients of blood and blood products, including factor VIII and immunoglobulins, were the first to be diagnosed with the illness.
.. Transmission has also been linked to needle stick injuries and transplanted organs. Transmission in drug abusers, dialysis patients, and surgery patients has been documented. The transmission of HCV has also been linked to sexual interaction [3]. Additionally, there is mounting proof of vertical transmission (from mother to child). On the other hand, a significant percentage of HCV infections have a "undefined" mode of transmission [4].It has been demonstrated that the hepatitis C virus is present everywhere in the world and affects people of various ages, genders, races, and geographical locations [6]. According to a World Health Organization (WHO) assessment, 170 million people, or around 3% of the global population, have HCV infection and run the risk of acquiring cancer, liver cirrhosis, or both [7].

. Various global regions have reported slightly differing prevalence rates. America recorded a prevalence of 1.7%, Europe reported 1.03%, the Western Pacific reported 3.9%, the Eastern Mediterranean reported 4.6%, South Asia reported 2.15%, and Africa reported 5.3% [8].Antibodies to different hepatitis C viral antigens are indicative of viral infection and, in most cases, suggest a chronic illness [9]. People who have chronic hepatitis may not exhibit symptoms for many years after the infection begins, as the disease can have a protracted and sneaky course [10].The utilization of enzyme immunoassays (EIAs) or enzyme linked immunosorbent assays (ELISA), which are currently offered for sale, is the foundation for the detection of anti-HCV antibodies in plasma or serum.

HCV antibodies develop slowly and variably after infection, and there is a window of three months before the tests show a positive result. [11] Antibodies directed against non-structural (NS3 and NS4) and structural (Core) proteins are detected using second generation EIAs.Together with antibodies targeted at the NS5 protein, third generation EIAs are more sensitive in detecting the same antibodies [12].Post-transfusion hepatitis cases have significantly decreased since the developed world's blood donors are now routinely screened for hepatitis C. For example, screening standards that excluded donors with human immunodeficiency virus infection and donors with surrogate indicators for non-A, non-B hepatitis contributed to a greater than 50% drop in transfusion-associated non-A, non-B hepatitis cases in the United States between 1985 and 1990.

[13.14].

In 1990, the probability of HCV infection linked to transfusions was roughly 1.5% for recipients, or 0.02% for each unit transfused. Regular testing for signs of HCV infection was started in May 1990, and more sensitive multiantigen testing was used in July 1992, bringing the probability of infection down to 0.001% per unit transfused. [15]

The hepatitis C virus poses a serious threat to public health as it is the primary cause of chronic liver disease worldwide. Due to its contagious nature, HCV is becoming more common; yet, due to a lack of data and underdiagnosis, it is still uncommon, especially in Nigeria (with the exception of Egypt).

The majority of data on HCV prevalence in Nigeria that are currently available are from the country's north, south-west, and south-south geopolitical zones; the few data that are available from the southeast were obtained from pregnant women [16, 17]. Based on the reviewed literature, there is a dearth of information regarding the prevalence of the Hepatitis C virus among blood donors in Nigeria, particularly in Anambra State.

MATERIALS AND METHOD

Population and Sample Size

This study was conducted in some selected medical laboratory units of both private and public hospitals in 3 geopolitical zones of Anambra State, South-East, Nigeria.

Names of the Laboratories:

Population of Study

The target population for this study was individuals of various ages and both sexes who will be coming to the selected study centers within the period of this study. They were made up of blood donors and patients/ clients who may consented to this study.The control was other clients presenting to the study centers for other laboratory services other than blood donation.

The calculated minimum sample size was 246 subjects. However, a sample size of 420 subjects was used for this study. A convenience sampling technique will be adopted for selecting participants to form the sample size of this study.

Validation and Reliability of Instrument of Data Collection

Three experts from the department of Medical Laboratory Science of Nnamdi Azikiwe University Awka were used to establish the face content validity of the instrument. The instrument was given to the experts for their correction and suggestion. Which was ensuring that the language used was clear, simple and understandable they also made correction on the questionnaire items ensuring that such are adequate, suitable and in line with stated objectives. Based on the correction and approval of these experts, a final copy of the questionnaire was produced and utilize for data collection.

Thirty copies of the questionnaire were administered to thirty respondents from Enugu State in a community which is not part of the study area. Cronbach alpha was used to determine the reliability co-efficient of each aspect of the study (knowledge of information of Hepatitis C virus r = 0.847) being investigated was computed separately and the overall reliability co-efficient obtained was 1.808. This was considered high enough to judge the instrument as being highly considered so it was used for the study.

Ethical Considerations/Informed Consent

In line with the Helsinki Declaration, ethical approved for the study was obtained from the Human Research and Ethics committees of the Nnamdi Azikiwe University Teaching Hospital, (NAUTH), Nnewi, Anambra State, Nigeria.

Collection of Data and Sample Collection

A structured questionnaire was be administered one-to-one to the intended participants to generate data on the Participant's socio-demographic variables such as age, gender occupation, employment status educational status, marital status and number of persons per household. Participant's attitude and knowledge of hepatitis C, including any history of previous screening, risk factors as well as knowledge of the mode of transmission of hepatitis C.Participant's knowledge of mode of transmission of hepatitis C was evaluated on a four – item, three-point Likert Scale. The four- terms were Hepatitis C can be passed from mother-to-child. Hepatitis C can be spread through needle prick and sharp objects

Hepatitis C can be spread via blood. Hepatitis C can be contacted through sexually intercourse. The responses were (i) true ii) false (ii) don't know and (iii) I don't understand, which shall correspond to scores of 3, 2 and 1 respectively. The reliability and validity of the questionnaire was tested using pre-test pilot study before going to the field for data collection.

Inclusion criteria

i) Participants with age 18 years and above

ii) Those that can response the questions in the questionnaire.

iii) Those that visited the selected study centers within the period of this study, and will give their consent to be included in the study.

Exclusion criteria

i) Those younger than 18 years of age.

ii) Those that will provide incomplete information on the questionnaire.

iii) Those that will not give consent to be included in this study will be excluded.

Regarding the blood collection, processing and HCV antibody screening, about 5ml of venous blood from the median cubital vein was collected into ethylenediaminetaaccetic acid (EDTA) tubes; allowed to clot and about twomilliters of this blood was centrifuged at 300rpm for 5minutes to obtain the plasma. An aliquot of 500microliters of each plasma sample will be produced and screened for anti-HCV antibody using HCV rapid test strip (Acumen) and Genedrive anti-HCV- ID test (Genedrive Plc, Manchester, UK) following the instructions of the manufacture's manual . The sensitivity and specificity of the Genedrive HCV-ID Kit are 100% and 100% respectively . The resultant plasma samples of all anti-HCV antibody positive samples were aliquoted into two labeled cryovials and stored at -800C for HCV antigen and antibody testing.

Specification of the Genedrive; Size: (12cm X 18cm X 10cm), Weight: (600g), Power: (Input: (100 – 240V, 1.2A, 50/60Hz Ac, Output: (12V, 8.33A DC), Operating: (500C humidity < 75% temperature non- condensing), Software: (Operating System: Microsoft, net; Microframework, V4.1), thermal Ramp Rate: (400C/S heating, 1.20C/S cooling), Time to result: from 50minutes depending assay (Genedrive System, 2019).

Acumen HCV Rapid Test is done using the HCV test strip and it comes within a kit that contains the test strips, droppers, test buffer, test cards and package insert. Other materials required for the test but not provided in the kit – timer, specimen containers and centrifuge. The test strip has the following components – absorbent sample pad/ specimen area, conjugate release pad, detection zone, test line control line and wicking pad. the strip was placed on clean and level surface, held the dropper vertically and transferred 1 drop of specimen plasma (approximately 25microliter) to the specimen area of the strip, then 2 drops of the test buffer (approximately 120microliter) was added and started the timer. The coloured line(s) appear, the test result was read and interpreted at 10 minutes.

Method of screening

The samples that were frozen earlier were thawed and used. All blood units were tested for HIV I&II, HCV antibodies and HBsAg using 3rd generation ELISA (Merilisa hiv 1 & 2 gen 3,28 Merilisa HCV29 and Merlisa HBsAg30 kits manufactured by Meril diagnostics). In addition donor units were screening with 4th generation HIV Ag-Ab, HCV Ag-Ab and HBsAg ELISA (Genscreen ULTRA HIV Ag-Ab kits,32 monolisa HCV Ag-Ab ULTRA V2 kit33 and monolisa

Statistical Analysis

Data that will be obtain, will be categorized in line with the study objectives and analyzed using Statistical Package for Social Sciences (SPSS) Version 21 (SPSS in c. ILL USA). Both descriptive (Mean Standard deviation, Percentage and Frequency) Statistics will be used for statistical data analysis. The Pearson Chi-Square test will be used to compare the relationship, between categorical variables with statistically significant level set at $p < 0.05$.

RESULTS

In this study, a total of 420 subjects were included, consisting of potential blood donors at Anambra State University Teaching Hospital, Awka, who visited the laboratory to donate blood. Among these subjects, 255 were females (60.70%) and 165 were males (39.30%). The age range of the participants was 18 to 50 years, with a mean age of 34 years.

The overall prevalence of HCV in the study population was found to be 13.10%, with 55 out of 420 individuals testing positive for the virus. When stratified by gender, males exhibited a prevalence of 12.72% (12 out of 165), while females had a slightly higher prevalence of 13.33% (34 out of 255).

Among the positive cases, the age group of 31 to 35 years had the highest prevalence, accounting for 34.43% (21 out of 61) of positive cases within that age range. This particular age group also displayed the highest overall prevalence among the positive cases, with a prevalence of 38.18% (21 out of 55).

Table 1; Prevalence of HCV among gender by Gene Drive molecular platform

Gender	Number Examined	Number Positive (%)
Male	165 (100)	21 (12.72)
Female	255 (100)	34 (13.33)
Total	420 (100)	55 (13.10)

Gene Drive molecular platform was used as the gold standard to compare results obtained when Acumen, Biopanda and RapidResponse antibody test strips were used to detect HCV antigen. Using Gene Drive, HCV was detected in 55 subjects (13.10%). When compared to the serological test kits, 64 (15.24%), 78 (18.57%), and 96 (22.86%) were positive by Acumen, Biopanda and RapidResponse kits respectively while 356(84.76%), 342 (81.43%), and 324 (77.14%) were negative for the diagnostic test kits respectively.

Table 2 Prevalence of HCV among the age groups by Gene Drive Molecular Platform

Age range	Positive (%)	Negative (%)	Total (100%)
16 – 20	02(5.00)	38 (95.00)	40
21 – 25	02 (4.65)	41 (95.35)	43
26 – 30	07 (9.46)	67 (90.54)	74
31 – 35	21 (34.43)	40 (65.57)	61
36 – 40	12 (14.46)	71 (85.54)	83
41 – 45	05 (7.69)	60 (92.31)	65
46 – 50	06 (11.11)	48 (88.89)	54
Total	55 (13.10)	365 (86.90)	420

Cohen's kappa coefficient was used to compare the agreement between Gene Drive and rapid diagnostic tests using Gene Drive as the gold standard. This study found that when compared between Biopanda and Gene Drive, there was moderate agreement (k = 0.491). Among other test kits, Acumen showed good agreement with PCR (k = 0.611), while RapidResponse showed fair agreement (k = 0.172).

DISCUSSION

The findings from many employees and/or journalists showed that HBV and HCV infection differs throughout Nigeria. These data bolster the claim made by [18, 19], who stated that the prevalence of viral hepatitis infections differs around the world among nations, regions, and population groups within a nation.According to estimates, the prevalence of HBV and HCV in Nigeria is 2.4%–18.4% and 3.6%–5% of the population, respectively.According to the data, the prevalence of HBV ranges from 2.2 to 12.5%, while the range for HCV is from 0 to 18.2%.Nations that test positive for viral hepatitis are categorized as having low endemic rates (<2%), intermediate endemic rates (2–8%), or high endemic rates (8%).

The WHO data for Nigeria, which showed that the prevalence of HBV and HCV was more than 8% and 1.2%, respectively, corroborated the rate of viral infection as it was seen in this review. Similar prevalences of HBV virus infections in other regions of Nigeria, including Southeast Nigeria (6.3%), were also reported by other workers in that nation [20]. Hepatitis B seroprevalence in pregnant women was estimated to be 2.9% in south-south Nigeria [21], according to another researcher [21], while it was reported to be between 18.3% and 12.5% by another group of workers in Nigeria [22, 23]. Table 1's prevalence of HBV among expectant mothers revealed that different professionals have reported low, intermediate, and high endemicity levels of the virus [24, 25].

The seroprevalence of HBV reported in this evaluation is consistent with other research on expectant mothers conducted in other nations with intermediate endemicity. (5.0%), Addis Ababa, Jimma, Ethiopia, [27]. Turkey (4.2%), Jeju Island of Korea (4.9%), Sierra Leon (6.2%), Zambia (6.5%), the USA, only for Asian Americans (5.6%), and [27, 30].Countries like Mali (15.5%), Hong Kong (10.0%), Papa New Guinea (11.0%), Taiwan (12.0%), Oman (7.1%), and Brazil (18.5%) were found to have a high prevalence of HBV, with the range of 7.2% to 38.5%. [28, 29]Reports from many nations have likewise indicated a low prevalence of HBV in the same research population. With the exception of Asian Americans, Mexico (1.65%), the northern region of Kerala State in South India (0.21%), Qatar, and the United Arab Emirates (1.0-1.5%), the USA had a prevalence of 0.14% to 0.97%.[30]

There have also been reports comparing the prevalence of HCV in Nigeria to other nations. The prevalence of HCV was reported to be 1.03% by Warda and colleagues, 2.1% in Gabon [31], 7.3% for anti-HCV, 2.2% for HBV, and 0.08% for co-infection of HBV and HCV by Batool and colleagues.

One of the main factors influencing the incidence and prevalence rates of HBV and HCV infections is the age at which an infection first appears. The age group (31-35 years) was found to have the highest prevalence of HBV (36.36%) and HCV (66.67%).

The age range of 26 to 30 years old came next, with prevalence rates for HBV and HCV of 24.53% and 22.22%, respectively. [18] found that the age range of 20–29 years had the highest prevalence of HBsAg (9.7%) and the age group of 30-35 years had the highest prevalence of HBeAg (4.5%). However, other studies that looked at HBsAg, HCV-A, and other infections in pregnant women covered the age range of 15–44 years and found that the majority of them were in the 25–29 years age group, with a prevalent rate of 32.8%. In a related study by [31], the age group of 26–30 years old had the highest incidence of HBV (7.5%), but no prevalence of HCV or co-infection was found.

The age group (30–34 years) had the highest prevalence (55.0%) out of 10 pregnant women who tested positive for HCV infection, followed by the age group (25–29 years) with 4 (40.0%). The age range of 15–20 years had the highest prevalence rate of HBsAg, whereas the age group of 21–26 years had the highest prevalence rate of Anti-HCV, at 20%, 20.0%, and 9.1%, respectively. It was shown that pregnant women between the ages of 15 and 20 had the highest prevalence of HCV antibodies. According to age, the prevalence rates of HBV and HCV among expectant mothers are consistent with research conducted in a few other nations.

According to reports, HBV was more common in women over 25 than in women under 25, yet the difference was not statistically significant from Egpt. Most of the women who tested positive for HBV in another study conducted by [32] from Pakistan were between the ages of 25 and 35. According to reports, pregnant women between the ages of 25 and 49 had a higher risk of contracting HCV than younger women (15–24 years old). The bulk of pregnant women who attended prenatal clinics at different hospitals in Nigeria belonged to the age groups that were the subject of the numerous research conducted there.

CONCLUSION

Acumen, Biopanda, and RapidResponse are the serologic diagnostic test kits used for Hepatitis C virus identification, and this study tested the Gene Drive molecular platform and these kits. The study discovered a high HCV prevalence of 13.10 percent. The serologic test agreements with HCV were measured using Cohen's kappa coefficient. A significant fraction of chronic viral hepatitides are caused by this widely distributed infectious pathogen. The comprehensive knowledge of the virus's structure, replication process, and precise roles for the different proteins may hold the key to stopping the hepatitis C pandemic. A potential vaccine and novel antivirals directed against the hepatitis C virus could be made possible by this insight.

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