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Research Article

Comparative Study Using Genedrive Molecular Platform and Acumen Antibody Strip in Diagnosis of Hepatitis C Virus Among Blood Donors in Anambra State.

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Abstract

Long-term government and stakeholder inertia has contributed to the quiet and neglected nature of chronic Hepatitis C virus (HCV) infection. The hepatitis C virus (HCV), discovered in 1988, has attracted the attention of numerous research groups worldwide. A comparative study was conducted in Anambra State to diagnose blood donors with hepatitis C virus using the Acumen antibody strip and the Genedrive molecular platform. A significant fraction of chronic viral hepatitides is caused by this widely distributed infectious pathogen. The comprehensive knowledge of the virus's structure, reproduction process, and precise roles for 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. Fifteen years after its discovery, the current understanding of the HCV genome is summarized in this paper. Described are the specific viral structural (core, E1, E2) and nonstructural (NS2, NS3, NS4, NS5) proteins and noncoding sections that are currently known to exist, along with their functions. Results that can be consistently verified by independent researchers are prioritized in light of the numerous contradictory reports from various study groups. Due to several challenges and restrictions associated with studying this remarkable virus, the existing understanding is insufficient, and the solutions to numerous significant issues are predictable.

Keywords: comparative, genedrive molecular platform, acumen antibody strip, hepatitis c virus.

INTRODUCTION

Donating blood should only be done voluntarily, without payment, and with the sole intention of helping an unidentified receiver. Thus, in developing nations, blood safety continues to be a key problem in transfusion medicine [1]. The World Health Organization (WHO) assists nations in establishing their own national blood systems to guarantee prompt access to adequate and safe supplies of blood and blood products by adhering to recommended transfusion practices to fulfill patient demands. The initiative promotes voluntary, unpaid blood donation as a means of achieving self-sufficiency in safe blood and blood products. It offers technical support and policy recommendations to guarantee that everyone has access to safe blood and blood products, achieving universal health care in the process [2]. WHO recommends that all donations be required to be checked for extremely prevalent illnesses that are likely to be spread through blood transfusions, in addition to encouraging unpaid voluntary blood donation. Every unit of blood collected in India must be tested for HIV, malaria, syphilis, hepatitis B, and hepatitis C. A donor's blood is deemed contagious and is disposed of if they test positive for any one of these five infections. Since infectious agents are carried by blood units provided by seemingly healthy and asymptomatic blood donors, it is even more crucial to thoroughly screen for these infections [4,5]. In order to improve the standards of Blood and its components, the Central Govt. through Drugs Controller General of India, has formulated a comprehensive legislation to ensure better quality control system on collection, storage, testing and distribution of blood and its components [6]. Central Govt. amended from time to time the existing requirements of

Blood Banks in the Drugs & Cosmetics Act, 1940 and Rules there under 1945 to meet the latest standards. Transfusion-transmissible infections such as HIV, HCV and HBV are among the greatest threats to blood safety and potential serious chronic sequelae associated with readily transmitted agents. Screening of blood donors first started in 1947.21Government of India has made mandatory to screen donated blood for HBV (since 1971), HIV (since 1989) and HCV (since 2001) [7]. Hepatitis C virus (HCV) infection can lead to chronic disease that may progress for decades without being noticed until symptoms of advanced liver disease appear.

Globally, an estimated 71 million individuals suffer with chronic HCV infection, with over 80% residing in low- and middle-income countries (LMICs). HCV should be eradicated by 2030, according to the World Health Organization (WHO). With the release of generic formulations and the development of short-course oral direct-acting antiviral (DAA) regimens with greater tolerability and cure rates, treatment for HCV is becoming more accessible globally [8]. Unfortunately, due to inadequate diagnostic services and inadequate care coordination, access to treatment is still restricted. High-throughput platforms are mostly used in specialized laboratories for nucleic acid testing for quantitative HCV RNA determination [9,10].

These tests can be expensive in general, and it may take several weeks for the results to be sent to the patients. HCV testing in LMICs may become more accessible and decentralized with the adoption of more transportable, reasonably priced, and user-friendly platforms that can be implemented in district hospitals or clinics. The Genedrive HCV ID Kit (Genedrive Diagnostics Ltd, Manchester, UK), henceforth called Genedrive, is one HCV RNA assay that can be used with less technical expertise. The majority of the technical specifications for a virological test are met by this test, according to the target product profile created by the WHO and FIND (Foundation for Innovative New Diagnostics).

Genedrive uses reverse transcription polymerase chain reaction (RT-PCR) to identify HCV RNA in a tiny amount of plasma (30 _L). The system does not require specialized knowledge or data interpretation in order to produce a straightforward, qualitative outcome. Due to its compact size and ease of use, the Genedrive instrument can be utilized in many decentralized laboratory environments with minimal need for additional equipment or test materials. The testing process for the Genedrive HCV ID Kit entails 12 manual steps and is comprised of lyophilized PCR reagents packaged into a single-use, disposable cartridge. The test takes roughly ninety minutes to complete [11].

After a positive HCV antibody test, it is meant to serve as a confirmation test for active HCV infection. The exam has a WHO prequalification and a CE mark. According to one study, Genedrive has a 2362 IU/mL analytical sensitivity, 98.6% sensitivity, and 100% specificity. Nevertheless, frozen plasma leftovers from a European research facility were used to assess the performance. It is not yet known how well Genedrive performs when used by intended users in real-world LMIC scenarios with recently obtained samples. For the purpose of confirming HCV viremia, Genedrive has the ability to decentralize and streamline testing procedures for HCV RNA testing [12]. The aim of this study was to evaluate the comparative studies usability of Genedrive molecular platform and acumen antibody strip in diagnosis of hepatities C virus among blood donorws in Anambra state.

MATERIALS AND METHOD

Research Design

The research design for this study shall be experimental and descriptive cross-sectional design, in which all intended participants that will consent to the study and will be screened for hepatitis C virus using standard protocols and parameters.

Population and Sample Size

This study will be 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:

The calculated minimum sample size was 246 subjects (Appendix Two). 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.

Population of Study

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

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 will be obtained from the Human Research and Ethics committees of the Nnamdi Azikiwe University Teaching Hospital, (NAUTH), Nnewi, Anambra State, Nigeria. Permission to use the laboratory units of the selected study centers shall also be obtained from the management of the study centers. Written and verbal consents will be properly sought and obtained from the intended participants. All information that will be obtained from participants including the findings of the laboratory test, will be treated with high level of confidentiality and used for the purpose of study only.

Collection of Data and Sample Collection

A structured questionnaire will 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 will be evaluated on a four – item, three-point Likert Scale. The four- terms will be 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 will be (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 will be 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.

Data 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 $p < 0.05$.

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 HBsAg ULTRA ELISA kits34 manufactured by Bio Rad).

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.

Table 4.3: Comparison of the Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and kappa values of Acumen, Biopanda and RapidResponse test kits versus Gene Drive Molecular Platform

Method	Sensitivity (95%C.I)	Specificity(95%C.I)	PPV (95%C.I)	NPV(95%C.I)	Kappa
Acumen	50.00 (26.45 – 75.35)	70.59(52.52-84.90)	44.44 (21.53-69.24)	75.00(56.60-88.54)	0.491
Biopanda	25.00 (7.27-52.38)	85.29(68.94-95.05)	44.44 (13.70-78.80)	70.73 (54.46-83.87)	0.611
RapidResponse	25.00 (7.27-52.38)	94.12 (80.32-99.28)	66.67 (22.28-95.67)	72.73 (57.21-85.04)	0.226

Key:

PPV: Positive Predictive Value

NPV: Negative Predictive Value

C.I: Confidence Interval

When the agreements between the diagnostic tests' kits were compared, Acumen and RapidResponse had poor agreement (k = 0.169), Biopanda and RapidResponse had a fair agreement (k = 0.257) while Acumen and Biopanda had a morderate agreement (k = 0.472).

Haematological parameters were also analyzed, these parameters include total white blood cell count (WBC), total red blood cell count (RBC), Haemoglobin (Hb), Packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total platelets count (PLT).

When the values of the haematological parameters obtained were compared between subjects that were positive for HCV using Gene Drive and those that tested negative for Gene Drive molecular platform, it was found that there was decreased WBC, Hb, PCV, RBC, MCV and MCH among the HCV positive cases while there were no observed changes in the values of MCHC and PLT among HCV positive and negative subjects. When the difference between these values and among the HCV positive and HCV negative cases were compared, it was found that there were statistically significant differences (P = 0.048, 0.045, 0.036, 0.048, 0.031 and 0.034) for WBC, RBC, Hb, PCV, MCV, and MCH respectively, whereas the differences were statistically non – significant for MCHC and PLT (p = 0.614 and 0.282) respectively. The sensitivity, specificity, Positive Predictive Value, Negative Predictive Value of Gene Drive molecular platform, and the test kits (Acumen, Biopanda and RapidResponse) are presented in table 4.3 above.

The knowledge of HCV was evaluated among the subjects studied. It was observed that 63.8% of the subjects were resident in urban areas while 36.3% were resident in rural places. Among the subjects studied, 58.1% knew HCV to be a virus while 41.9% did not know if it is a virus. Also, 43.3% had responded that vaccination is available for HCV, 32.2% responded that there is no vaccination while 24.5% did not know if vaccination was available or not. In the same way, 43.3% knew that Hepatitis C and Hepatitis B virus can be transmitted by sexual intercourse while 56.7% believed it cannot be transmitted by sexual intercourse. The indices that were used to measure the knowledge of HCV among the subjects is presented in table 14.

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 [19], who stated that the prevalence of viral hepatitis infections differs throughout the world among nations, regions, and population groups within a nation. In order to assess the sensitivity of third and fourth generation ELISA kits and to determine the prevalence of HIV, HCV, and HBsAg, 373 willing blood donors were examined for the current cross-sectional study.

The risk of transfusion-transmitted infection is increased in voluntary blood donors who have asymptomatic HIV, HCV, or HBsAg infections during the window period.The only source of blood that our blood bank receives is voluntary donations. Every donor that was a part of the study gave their consent to donate blood.There were 53 (14.2%) and 320 (85.8%) female participants in the current study. In a similar vein, 135 86.6% of the donors in the study conducted by [20] were men, while 13.4% were women. However, the overall sex distribution in the research [21] 127 was 4.6% female and 95.4% male. The primary causes of the low number of female donors were the high rate of anemia among Indian women and the poor level of knowledge, inspiration, and instruction surrounding voluntary blood donation among women.

The seroreactive levels of HCV and HBsAg in the current investigation were 0.8% and 0.27%, respectively. It was discovered that none of the willing blood donors tested positive for HIV.Arora et al. conducted a similar study in which they found that among 136 replacement donors in the state of Haryana, the seropositive rates for HBsAg, HCV, and HIV were 1.4%, 0.9%, and 0.3%, respectively. In the same study, voluntary blood donors were shown to be less seroreactive for HCV and HBsAg—0.3 and 1%, respectively—while none tested positive for HIV. Using third- and fourth-generation ELISA, the current study's estimation of the HIV seroreactive rate was 0 per 373 donors (0%).

The prevalence of HIV seroreactivity in 137 donors was estimated by Sheetal Malhotra et al. to be 1.37 per 1000 contributions (0.13%) using third-generation ELISA and 3.62 per 1000 donations (0.36%) using fourth-generation ELISA. Similar to our study, theirs involved only willing blood donors; however, it was discovered that a small increase in seroreactivity as determined by 4th generation ELISA was the result of more false positive results (33% of samples found to be seroreactive by 4th generation ELISA were found to be nonreactive by western blot study).In their study of volunteer blood donors from rural Andhra Pradesh, Kola Sujatha et al., 130 discovered that 1% of the donors were HIV seropositive by 4th generation ELISA and 0% by 3rd generation ELISA.

In the present study, HBsAg seroreactive was estimated to be same (0.8%) by both 3rd and 4th Generation ELISA. In a study among blood donors in Mumbai (both replacement and Voluntary), Sachin Shivaji kapse and the others, 135 found 1.1% HBsAg seroreactive by 3rd generation ELISA. It was stated in their study among voluntary blood donors in Gujarat, by 4th generation HBsAg ELISA found 0.63% HBV seroreactive [21]. In the present study, HCV seroreactive was estimated to be 0 per 373 donations using 3rd generation ELISA and 0.27 % (1 per 373 donors) HCV by 4th generation ELISA. Makroo and the others,5 studyamong blood donors (96.9% replacement and 3.1% voluntary) in New Delhi revealed 0.43% seroreactive by 3rd generation ELISA. The study in Gujarat found 0.21% HCV seroreactive by 3rd generation ELISA.138 in their study among blood donors in New Delhi (25.4% replacement and 74.6% voluntary) found 0.73% HCV seroreactive by 4th generation [22].

The wide variations in the seroreactive of HIV, HCV and HBV among the voluntary blood donors in different studies conducted in India is most probably due to the use of different methods of testing and use of different generation of ELISA test kits having different sensitivities and specificities.In the present study, the most common age group of voluntary blood donors were less than 18-20 years (49.1%) and 32.4% in 21-30 years, 9.4% in 31-40 years, 6.7 % in 41-50 years, 2.4% in >50 Years. However the age distribution of seropositivity among the voluntary blood donors is seen in 21-30 years of age which is 3.3% and these is similar to the following studies,In the study by Sachin Shivaji kapse and the others, 135 Majority of TTI positivity is among 18 – 27 years about 0.59%.(3rd generation). In the study by [23].,139 had 69.8% positive donors belonged to the age group of 18-30 years, and 28.3% belong to 31-45 years age group.

In the study conducted by Solomon Bisetegen and the others,140 65 (23.6%) of donors less than or equal to 30 years and 50 (43.9%) of donors greater than 30 years have evidence of at least one blood borne pathogen.(4th generation). The present study had all seroreactive donation belonged to male gender which implies no female donors had seroreactivity [24,25]. In which 3 donations belonged to HBV and 1 had HCV positivity. The 3rd generation ELISA showed 3(0.94) out of 4 positive donations and did not pick up 1(0.31%) out of 4HCV positive donation. Whereas 4th generation ELISA picked all 4 (1.25%) out of 4 as positive reactions (3 HBsAg and 1HCV). In the present study among the total of 373 voluntary blood donations 233 were first time donation and the remaining 140 were repeat donations. In which all3 HBsAg seroreactive donations, 2 (233) HBsAg seroreactive donations (0.86%) belonged to first time donors and the remaining 1 (140) 0.71% seroreactive donation belonged to a repeat voluntary blood donation which showed

seroreactivity in 3rd generation ELISA. However, 3rd generation ELISA did not pick up 1 HCV seroreactive donation. Further, in 4th generation ELISA picked up all 4 /4 seroreactive donations in which 3 HBsAg seroreactive donations, 2 (233) HBsAg seroreactive donations (0.86%) belonged to first time donation and the remaining 1 (140) 0.71% seroreactive donation belonged to a repeat voluntary blood donation and further, it also picked up 1 seroreactive HCV belonged to first time donation. Similarly in the study conducted by Sachin Shivaji kapse et al,135 1.01% seroreactivity among 1st time donors and 0.38%seroreactivity in repeat donors the study was done by using 3rd generation ELISA kits.In the study done by [26].,137 had 1.61 /1000 1st time donors and, 1.23/1000 repeat donors found seroreactive in 3rd generation ELISA however in 4th generation ELISA 2.94/1000 showed seroreactive results and 0.69/1000 found to be in grey zone in both 1st time and repeat donors.

In this study, to confirm seropositive samples RT-PCR was done. All 3 samples which were HBsAg seroreactive by both 3rd and 4th generation ELISA showed the presence of HBV DNA by RT PCR, thereby ruled out any false positive reactions. Only one sample was seroreactive by 4th generation ELISA, that too was not detected by 3rd generation ELISA. RT PCR done on this sample revealed the presence of HCV RNA (2.8x105IU/ML, Genotype 1b), thereby ruled out false positive reaction by 4th generation ELISA.

The sample which was nonreactive by 3rd generation ELISA found to be reactive by 4th generation ELISA for HCV is due to the ability of 4th generation ELISA to detect HCV core antigen much earlier than HCV antibody by 3rd generation ELISA. The Syria Laperche and the others, 134 study on simultaneous detection HCV core antigen and anti-HCV antibodies on voluntary blood donors revealed 33 to 46% reduction in the HCV related TTI. All the four seroreactive donors were given post-test counselling and were advised to refrain from high risk behaviour and also to self-exclude from future donations. All the four seroreactive donors was referred to medical gastroenterology department at a higher centre for counselling, Management and further follow up.

This study was conducted to compare detection of HBV, HCV and HIV among voluntary blood donors in Chennai by 3rd and 4th generation ELISA. Among total 373 voluntary blood donor samples, 3 were found to be seroreactive for HBsAg by 3rd and 4th generation ELISA. Only one sample was found to be seroreactive for HCV by 4th generation ELISA, this was found to nonreactive by 3rd generation ELISA. None of the samples were reactive for HIV by both 3rd and 4th generation. 233 donors were first time donors and the remaining 140 were repeat donors.2 of the first time donor samples and one of the repeat donor samples were found to be reactive for HBsAg by both 4th and 3rd generation. The sample which was reactive for HCV by 4th generation ELISA was from a first time blood donor.

The 3 samples which were found to be reactive for HBsAg by 3rd and 4th generation ELISA and only one sample which was reactive for HCV by 4th generation ELISA were confirmed by RT PCR.

Conclusion

Acumen, Biopanda, and Rapid Response 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. Of the three diagnostic test kits that were examined, Acumen was determined to have the best agreement among them, with a moderate score. The gene drive molecular platform, which detects the presence of HCV nucleic acids directly in the samples and is therefore very sensitive and highly specific, was utilized as the gold standard to evaluate the three diagnostic test kits that were examined.

In comparison to samples that tested negative for HCV, this study discovered a drop in a few haematologic markers in samples that tested positive. Total white blood cells, hemoglobin concentration, packed cell volume, mean cell volume, and mean corpuscular hemoglobin are among the metrics that dropped; however, platelets and mean corpuscular hemoglobin concentrations remained unchanged. To lower the prevalence to the lowest possible level, this study recommends increasing public education about HCV, its routes of transmission, and preventative techniques.

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