

COMPARATIVE ANALYSIS OF SENSITIVITY AND SPECIFICITY OF ANTI-HCV RAPID DIAGNOSTIC TEST (RDT) WITH HCV QUANTITATIVE POLYMERASE CHAIN REACTION (PCR): A LONGITUDINAL STUDY OF PUNJAB, PAKISTAN

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Abstract

With an extensive burden of chronic viral Hepatitis C, Pakistan is ranked second in the World in terms of disease burden after China and second in prevalence after Egypt. The Punjab Hepatitis & Infection Control Program has established a network of Hepatitis Clinics across the province in district, tehsil, teaching and partner organizations hospitals with provision of services in a standardized manner. Anti-HCV Rapid diagnostic test kits (RDTs) are convenient method of serological testing for screening for Hepatitis C. In this study, the sensitivity, specificity and diagnostic practicality of SD Biotec anti-HCV RDTs are assessed and compared with Quantitative HCV Polymerase Chain Reaction (PCR) for the detection of HCV through analysis of year-wise screening vs PCR data extracted from the electronic medical record (EMR) of the Hepatitis and Infection Control Program, Punjab from 2017-2022. After analyzing the complete data of 2,181,212 people who visited the Hepatitis Clinics and were screened for HCV on anti-HCV RDTs from August 2017 to December 2022, a total of 699,705 were screened positive for anti-HCV RDTs. Out of 699,705 individuals, only 376,467 were confirmed through HCV PCR meaning thereby confirmation rate for HCV RDT screened positive individuals stand at 50-60%. The data also indicated that 1,481,507 people were detected as negative. Based on the accuracy of HCV RDTs versus HCV PCR tests, there is a probability that the screened negative individuals might be carrying HCV and could not be detected. This is because of the low accuracy rate of HCV RDTs which might be a symptomless and silent source of HCV spread among the population. Keeping in view the findings of the study, it is recommended that manufacturers manufacturing anti-HCV RDTs should improve sensitivity by 95%. Secondly, the widespread use of anti-HCV RDTs may be curtailed and more sensitive methods of screening may be adopted at mass level. Thirdly, data health facility level data indicates that anti-HCV positivity at 17%, therefore, a fresh population level prevalence survey may be conducted or the Egyptian model may be replicated in order to eliminate Hepatitis C from Pakistan by 2030.

INTRODUCTION

Viral Hepatitis is an alarming public health problem around the World (Mustafayev & Torres, 2022). According to WHO, chronic Hepatitis B and C viruses affects 257 million and 71 million people worldwide respectively, resulting in millions of deaths annually. Hepatitis B, C & D are blood-borne infections, while Hepatitis A and Hepatitis E are

transmitted through the oral-fecal route with water and food as the main sources of infection (Lanini et al., 2019). Both Hepatitis B and C, if left untreated, can lead to end-stage liver diseases including liver cirrhosis, hepatocellular carcinoma, and liver-related deaths (Wiegand & Berg, 2013).

With an alarmingly high burden of chronic viral Hepatitis C, Pakistan stands second to China Vis a vis disease burden and second to Egypt in terms of prevalence (Khan et al., 2019; Sievert et al., 2011). The province of Punjab accounts for almost 60 to 70% of all Hepatitis cases in Pakistan. In 2008, Hepatitis C prevalence in the province of Punjab stood at 6.7% well above the national prevalence of 5% and that of Hepatitis B was 2.4% while the national prevalence was 2.5% ("Pakistan Hepatitis B and C Prevalence Survey 2007-2008,"). Come 2018, Hepatitis C prevalence in Punjab surmounted to 8.9% while that of Hepatitis B stood at 2.2% ("Seroprevalence Survey by Bureau of Statistics, Government of Punjab 2018,"). Pakistan is facing a public health emergency, while under SDG (3.3) ("WHO Guidelines Approved by the Guidelines Review Committee," 2014), it is committed to eliminating Hepatitis C by treating 90% of patients by 2030. It is estimated that up to 11 million individuals have active HCV viremia, and 240,000 new patients' contract HCV infections each year in Pakistan. With up to 11 million individuals with active HCV viremia, the province of Punjab needs to treat 850,000 individuals annually as per Bristol Modelling Study.

Hepatitis & Infection Control Program working under Primary and Secondary Healthcare Department, Government of the Punjab has established a network of 281 Hepatitis Clinics, and the program is implementing a 4-prong strategy of 'educate, prevent, test and treat' to tackle the menace of viral Hepatitis B and C across the province of Punjab. Rapid diagnostic tests (RDTs) serve as a convenient method for serological testing to screen individuals for HCV infection. The RDTs for HCV screening, SD Bioline HCV (Standard Diagnostics, Kyounggi-do, Korea), are based on immuno-chromatographic testing for the detection of anti-HCV, against HCV recombinant core, NS3, NS4, and NS5 antigens, in human serum, plasma or whole blood (Narasimhan et al., 2020; Kim et al., 2013). In this study, the sensitivity, specificity and diagnostic practicality of SD Bioline anti-HCV RDTs was assessed and compared with quantitative PCR for the confirmation of HCV through analysis of year-wise screening and PCR testing data from the electronic medical record (EMR) of Hepatitis and Infection Control Program, Punjab from 2017-2022. This EMR system is functional across all Hepatitis clinics working under Hepatitis and Infection Control Program, Punjab since August 2017.

METHODS

Data Extraction and Analysis

The analysis was based on secondary data retrieved from the Electronic Medical Record (EMR) of the Hepatitis & Infection Control Program, Punjab from 2017-2022. The number of screened positive patients for HCV, through RDTs is compared with the PCR confirmation results for HCV being positive or negative. The Electronic Medical Record (EMR) system was developed in 2017, to serve as a data repository for clients facilitated

by Hepatitis & Infection Control Program, Punjab, and became fully operational in August 2017 (dashboard shown in **Figure 1**). The three-tier management system is being implemented through Hepatitis Control Program, Punjab EMR, and through a proper referral module, patients can be referred from the first tier (Hepatitis B and C generic cases already being dealt with at Hepatitis Clinics of DHQ and THQ hospitals), second tier (referral component for referring patients to Gastroenterology and endoscopy units for complicated/ advanced cases; **Figure 2**) and third-tier (referral module for referring cirrhotic/ cancer cases to PKLI for liver transplantation).

HCV Screening and PCR Protocols

The sample for screening was obtained by the prick method and analyzed using the standard procedure as outlined in the respective HCV RDT manual. Blood samples drawn for quantitative PCR were analyzed using Roche Cobas 6800 and Abbott m-2000 HCV PCR systems using standard procedures.

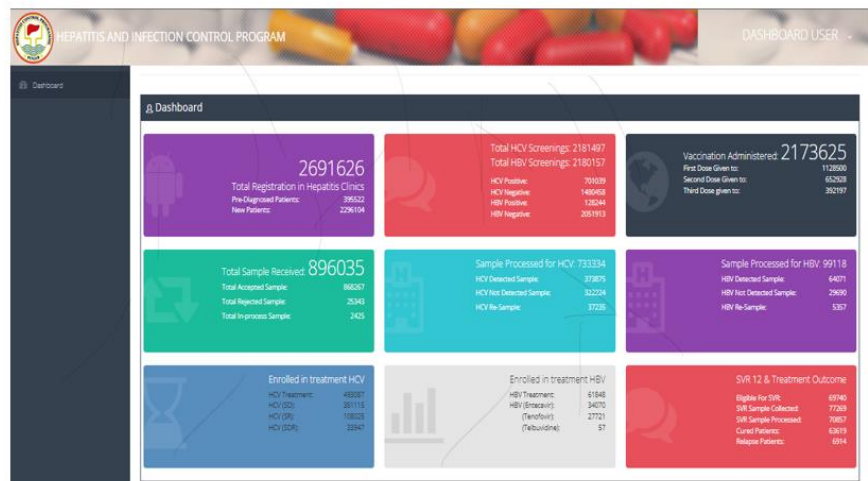


Figure 1: Dashboard of Electronic Medical Record (EMR)

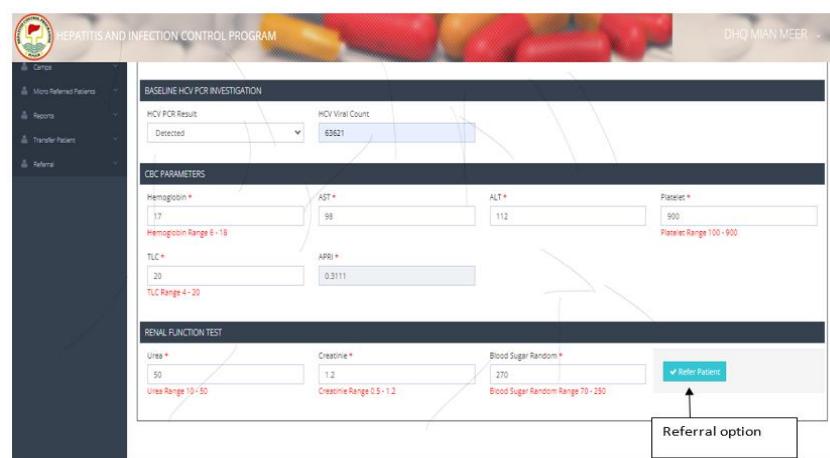


Figure 2: Referral option of HCV patient to 2nd tier of care

RESULTS

Total data extracted from EMR from 2017-2022 comprised of 699,705 individuals who were found positive on HCV RDT. **Figure 3** represents the breakdown of number of screened positive individuals on HCV RDT (i.e. 699,705) year-wise from 2017-2022. Among all the years, the highest number (190,995 individuals) were screened positive for HCV in the year 2022, followed by the year 2018 in which 186,419 individuals were screened positive on HCV RDT.

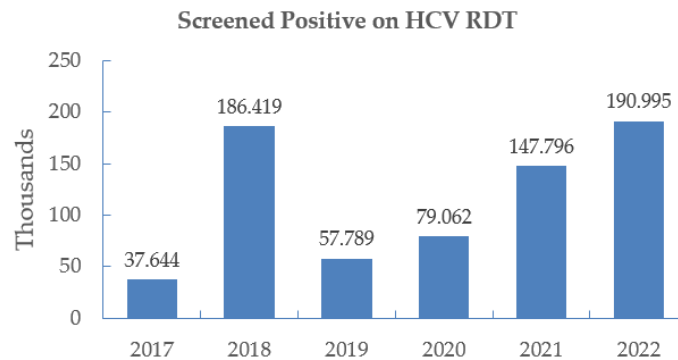


Figure 3: Number of HCV screened positive individuals on HCV RDT from 2017-2022

Figure 4 shows the numbers of HCV detected positive on HCV RDT but confirmed negative on PCR from 2017-2022. It was noticed that a significant number of individuals who were previously screened positive on HCV RDT were found negative on PCR every year from 2017-2022. In total, out of 699,705 screened positive on HCV RDTs, 376,467 were actually confirmed positive upon quantitative PCR and the remaining 323,238 were found negative on quantitative HCV PCR which amounts to almost half of the total screened positives.

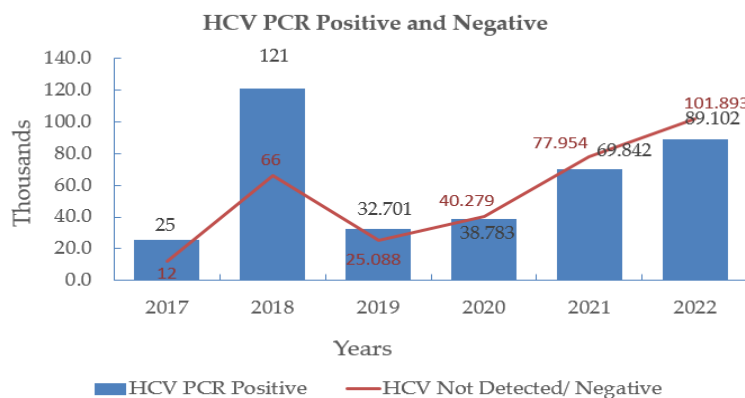


Figure 4: Numbers of HCV detected positive and detected negative on PCR from 2017-2022

The trend of % PCR positivity out of screened positive from 2017-2022 is depicted in **Figure 5** which shows a decreasing trend starting from 67% in 2017 to 47% in 2022. **Figure 6** shows comparison between ratios of HCV PCR positive and negative individuals out of screened positive from 2017-2022. For the years 2017-2019, the PCR positives were greater in number than negatives; however, from 2020-2022, the percentage of positives became lesser than negatives, showing the increasing effect of false positives in screening results using HCV RDT. The overall data of screened individuals for HCV from 2017-2022 has been shown in Table 1. Pie charts representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative for each year are shown in **Figures 7-12**.

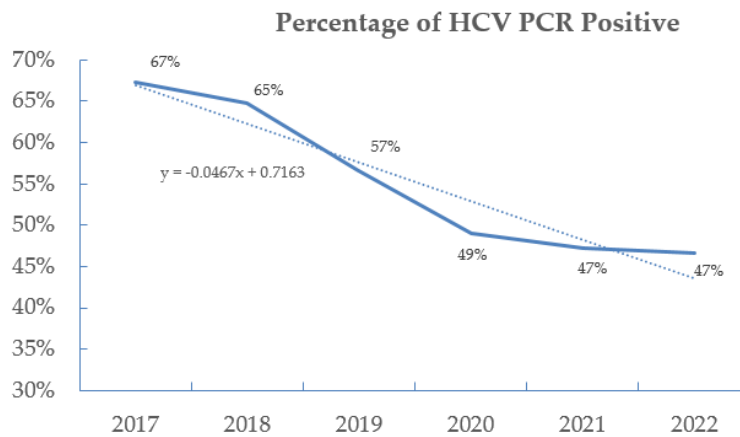


Figure 5: Percentage of HCV PCR positive out of screened positive individuals from 2017-2022

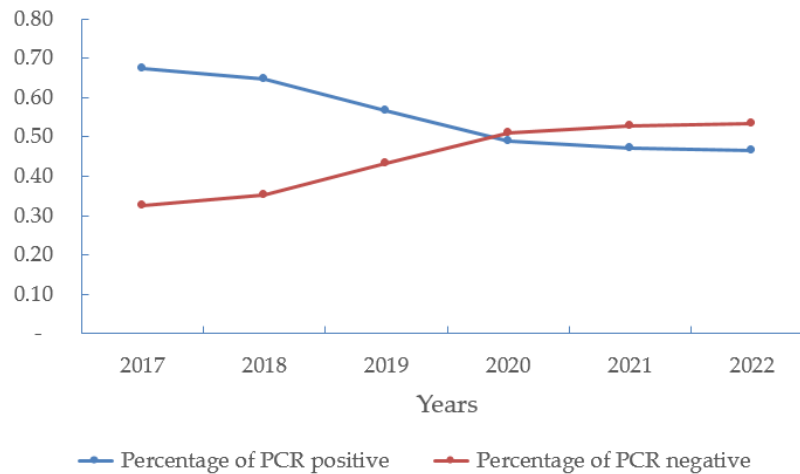


Figure 6: Comparison between ratios of HCV PCR positive and negative individuals out of screened positive from 2017-2022

Table 1: HCV Screened Individuals Data Extracted from Electronic Medical Record (EMR)

Timeline	Total Screened for HCV	Screened Positive on HCV RDT	HCV PCR Positive	HCV Not Detected/ Negative	Percentage of PCR positive	Percentage of PCR negative
Aug 2017- Dec 2017	152,279	37,644	25,358	12,286	67%	33%
Jan 2018- Dec 2018	541,440	186,419	120,681	65,738	65%	35%
Jan 2019- Dec 2019	313,775	57,789	32,701	25,088	57%	43%
Jan 2020- Dec 2020	273,403	79,062	38,783	40,279	49%	51%
Jan 2021- Dec 2021	385,107	147,796	69,842	77,954	47%	53%
Jan 2022- Dec 2022	515,208	190,995	89,102	101,893	47%	53%
Total	2,181,212	699,705	376,467	323,238	54%	46%

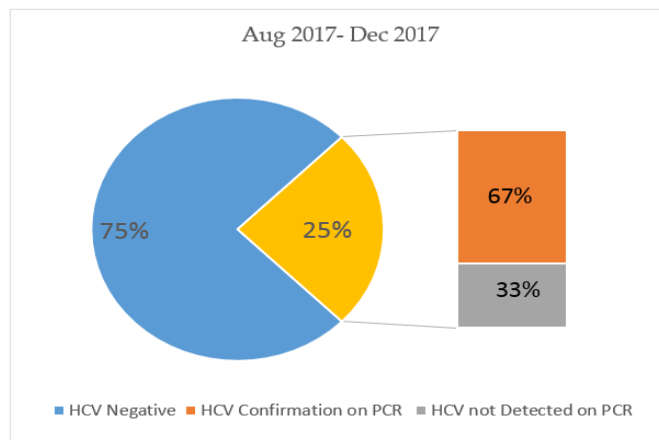


Figure 7: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Aug 2017- Dec 2017

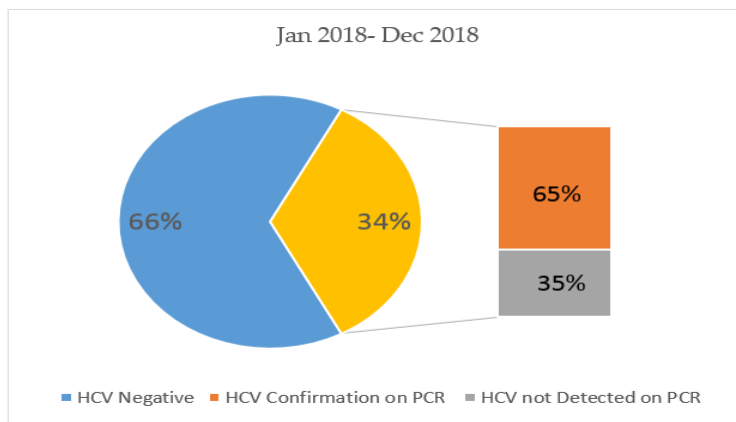


Figure 8: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Jan 2018- Dec 2018

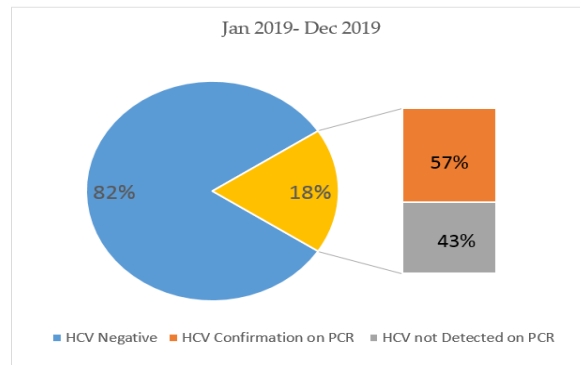


Figure 9: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Jan 2019- Dec 2019

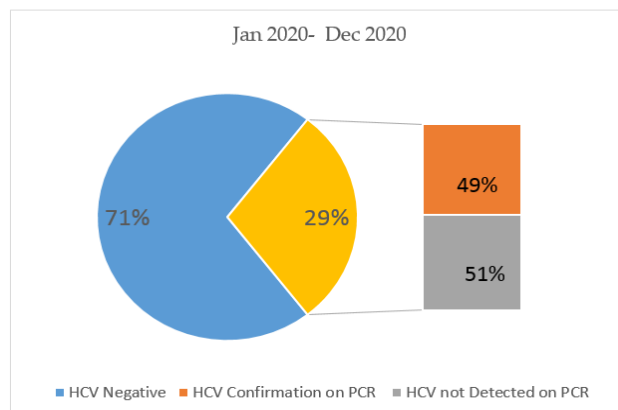


Figure 10: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Jan 2020- Dec 2020

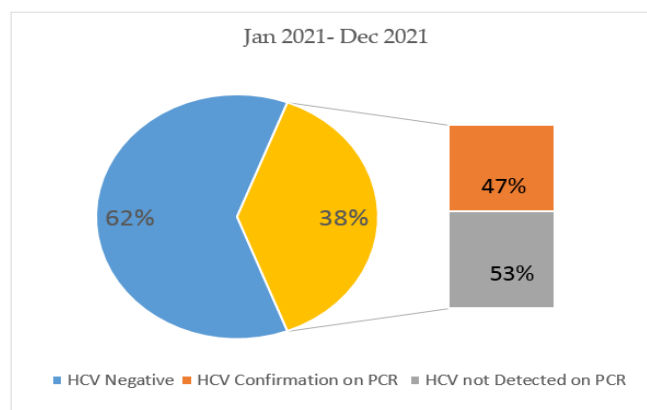


Figure 11: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Jan 2021- Dec 2021.

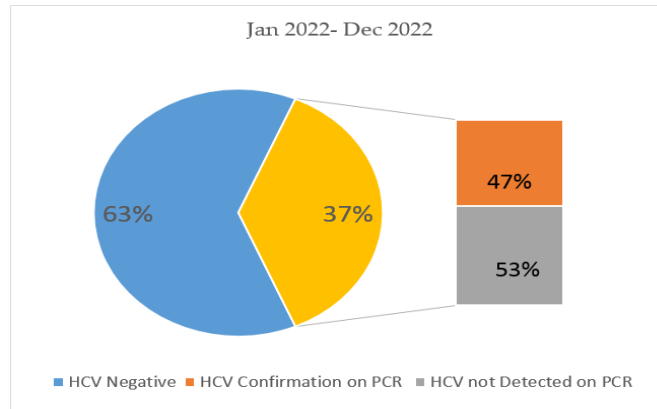


Figure 12: Pie chart representing percentages of individuals screened positive and negative on HCV RDT and HCV PCR positive and negative from Jan 2022- Dec 2022

DISCUSSION

This study analyzed the longitudinal data of 2,181,212 individuals from August 2017 to December 2022 who visited the 281 Hepatitis Clinics across Punjab and got screened for HCV. To identify the positive individuals, Hepatitis Control Program designed a two-step strategy for detection of Hepatitis C. At first step the reported person is screened on HCV RDTs to identify that whether the individual is suffering from Hepatitis C or not. At the second step, the blood sample of the HCV screened positive individual is sent to the Hepatitis Reference cum Public Health Lab and Research Centre for PCR to confirm the positive case and reduce false positive cases.

The current study used the data taken from the Electronic Medical Record (EMR) of the patients, Hepatitis Control Program Punjab, Primary & Secondary Healthcare Department, Punjab. The data entry of patients is quite accurate and systematic. The system was initially designed by Punjab Information Technology Board (PITB) and later on managed by Health Information and Service Delivery Unit (HISDU), P&SHD, Punjab thereafter. The Hepatitis Control Program, Punjab established 281 Hepatitis Clinics at District Headquarter (DHQ) and Tehsil Headquarter (THQ) Hospitals to record the particulars of the patients and screen them through Rapid Diagnostic Tests (RDTs) to find out whether the patients are Hepatitis C positive or negative. The Department used FDA approved WHO pre-qualified HCV RDTs procured from M/s Abbott Laboratories. The program screened 152,279 individuals for HCV and found 37,644 screened positive for HCV during August, 2017 to December, 2017. Then the patients' blood samples were sent to Provincial Lab of Hepatitis Control Program for HCV PCR tests and found that 25,358 patients were confirmed with Hepatitis C positive representing 67% accuracy of the HCV RDTs. Whereas, during the period of Jan 2018 to December 2018, 541,440 individuals visited Hepatitis Clinics and found 1,86,419 individuals screened positive on HCV RDTs. To get the confirmation and to check the accuracy of tests, the samples were

sent to PCR lab and evaluated that 1,20,681 were confirmed with Hepatitis C positive ensuring the 65% accuracy of RDTs.

Moreover, during the period of January, 2019 to December, 2019 313,775 people visited the clinics and found that 57,789 screened positive on HCV RDTs. The blood samples of these patients were re-evaluated through PCR and found that 32,701 were correctly reported positively on HCV RDTs which indicates the sensitivity of 57%. Further, it was reported that 273,403 people visited the Clinics during the period of January, 2020 to December, 2020 for HCV screening. According to EMR, 79,062 individuals were screened positive on HCV RDTs. Their blood samples were re-evaluated through PCR and found that 38,783 were correctly reported positively on HCV RDTs which showed the accuracy rate up to 49%. It is pertinent to mention here that due to COVID-19 pandemic; a lesser number of people attended the Hepatitis Clinics as compared to previous year. But the ratio of visits increased during January, 2021 to December, 2021 and reached up to 385,107 due to two reasons. Firstly, COVID-19 pandemic was going to reduce, and Primary & Secondary Healthcare Department, Punjab took the administrative control of Hepatitis Clinics in One hundred nineteen Tehsil Headquarter hospitals, twenty-six District Headquarter hospitals, three dispensaries, sixty Rural Health Centers, and twenty Hepatitis Clinics of Specialized Healthcare and Medical Education Department and in partnership with four private-partner organizations. It was found that 147,796 individuals were screened positive for HCV out of 385,107. For step-2 confirmation, their blood samples were re-evaluated at PCR Lab and found that only 69,842 were confirmed positive out of 147,796 individuals. The accuracy of HCV RDTs in 2021 remained 47% which was alarming.

Afterwards, after the end of Covid-19 pandemic i.e. in 2022, 515,208 individuals visited 232 Hepatitis Clinics across Punjab out of which 190,995 were screened positive on HCV RDTs. Relying on the previous accuracy of the HCV RDTs, samples of patients transported to PCR lab for confirmation and found that 89,102 patients were suffering from Hepatitis C disease which indicated 47% accuracy of the RDTs. The data indicated that overall reliability of HCV RDTs was almost 50% which is alarming from two aspects. Firstly, 50% people may not be accurately detected by HCV RDTs. It was noted with grave concern that if the RDTs were wrongly screening people positive, then there is probability that these kits were also reporting false negatives, who could actually be HCV positive and could be a continuous source of HCV spread across Punjab. It is pertinent to mention here that 1,481,507 individuals were screened negative for HCV from August, 2017 to December, 2022. As per the data, it can be assumed that accuracy of RDTs is 50-60% and there is a probability that the screened negative individuals might be carrying HCV and could not be detected due to low accuracy rate of HCV RDTs and might be a symptomless and silent source of HCV spread among their family, dear ones and colleagues.

CONCLUSION

After analyzing the complete data of 2,181,212 people who visited the Hepatitis Clinics and were screened for HCV on RDTs from August 2017 to December 2022, a total of

699,705 were screened positive on RDTs. out of 699,705 individuals, 376,467 were confirmed through HCV PCR testing thus showing HCV RDT accuracy of 50-60%. The data also indicated that 1,481,507 people were detected as negative. Based on the accuracy of HCV RDTs versus HCV PCR tests, we can suspect that almost 7 hundred thousand people might be declared false negative by the RDTs and those individuals would possibly be transmitting HCV in more people.

Recommendations

First and the foremost is that the manufacturers manufacturing anti-HCV RDTs should improve test sensitivity. Second recommendation is to reduce the reliance on RDTs owing to their low sensitivity and specificity. Thirdly the data indicates that anti-HCV positivity rate is up to 17%; therefore, a fresh survey may be conducted to ascertain the exact situation so as to correctly design and execute the elimination strategy or the Egyptian model may be replicated in order to eliminate Hepatitis C from Pakistan.

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