

Investigation of Hepatitis B and Hepatitis C in the blood of hemodialysis patients from Peshawar, Pakistan.

Abid ali (✉ abid.biosci@gmail.com)

Islamia College University Peshawar Pakistan <https://orcid.org/0000-0002-7646-6304>

Gul Nabi Khan

Department of Zoology, Islamia College University Peshawar Pakistan

Muhammad Salman

Department of Biotechnology and microbiology, Abasyn University Peshawar Pakistan

Muhammad Numan Khan

Endocrinology lab, department of Zoology, Quaid i Azam University Islamabad Pakistan

Asad Ullah

Zoology department, University of Agriculture Faisalabad Pakistan

Abdul Haleem Khan

Zoology department, Islamia College University Peshawar Pakistan

Shoib Wazir

Zoology department, University of Agriculture Faisalabad Pakistan

Fahad Shamim

Zoology department, Islamia College University Peshawar Pakistan

Samra Noreen

Microbiology department, Quaid-i-Azam University Islamabad Pakistan

Sher Akbar

Statistic department, Quaid-i-Azam University Islamabad Pakistan

Research

Keywords: HBV, HCV, PCR, Hemodialysis

Posted Date: July 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-36829/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Aim: High prevalence of Hepatitis C virus (HCV) and Hepatitis B (HBV) has reported among dialysis patients throughout the world. The aim of this study was to find out the frequency of HCV and HBV both in Hemodialysis (HD) at District Peshawar, the capital of Khyber Pakhtunkhwa Province of Pakistan.

Methods: A cross-sectional study was conducted from July 2018 to March 2019. The infections were investigated through serological and molecular techniques. The polymerase chain reaction was performed for only HCV RNA detection in HD patients. The data obtained were compared with available past studies from Pakistan.

Results: To evaluate the frequency of HCV, HBV, and co-infection in HD patients. 200 blood samples were collected from dialysis patients who visited Lady Reading Hospital Peshawar. Among 200, HCV infected were diagnosed in 22%, HBV in 8%. The percentages of male to female diagnosed patients with HCV were 21% and 20% respectively. Moreover, the chi-square test of association among variables based on our empirical findings, it is clear that odds of HCV positive among males is 3.467 times greater than female, with statistically significance ($P= 0.012^*$) against 50 and above age group..

Conclusion: The percentage of HCV positive was found higher in the overall HD patient population. Only 7.5% of patients were found Negative of HBV and HCV in both males and females visited hospitals for dialysis. The high prevalence of hepatitis viruses among HD patients indicates a close relation between HD centers and hepatitis virus transmission. Therefore, preventive control measures are essential to reduce hepatitis transmission in HD centers

1. Background

Patients undergoing HD have an excess risk of acquiring blood Borne virus infections. The prevalence of hepatitis B virus and hepatitis C virus (HBV and HCV) infections in dialysis- treated patients is commonly higher than the general population [1]. HBV and/or HCV infections in dialysis patients increase morbidity and mortality risks due to liver disease and reduced immunity. The prevalence and incidence of HBV and HCV infections among dialysis patients vary widely between countries and also within the same country and it correlates with the prevalence in the general population [2.] Global data indicate that the prevalence of HBV and HCV infection is high in the populations of Africa and the Middle-East regions [3]. The overall prevalence of both infections in maintenance dialysis patients is decreasing in many countries. The prevalence of HBV infection in dialysis patients in Western Europe and the United States ranges between 0% and 7% [4] Burdick et al., 2003. Reports from Hong Kong and Brazil showed a higher prevalence of approximately 10% [5]., 2004. Data from Saudi Arabia showed a prevalence rate of 4.6% (SCOT. Saudi Centre for Organ Transplantation- Annual Report, 2009) and from Jordan of 5.9% [6]. A low rate of 1.6% was reported in Japan. The prevalence of HCV seropositivity, on the other hand, is generally higher than HBV in dialysis populations though it differs widely between countries. Previous epidemiological studies have shown that the prevalence of HCV seropositivity among patients receiving HD varies from as low as

6.1% in Germany [7]. Reports showed that the prevalence of HCV seropositivity in HD patients was 50% in Saudi Arabia [8], 42% in Tunisia [9], 20.2% in Turkey [10] and 21 % in Jordan [11]. Multiple factors contribute to the increased risk of HBV and HCV infections among dialysis patients. Observational studies carried out in various centers worldwide have shown that the number of blood units transfused [12] the duration of HD a history of previous renal transplant [13].

Healthcare-related HCV transmission can be eradicated with control measures planned to avoid transmission of blood-borne pathogens [14]. No documented data have been reported on the prevalence of hepatitis viruses among the HD patients in Peshawar, Pakistan, and to the best of our knowledge, this report is the first to address both HCV and HBV issues. Therefore, the specific objectives of this study were to investigate the frequency of HBV and HCV among the HD patients of Peshawar, Pakistan, and to assess the major risk factors for transmission of these viruses among HD populations [15].

2. Methods

The study included individuals from all over the Peshawar District of Khyber Pakhtunkhwa province. Informed consent was taken from individuals under observation at Lady Reading Hospital Peshawar, one of the biggest and functional hospitals in the region. A cross-sectional study was conducted from July 2018 to March 2019 and a total of 200 blood samples were collected from the people who visited. We recorded a detailed history of the individuals on a questionnaire. The blood sample was collected from each patient by using a 5 ml syringe and used a centrifuge tube and the serum was separated from the blood.

Immuno-chromatographic tests (ICT)

For the detection of HBsAg version 2.0 ICT (Immuno Chromatographic Techniques) kit (Heiligen Scientific LLC, Houston, Canada) while for those anti-HCV antibodies detection, HCV version 3.0 ICT kit (Accurate Diagnostics, Healgen Scientific LLC, Houston, USA) was used for all the collected samples. A nonreactive sample was declared negative HCV. The sample diagnosed positive in the case of HCV only, we run PCR for further confirmation, and because of limited time and fund we were not able to run PCR for the positive HBV samples.

RNA Extraction and PCR

HCV RNA was extracted from 200 µl serum samples by using the Ana-gen RNA extraction kit (Ana-gen, USA) according to manufactures' instructions. cDNA was prepared by Reverse transcription PCR using M-MLV reverse transcriptase (Fermentas, USA). The amplified cDNA was further subjected to two rounds of PCR amplification using nested primers [18]. The conditions for the first round PCR were as follows; An initial denaturation step at 95°C for 2 minutes followed by 30 cycles of 94°C for 45 seconds, 54°C for 45 seconds, and 72°C for 1 minute performed in a thermal cycler (Eppendorf, Germany). The conditions for the 2nd round PCR were the same except that a different set of inner primers was used and the annealing temperature was raised to 62°C in order to amplify the 1st round product. [16; 17].

Gel electrophoresis and Documentation

The amplified product was subjected to electrophoresis. Agarose (2 %) gel was prepared in 0.5X Tris-Cl boric acid EDTA (TBE) buffer. Agarose and TBE was boiled in the microwave oven for 2 minutes and then was cooled up to 50°C, ethidium bromide was added and was run in an electric field. The amplified product was evaluated under ultraviolet light. Amplified products were determined by comparing with 100bp DNA ladder marker Ferment as the USA, was used as a DNA size marker.

Statistical Analysis

All the data taken from the different people during sample collection were put into R version 4.0 for chi square test among different age with the significant $P > 0.05$.

3. Results

A total of 200 samples were taken from the patients of HD at Lady reading hospital Peshawar. From the total, about 161 were collected from men while the remaining 39 from the female. Among those anti-HCV antibodies were diagnosed in 37%, anti-HBV antibodies in 8%, infection were documented in 3.5% using Immuno Chromatographic test (ICT). Nevertheless, we also recorded 15 HD patients with no Anti-bodies for any virus which remain high in male Figure 1.. Moreover, due to limited time and resources, we continue and only the anti-HCV positive samples were processed for RNA extraction and RT PCR, the result revealed that RNA were detected with 21% in male and 20% in female. The prevalence of anti-HCV antibodies and HCV RNA in the case of the male and female population as determined by the combination of Immuno Chromatography procedures and PCR is given in Table 1.

Moreover, the chi-square test of association among variables based on our empirical findings, it is clear from Table 2 that odds of HCV positive among males is 3.467 times greater than female, with statistically significance ($P= 0.012^*$) against 50 and above age group. The testing ability of both ICT and RNA methods for only HCV were compared, which revealed the best performance of RNA based tests. Up to this end, table 3 shows the odds of being tested positive using RNA for males is 2.246 times more than females, against 50 and above age group with the significance level of 0.05.

However, Table 4 shows the prevalence of males with HBV positive is 2.456 times more than females against 50 and above age group with significant ($P= 0.021^*$). Finally, it is concluded that the age group of 50 and above ages, were found statistically significant with more effected by the HCV and HBV.

Table 1: Sex wise distribution of Hepatitis in HD Patients.

Sex	No. of Samples	Anti-HCV +	HCV RNA +	Anti-HBV +	No. of co infected
Male	161	62 (38.5%)	34 (21%)	14 (8.6%)	6 (3.7%)
Female	39	13 (33.3%)	8 (20%)	2 (5%)	1 (2.5%)
Total	200	75 (37%)	44 (22%)	16 (8%)	7 (3.5%)

Table 2: HCV positive and negative sample frequency among different age groups and sex.

Age-Groups (years)	Sample Size	Male +/-	Female +/-	p-value	O.R	C.I
10 to 20	30	03/27	01/3	0.381	0.543	0.358-1.482
21 to 30	20	04/12	01/8	0.332	0.421	0.547-1.248
31 to 40	24	06/19	01/5	0.402	0.627	0.841-1.534
41 to 50	48	18/37	03/11	0.051	2.891	0.945-1.321
above 50	78	31/66	07/12	0.012*	3.467	0.974-1.188
Total	200	62/161	13/39	-	-	-

Table 3: RNA positive and negative sample frequency against different age groups and sex.

Age-Groups (years)	Sample Size	Male +/-	Female +/-	p-value	O.R	C.I
10 to 20	30	01/27	01/03	2.511	0.241	0.475-3.751
21 to 30	20	01/12	00/08	1.952	0.397	0.414-3.543
31 to 40	24	02/19	01/05	0.873	0.469	0.554-3.347
41 to 50	48	13/37	02/11	0.022*	1.973	0.578-1.921
above 50	78	17/66	04/12	0.049**	2.246	0.639-1.725
Total	200	62/161	13/39	-	-	-

Table 4: HBV positive and negative sample frequency among different age groups and sex.

Age-Groups (years)	Sample Size	Male +/-	Female +/-	p-value	O.R	C.I
10 to 20	30	00/27	00/03	1.351	0.491	0.285-3.825
21 to 30	20	01/12	00/08	1.275	0.478	0.314-3.540
31 to 40	24	01/19	00/05	0.987	0.469	0.654-3.534
41 to 50	48	02/37	01/11	0.225	1.893	0.778-2.921
above 50	78	10/66	01/12	0.021*	2.456	0.989-1.222
Total	200	62/161	13/39	-	-	-

4. Discussion

The present study was designed to evaluate the ratio of HBV and HCV in hemodialysis patients. As false positivity is a common problem associated with ICT devices [18] [19] so in order to refine the screening procedure, we analyzed all the ICT HCV positive samples by RT-PCR which indicated that 22% of the HD had antibodies against HCV which show significant decline because PCR is more sensitive to the viral genome [15]. These results reveal that screening of blood and blood products by ICT devices may not predict the true picture of anti-HCV prevalence in HD Patients. In KP Pakistan, screening of the blood and blood products at maximum health care units is carried out with ICT devices only [2]. We suggest that we should replace ICT procedures for screening in all health care units, especially those which are concerned with Dialysis.

The prevalence and incidence of HBV and HCV infections among dialysis patients vary widely between countries and also within the same country and it correlates with the prevalence in the general population [4; 11]. The World Health Organization (WHO) has compared hepatitis C to a “viral time bomb” and estimates that about 180 million people (some 3% of the world’s population) are infected with hepatitis C virus (HCV) [20] and HD is considered to be one of the major risk factors for HCV transmission [2]. Some earlier studies recorded 68% in Pakistan, 23.7% particularly in Quetta, 28.7% from Karachi and 24.7%, and 25.53% respectively from Lahore however 28% from Khyber Pakhtunkhwa. [21] While the study from India conducted by [22] from 1992 to 2000 reported the frequency of HCV in HD was 30% thus HCV among dialysis patients varies markedly from country to country and among dialysis centers within a single country [23]. However, the ratio of HCV is higher in either sex, male or female is not yet clearly understood

but we recorded 21% in male and only 20% in female and a similar trend had recorded by [15] with 20.57% in male and 18.18% only in female from Hazara division, Pakistan. While this show contrast with [24] recorded which indicated the ratio of HCV in HD was higher in female as compared to male with 62.50% and 40.13% respectively. The reason for this higher frequency in females than in males is that [24] conducted the study in Saudi Arabia while the present study was carried out in Pakistan and areas are matter [2].

In this study, the overall prevalence of positive Anti-HBV in HD recorded (8%) but it is not known whether these patients were Anti-HBV positive before the start of dialysis or became positive during the dialysis. However, it has been noted that the risk of HBV infection correlates with the sex for example grater were recorded in male with 8.6% in male and 5% in female. In the current study, the frequency of HBV in HD patients from Peshawar is similar to the study reported by [15] with 7.5% from five HD centers in the Hazara division of Pakistan. Thus, the HD does not increase but significantly increase hepatitis C infection rates only [25]. We recorded lover HBV in HD than Saudi Arabia 10% and Bahrain 11.8% [6; 26]. Nevertheless, the prevalence of HBV infection within dialysis units in developing countries appears higher 20% based on several reports [24]. The reason for differences may be the proper regular screening test for HBV and isolation of dialysis devices before the patient is going under dialysis. Several factors are also including exposure to contaminating types of equipment that have been noted [27].

The ratio of HBV and HCV co-infection were recorded higher in male 3.7% as compared to female, which is recorded 2.5% and what has been done by [24] showed the male HD patients were more susceptible to HBV and HCV co-infection. The reason for higher co-infection of HBV and HCV in male than in female may be the man is more involved in outdoor activities and could be due to their exposure to various HCV risk factors [28] particularly barber community and multiple sexual exposures [29].

The current study reported a significant relationship between HCV, HBV infection and age of the patients in a way that patients aged more than 40 years were found to be more susceptible to HCV and HBV than younger patients and this could be attributed to [2] results, Old age groups were found more infected with HCV. It has been suspected that fragile health structure, unsterilized instruments and use of contaminated razor by barbers may be contributing to the spread of HCV [2] while in contrast a study [29] recorded relationship between HBV infection and age of the patients in a way that patients aged less than 40 years were found to be more susceptible to HBV than older patients, however, Other studies [30; 20] have reported a higher prevalence of HBV or HCV seropositivity in older patients and the reason for this difference is not clear but the one reason may be the older age people immune system is not enough strong. Also, the current study revealed that HCV infection in HD patients is increasingly seen than HBV.

Conclusion

In hemodialysis patients, the percentage of HCV was found higher 21% in overall HD patient's population. The percentage of HBV were 8% while for those co-infected was found 3.5% in total samples. Thus it means that HD is also one the most cause of spreading the HCV, HBV, or even both.

List Of Abbreviations

HCV: hepatitis C virus

HBV: hepatitis B virus

HD: hemodialysis

ICT: Immuno-chromatographic Test

PCR: Polymerase chain reaction; RNA: Ribonucleic acid

RT PCR: Reverse transcription polymerase chain reaction

KP: Khyber Pakhtunkhwa

Declarations

Ethical Approval

The study has been approved by the research committee of Department of Zoology, Islamia College University Peshawar as satisfying the award of degree of BS in Zoology with the reference number 2014/ICP/2073.

Consent to publication

The study didn't contain any individual Person data.

Availability of Data and Material

The data was collected from the Dialysis Center of Lady Reading Hospital Peshawar under the legal permission of chief controller of the section.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Authors' contributions

Abid conceived the study, participated in its design and coordination, gave a critical view of manuscript writing and collected the prevalence data, performed screening, determined active HCV and analyzed the data statistically. GK and MS helped in molecular assays and gave a critical view of manuscript. SA

participated in data analysis. Remaining authors helped in writing of the report. All the authors read and approved the final manuscript.

Acknowledgements

We are very pleased to the staff of Lady Reading Hospital Peshawar for their kind of cooperation in collecting samples.

References

1. Fabrizi, F., Poordad, F. F., & Martin, P. (2002). Hepatitis C infection and the patient with endstage renal disease. *Hepatology*, 36(1), 3-10
2. Khan, S., Attaullah, S., Ali, I., Ayaz, S., Khan, S. N., Siraj, S., & Khan, J. (2011). Rising burden of Hepatitis C Virus in hemodialysis patients. *Virology journal*, 8(1), 438.
3. Frank, C., Mohamed, M. K., Strickland, G. T., Lavanchy, D., Arthur, R. R., Magder, L. S., ... & Sallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *The Lancet*, 355(9207), 887-891
4. Burdick, R. A., Bragg-Gresham, J. L., Woods, J. D., Hedderwick, S. A., Kurokawa, K., Combe, C., ... & Young, E. W. (2003). Patterns of hepatitis B prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney international*, 63(6), 2222-2229
5. Carrilho, F. J., Moraes, C. R., Pinho, J. R., Mello, I. M., Bertolini, D. A., Lemos, M. F., ... & Da Silva, L. C. (2004). Hepatitis B virus infection in Haemodialysis Centres from Santa Catarina State, Southern Brazil. Predictive risk factors for infection and molecular epidemiology. *BMC Public Health*, 4(1), 13.
6. Al Hijazat, M., & Ajlouni, Y. M. (2008). Hepatitis B infection among patients receiving chronic hemodialysis at the Royal Medical Services in Jordan. *Saudi Journal of Kidney Diseases and Transplantation*, 19(2), 260.
7. Hinrichsen, H., Leimenstoll, G., Stegen, G., Schrader, H., Fölsch, U. R., & Schmidt, E. (2002). Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. *Gut*, 51(3), 429- 433
8. Souqiyyeh, M. Z., Al-Attar, M. B. A., Zakaria, H., & Shaheen, F. A. (2001). Dialysis centers in the kingdom of Saudi Arabia. *Saudi Journal of Kidney Diseases and Transplantation*, 12(3), 293.
9. Jemni, S., Ikbel, K., Kortas, M., Mahjoub, J., Ghachem, L., Bidet, J. M., & Boukef, K. (1994). Seropositivity to hepatitis C virus in Tunisian haemodialysis patients. *Nouvelle revue française d'hématologie*, 36(5), 349-351.
10. Yakaryilmaz, F., Alp Gurbuz, O., Guliter, S., Mert, A., Songur, Y., Karakan, T., & Keles, (2006). Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. *Renal failure*, 28(8), 729-735
11. Batieha, A., Abdallah, S., Maghaireh, M., Awad, Z., Al Akash, N., Batieneh, A., & Ajlouni, K. A. (2007). Epidemiology and cost of haemodialysis in Jordan

12. Vladutiu, D. S., Cosa, A., Neamtu, A., Braila, M., Gherman, M., Patiu, I. M., & Dulau-Florea, I. (2000). Infections with hepatitis B and C viruses in patients on maintenance dialysis in Romania and in former communist countries: yellow spots on a blank map? *Journal of viral hepatitis*, 7(4), 313-319.
13. Dusheiko, G., Song, E., Bowyer, S., Whitcutt, M., Maier, G., Meyers, A., & Kew, M. C. (1983). Natural History of Hepatitis B Virus Infection in Renal Transplant Recipients—A Fifteen-Year Follow-*Hepatology*, 3(3), 330-336
14. Disease K. Improving Global Outcomes (2008). KDIGO clinical practiceguidelines for the prevention, diagnosis, evaluation, and treatment ofHepatitis C in chronic kidney disease. *Kidney Int*, 73(1)–99
15. Ali, N., Hussain, W., Hayat, A., Shah, T., Wen, R., Zeb, I., ... & Khan, I. (2019). Prevalence and risk factors of hepatitis B and C viruses among haemodialysis patients: a multicentric study. *European journal of gastroenterology & hepatology*, 31(1), 29-33.
16. Ali, A., Ahmad, H., Ali, I., Khan, S., Zaidi, G., & Idrees, M. (2010). Prevalence of active hepatitis c virus infection in district mansehra Pakistan. *Virology Journal*, 7(1), 334.
17. Ali, I., Siddique, L., Rehman, L. U., Khan, N. U., Iqbal, A., Munir, I., ... & Aslam, M. S. (2011). Prevalence of HCV among the high risk groups in Khyber Pakhtunkhwa. *Virology journal*, 8(1), 296.
18. Grobusch MP, Alpermann U, Schwenke S, Jelinek T, Warhurst DC: Falsepositive rapid tests for malaria in patients with rheumatoid factor. *Lancet* 1999, 353:297
19. Srivastava, A. V., et al. "267: High Rates of False-Positive Hepatitis C Antibody Tests Can Occur after Left Ventricular Assist Device Implantation." *The Journal of Heart and Lung Transplantation*2 (2009): S159-S160.
20. Umar, M., tul Bushra, H., Ahmad, M., Ahmad, M., Khurram, M., Usman, S., & Naeem, A. (2010). Hepatitis C in Pakistan: a review of available data. *Hepatitis monthly*, 10(3), 205.
21. Zarkoon, A. K., Shah, K., ur Rehman, H., Daud, A., & Ahmed, J. (2008). Hepatitis C virus infection in patients on long term hemodialysis. *Gomal Journal of Medical Sciences*, 6(1).
22. Jaiswal, S. B., Chitnis, D. S., Salgia, P., Sepaha, A., & Pandit, C. S. (2002). Prevalence of hepatitis viruses among chronic renal failure patients on hemodialysis in central India. *Dialysis & transplantation*, 31(4), 234-240.
23. Alavian, S. M. (2010). Hepatitis C in hemodialysis patients' needs more attention for control and review the risk factors. *Saudi J Kidney Dis Transpl*, 21(2), 357-8.
24. Al-Hegami, M. A., Al-Mamari, A., Al-Kadasse, A. S., Al-Gasha'a, F. A., Al-Hag, S., & Al-Hegami, A. A. (2015). Prevalence and Risk Factors of Hepatitis B and Hepatitis C Virus Infections among Patients with Chronic Renal Failure in Zabeed City, Yemen Republic. *Open J Med Microbiol*, 5, 136.
25. Xijie, L., & Liuqin, L. (1998). Relationship between hemodialysis and viral hepatitis. *J NephrolDialy Transplant*, 7(3), 245-246
26. Almawi WY, Qadi AA, Tamim H, Ameen G, Bu-Ali A, Arrayid S, et al. Seroprevalence of hepatitis C virus and hepatitis B virus among dialysis patients in Bahrain and Saudi Arabia. *Transplant Proc* 2004; 36:1824–1826.

27. Nasir khokhar, A. Y., & NAZ, F. (2004). Hepatitis B surface antigenemia in patients on hemodialysis. *Rawal Medical Journal*, 29(1), 18-21.

28. Raja, N. S., & Janjua, K. A. (2008). Epidemiology of hepatitis C virus infection in Pakistan. *Journal of microbiology, immunology, and infection= Wei mian yu gan ran za zhi*, 41(1), 4-8.

29. Lyra AC, Ramrakhiani S, Bacon BR, Adrian DB: Infection with Hepatitis CVirus Genotype 4 in the United States. *Journal of Clinical Gastroenterology*2004, 38(1):68-71

30. Taal, M.W. and van Zyl-Smit, R. (2000) Hepatitis C Virus Infection in Chronic Hemodialysis Patients- Relationship to Blood Transfusions and Dialyzer Re-Use. *South African Medical Journal*, 90, 621-625

Figures

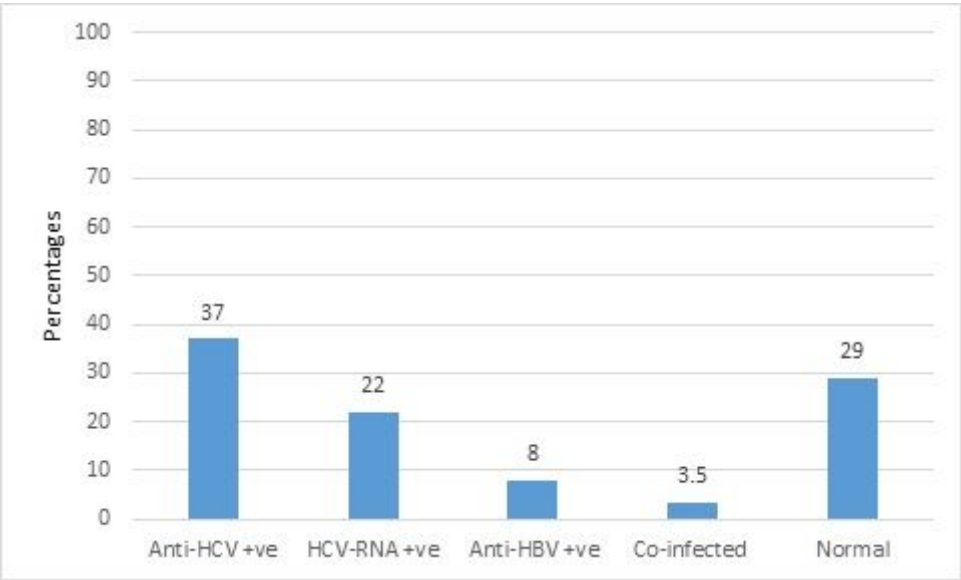


Figure 1

Percentages of different disease in total collected samples.