Prevalence of Hepatitis C Virus and its risk factors in blood donors in district Peshawar

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Abstract: The current study was designed in order to elucidate the most sensitive method for daily practice as well as to evaluate the risk factors for HCV infection associated with blood transfusion in District Peshawar. A total of 1400 healthy volunteer blood donors were tested for Anti-HCV. A questionnaire was used to evaluate the risk factors. Initial testing of all blood samples was done by Immuno Chromatographic Technique (ICT) and confirmed by micro particle enzyme immunoassay (MEIA) and Enzyme Linked Immunosorbent Assay (ELISA). The comparison among ICT, ELISA and MEIA techniques was also evaluated for the purpose of sensitivity. Among 1400 blood donors, 26 (1.85%) cases were found positive for Anti-HCV. These 26 cases were positive on MEIA, 16 individuals were positive on ELISA while 14 were positive on ICT. These 26 cases had different histories of dental treatment (50%), traveled abroad (23.07%), surgery (11.53%), blood transfusion (7.69%) and unknown reason (7.69%). Among all these different histories of dental treatment and blood transfusion were the main risk factors for HCV infection. The results revealed that MEIA ia a quick and reliable technique for routine screening of blood donors particularly for controlling the spread of HCV.

Keywords: Blood donors, Hepatitis C Virus, Risk factors, ICT, ELISA and MEIA.

INTRODUCTION

Hepatitis C is an infectious viral disease that primarily infects liver, caused by HCV belongs to Flaviviridae family. It is enveloped virion containing a genome of single stranded; positive polarity RNAans has no virion polymerase (Warren and Francisco 2004). Initially the disease is often asymptomatic. However, infection with HCV can lead to chronic liver disease in which scarring of liver (fibrosis) can occur and if it continues to progress then cirrhosis and in some cases hepatocellular carcinoma is the end result that appears after years (Tong *et al.*, 1995). HCV worldwide prevalence is estimated to be approximately 3% by the World Health Organization (WHO) which corresponds to about 170 million persons infected with this virus. Almost about 3 to 4 million infected persons are diagnosed annually (Wkly, 1997). In Pakistan the data is not well organized but approximately 10 million cases have been reported (Hamid *et al.,* 2004). Previous studies of clinical cases have identified the blood products transfusion as main factors in the spread of HCV infection (Alam and Ahmad, 2001). Apart from all these face or armpit shaving at community barber shops, ear piercing and tattooing can be possible means of HCV transmission (Butt *et al.,* 2003). Since 1930, blood has been used for various indications (Zafar 2000). Introduction of blood banks and various improved storage techniques increased its use in patients. Each year more than 1.5 million pints of blood are collected in Pakistan from various donors including replacement donors (65%), volunteer donors (25%) and professional donors (10%) (Asif *et al.,* 2004 and Rehman *et al.,* 2003). The prevalence of transfusion transmitted diseases in healthy volunteer blood donors is much lower than professional blood donors. The attitude of physicians and patients about blood transfusion dramatically changed after discovery of various problems related to it, thus becoming more concerned about safer blood transfusion. These problems can be pointed out and controlled by proper screening and selection of donors before blood collection (Mujeeb 1997). Assessment and analysis of donor selection processes suggest that careful donor selection can control and decrease the rate of Hepatitis C and transfusion transmitted infections (TTVIs) (Gimble and Friedman, 1992 and Whyte and Savoia, 1997). The detailed medical history and examination of the receptors should be carried out (Chaudhary *et al.,* 2005). A number of published studies conducted across the country have concluded that the main risk factors for HCV transmission in Pakistan are the use of unnecessary injections and reuse of contaminated needles. Standard sterilization procedures are not known by majority of health workers as they are not medically well qualified or scientifically trained (Muhammad and Jan 2005 and Khan *et al.,* 2000). In the United States, blood transfusion, other blood products and organ transplantation are now considered decreasing risk factors for hepatitis C because of implementation of a valid HCV screening test in 1992. But the status of blood born transfusion of HCV in many developing countries including Pakistan is still alarming despite the

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introduction of modern laboratory apparatus and screening techniques. Thus HCV is found to be the most common blood-borne infection in these countries as it has been found to be asymptomatically occurring which remains undetected(Lo and Kostman, 2005).When look at possible reasons for this fact there are a number of such kind; lack of potential resources both on public as well as private sectors, low infrastructure, especially in government hospitals and organizations, ill-equipped labs, poorly trained technical staff, corruption and irregularities at governmental blood banks, applying insufficient policy and inadequate methods of screening of blood donors (Aslam and Syed 2005). Keeping in view the importance of HCV, this study has been designed to achieve the objectives regarding HCV in blood donors.

MATERIALS AND METHODS

A total of 1400 blood samples were collected from healthy volunteer blood donors and screened for Anti-HCV. The data was collected on a (prescribed) structured Performa to evaluate the risk factors for HCV infection associated with blood transfusion in Peshawar. For this purpose blood donors are carefully examined by Medical Lab Technologist by evaluating their detailed medical history. After that 5 ml blood sample was collected from each donor in a disposable syringe under aseptic conditions and centrifuged to separate serum from it in a sterile test tube. Serum was then used for further screening.

Immunochromatography technique (ICT)

The ICT acts on same principle as ELISA. The only difference is that immunological reaction is carried out on the chromato-graphic paper by capillary action.

Procedure

Before proceeding with the assay, all reagents and specimens were brought to room temperature. Briefly, the test strip was removed from the foil pouch and placed on a clean dry surface. 5µl serum sample was dispensed on the sample pad and two drops of buffer were added to it. The results were interpreted after 15 minutes according to presence of color band. Control was also run to check the validity of the kit.

Interpretation of results

Positive: Both purplish red test band and purplish red control appeared on the membrane.

Negative: Only the purplish red control band appeared on the membrane and no band of sample is formed.

Micro particle enzyme immunoassay (MEIA)

The Architect Anti-HCV assay is a two steps immunoassay, using chemiluminescent micro particle immunoassay technology, for the quantitative detection of anti-HCV in human serum and plasma. In the first step sample, recombinant HCV coated paramagnetic microparticles and assay diluent are combined. Anti-HCV present in the sample binds to the HCV coated microparticles. After washing, anti-human acridinium-labeled conjugate is added in second step. Following another wash cycle, pre-Trigger (13.2% hydrogen peroxide) and Trigger (0.35 N sodium hydroxide) solutions are added to reaction mixture. The resulting chemiluminescent reaction is measured as relative light units. A direct relationship exists between the amount of anti-HCV in sample and relative light units detected by Architect System optics. The presence or absence of anti-HCV in the specimen determined by comparing the chemiluminescent signals in the reaction to cutoff signals determined from a previous Architect Anti-HCV calibration. If the Chemiluminescent signals in the specimen is greater or equal the cutoff signals, the specimen is considered reactive for anti-HCV.

Procedure

- Samples were loaded into the sample carrier and the sample carrier was placed in the sample load queue.
- Run button was pressed. The Architect System performed the following functions:
- Moves the sample carrier to the sample processing queue.
- Loads a reaction vessels into the process path.
- Aspirates and transfer sample into the reaction vessels.
- Advances the reaction vessels one position and transfers micro particles and assay diluent into the reaction vessels.
- Mixes, incubates (37°C) and washes the reaction mixture.
- Adds pre-Trigger and Trigger solutions.
- Measures chemiluminescent emission to detect the presence of ant-HCV in the sample.
- Aspirates contents of reaction vessels to liquid waste and unloads reaction vessels to solid waste.
- Results are available on monitor screen of Architect system.

Interpretation of results

The presence or absence of anti-HCV in the specimen determined by comparing the chemiluminescent signals in the reaction to cutoff signals determined from a previous Architect Anti-HCV calibration. If the Chemiluminescent signals in the specimen as greater or equal the cutoff signals (1.00), the specimen is considered reactive for anti-HCV. If the Chemiluminescent signals in the specimen less than cutoff signals (1.00), the specimen is considered non-reactive for anti-HCV.

Enzyme linked immunosorbent assay (ELISA)

During the first incubation step, HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to horseradish per oxidase (HRP) is

added. During the second incubation, these HRP-conjugated antibodies will be bound to any antigen-antibody complexes previously formed and the unbound HRP-conjugate is then removed by washing. The antigen-antibody-anti-IgG (HRP) immunocomplex then will be turned into yellow substances with certain treatment with the reagents in the anti-HCV ELISA testing SD (Standard Diagnosic Kit, Korea) ELISA kit. The degree of the color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colorless.

Procedure

1. All reagents and specimens were brought to room temperature (20-30°C) before beginning the test assay.
2. Three wells coated with specific antigen were taken, 100µl of sample diluent and 10µl of sample each positive control, negative control and sample were added into appropriate well respectively. The adhesive slip was applied to each well.
3. The wells containing samples and controls were incubated at 37°C for 30 minutes.
4. After incubation, the adhesive slip was removed from wells and washed 3-5 times.
5. Then 100 µl of Enzyme Conjugate was added into each reaction well.
6. The adhesive slip was applied again to each well.
7. The wells containing samples and controls were incubated at 37°C for 30 minutes.
8. The step No. 4 was repeated.
9. 50 µl of TMB substrate solution A was added and then 50 µl of TMB substrate solution B was added into each well including.
10. All the wells were kept in the dark at room temperature for 10 minutes after applying adhesive plastic slip.
11. Stop solution of 100 µl was added to stop the reaction.
12. The absorbance of controls and test specimens was determined within 15 minutes with a spectrophotometer.
13. The blue color turns yellow after reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen in the samples. Wells containing samples negative Anti-HCV remained colorless. The result was read on spectrophotometer (Strip Reader, America).

Interpretation of results

Positive: Specimen with absorbance values equal to or greater than the cut-off value (1.00) was considered Anti-HCV positive (reactive).

Negative: Specimen with absorbance values less than the cut-off value was considered Anti-HCV negative (non-reactive).

RESULTS

A total of 1400 blood donors were tested for anti-HCV. Out of 1400 blood donors 26 (1.85%) were found positive for HCV as shown in table 1. The initial screening was done by ICT and then tested by MEIA. These 26 cases were positive on MEIA, while 14 cases were found positive on ICT and remaining (10) were negative. All the positive samples on MEIA were also tested on 3rd Generation ELISA to compare the sensitivity of techniques and detected 16 positive cases for HCV (fig. 1). These 26 cases had different histories of dental treatment (50%), traveled abroad (23.07%), surgery (11.53%), blood transfusion (7.69%) and unknown reason (7.69%) as presented in fig. 2.

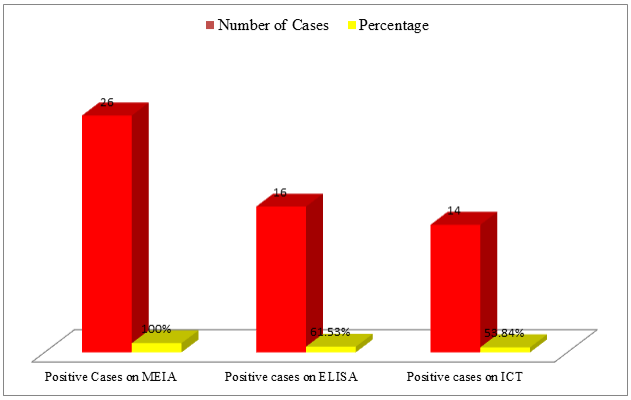

Fig. 1: Results distribution of HCV infection using ICT, MEIA and ELISA in blood donors.

DISCUSSION

Epidemiological studies are required for preventive strategies of diseases. Replacement donors are the main source of blood donation (Mujeeb, 1997). Safe blood donors are believed to be without any risk factor in their medical history and whose donations are repeatedly negative when screened for HCV (Rahman *et al.*, 2003). The positive cases of Hepatitis C in blood donors observed in this study were 26 (1.85%). Ali *et al.* 2003 reported the same incidence of HCV (1.87%) in healthy blood donors from Quetta. The minor decline in HCV incidence rates might be due the accumulative effect of increasing public awareness of the infection with virus, leading to a decrease in new cases, implementation of stringent donor selection and self-deferral by high risk individuals. In contrast to this study, Ali *et al*. 2003 observed the high seroprevalence of 6.8% from Karachi in blood donors. This study documented the most common identified potential risk exposures that might be the cause of Hepatitis C in blood donors. These included the history of dental treatment (13), traveling abroad (6), surgery in the past (3), blood transfusion (2) and unknown reason (2). There were two donors (7.69%) who had history of blood transfusion. In both cases the blood transfused was declared free of any infection at the time

Table 1: Total Number of Hepatitis C Positive Cases in blood donors

Parameter	Total Samples	No. of negative Cases (%)	No. of Positive Cases (%)
Anti-HCV	1400	1374 (98.15)	26 (1.85)

Table 2: Percentage of Different Risks Factors among Blood Donors for HCV

Risk Factors	Number of Positive Cases	Percentage (%)
Dental Treatment	13	50
Travel Abroad	06	23.07
Surgery	03	11.53
Blood Transfusion	02	07.69
Unknown Reason	02	07.69

of transfusion. The reason might be the lower sensitivity of the screening tests or in-efficiency of the test operator. The same observations were reported in two other studies; 19 and 3.2% Hepatitis C infected blood donors, respectively had the history of receiving blood products (Polizzotto *et al.,* 2008; Gregory *et al.,* 2002). Another study has also observed 2.7% blood donors in Egypt, who had previous history of blood transfusion (Medhat *et al.*, 2002). These observations clearly show the severity of matter and raise many questions on the sensitivity of the test. Keeping in view these facts, all positive cases (confirmed by MEIA) were tested by ICT and ELISA from different known laboratories in the city. The results of these tests affirmed that lower sensitivity of the test may lead to the skipping of virus and result into false negative. ICT missed 12 (53.84%) samples out of 26 and give false negative results. Similarly, 3rd Generation ELISA sensed 16 samples (61.53%) out of 26 and missed the remaining one. It was observed in this study that MEIA technique is more sensitive and reliable than ICT and ELISA. A well reported study showed that MEIA is more sensitive and reliable technique than ELISA (Dietemann *et al.,* 2001). Another study also observed the same results by using MEIA technique. They found that the specificity of MEIA is more than 99% and sensitivity of this technique is 100%.

CONCLUSION

The prevalence of Hepatitis C virus is 1.85% in 1400 healthy volunteer blood donors in district Peshawar. It can be concluded that MEIA Method is more sensitive and reliable than ICT and 3rd Generation ELISA. One of the major causes of its spread is contaminated blood transfusion. The blood donors with histories of previous blood transfusion, dental treatment and surgery were the risk factors most strongly associated with HCV infection.

RECOMMENDATIONS

It was observed in this study that MEIA is more sensitive and reliable than ICT and ELISA, so MEIA is very useful for screening of blood donors to minimize the spread of HCV; may not be sensed by ICT and ELISA. A combination of preventive strategies; safe injection practices and proper sterilization of medical appliances and appropriate counseling of blood donors by giving proper guidelines can better reduce the incidence of HCV infection. Selection of healthy blood donors is mandatory. Alongside public awareness and health education programs is also necessary to control its spread. In most of the Blood Banks of Government hospitals in Pakistan lack the facilities of MEIA. So, these Blood Banks should be well equipped with reliable and sensitive assays like MEIA in order to prevent the spread of HCV infection.

REFERENCES

Alam M and Ahmed D (2001). Prevalence of antibodies to Hepatitis C virus in blood donors in Sialkot. *J. Coll. Physicians. Surg. Pak*., **11**: 783.

Ali N, Nadeem M, Qamar A, Qureshi AH and Ejaz A (2003). Frequency of Hepatitis-C virus antibodies in blood donors in Combined Military Hospital, Quanta. *Pak. J. Med. Sci*., **9**: 41-44.

Asif N, Kokhar N and Ilahi F (2004). Seroprevalence of HBV, HCV and HIV infection among voluntary non remunerated and replacement donors in Northern Pakistan. *Pak. J. Med. Sci.,* **20**: 24-28.

Aslam F and Syed JA (2005). Seeking a safer blood supply. *Lancet*. **365**: 1464.

Butt AK, Khan AA, Khan SY and Sharea I (2003). Dentistry as a possible route of Hepatitis C Transmission in Pakistan. *Int. Dent. J.,* **53**: 141-144.

Chaudhary A, Samiullah S, Khan S, Masood R, Sardar MA and Mallhi AA (2005). Seroprevalence of Hepatitis B and C among the healthy blood donors at Fauji Foundation Hospital, Rawalpindi. *Pak. J. of Med. Sci*., **3**: 1618-1624.

Dietemann, J, Berthoux P, Gay JP, Montchamp P, Batie M and Berthoux F (2001). Comparison of ELISA method versus MEIA method for daily practice in the therapeutic monitoring of tacrolimus Nephrol. *Dial. Transplant*, **16**: 2246-2249.

Gimble JG and Friedman LI (1992). Effects of oral donor questioning about high-risk behaviors for human immunodeficiency virus infection *Transfusion* **32**: 446-449.

Gregory L, ArmstrongA, Wasley A, Edgar PS, McQuillan GM, Wendi LK and Miriam JA (2002). The Prevalence of Hepatitis C Virus Infection in the United States. *Annal. of Int. Med*., **144**: 705-714.

Hamid S, Umar M, Alam A, Siddiqui A, Qureshi H and Butt J (2004). Pakistan Society of Gastroenterology. PSG consensus statement on management of hepatitis C Virus infection. *J Pak. Med. Assoc.,* **54**: 46-50.

Khan AJ, Luby SP, Fikree F, Karim A, Obaid S, Dellawala S (2000). Unsafe injections and the transmission of hepatitis B and C in a periurban community in Pakistan. *Bull. World Health Organ*. **78**: 956-963.

Lo RV and Kostman JR (2005). Management of chronic hepatitis C. *Postrad. Med. J*., **81**: 376-382.

Medhat A, Shehata M, Magder LS, Mikhail N, Nafeh LAM, Hamid MA, Strickl GT and Alan D (2002). Hepatitis C in a community in Upper Egypt: Risk Factors for Infection. *Am. J. Trop. Med.*, **66**: 633-638.

Muhammad N and Jan MA (2005). Frequency of hepatitis C in Buner, NWFP. *J Coll. Physicians. Surg. Pak*., **15**: 11-14.

Mujeeb SA (1997). Single unit blood transfusion, a bad clinical practice. *Transfusion Today,* **36**: 5-7.

Polizzotto MN, Wood EM, Ingham H, Keller AH, Australian Red Cross Blood Service Donor and Product Safety Team (2008). Reducing the risk of transfusion-transmissible viral infection through blood donor selection. The Australian experience 2000 through 2006. *Transfusion*, **48**: 550-563.

Rahman M, Akhtar G, Qadeer M, Shams T, Usmani A and Lodhi Y (2003). Safe blood begins with safe donors. *Pak. J. Med. Sci.,* **19**: 161-8.

Tong MJ, El-Farra NS and Reikes AR (1995). Clinical outcomes after transfusion-Associated Hepatitis. C. *N Engl. J. Med*., **332**: 1463-1466.

Warren L and Francisco S (2004). Eight editions. Book of Medical Microbiology & Immunology. **8**: 541.

Whyte GS, Savoia HF (The risk of transmitting HCV, HBV or HIV by blood transfusion in Victoria. *Med. J Aust* 665846 Wkly (1997). World Health Organization Hepatitis C: global prevalence. *Epidemiol. Rec*., **72**: 341-344.

Zafar N (2000). A survey of blood transfusion practices. *J Coll. Physicians. Surg. Pak*., **10**: 90-92.