

PERCUTANEOUS ROUTE AS A RISK FACTOR FOR SEROPOSITIVITY OF HEPATITIS C VIRUS INFECTIONAnuj Sharma¹, Sharanjit Kaur², Neeraj Sharma³**HOW TO CITE THIS ARTICLE:**

Anuj Sharma, Sharanjit Kaur, Neeraj Sharma. "Percutaneous Route as a Risk Factor for Seropositivity of Hepatitis C Virus Infection". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 05, January 15; Page: 843-852, DOI: 10.14260/jemds/2015/121

ABSTRACT: BACKGROUND: Hepatitis C seropositivity is a chronic infection in India which remains silent in the blood over the years and can pose a threat to public health. Paucity of the data on the part of patient during pre-donation questioning about various risk factors is likely to have an impact on safe blood transfusion programme. **AIM:** The objective of this study was to identify percutaneous route as a risk factor associated with HCV infection in volunteer blood donors in Amritsar Punjab. **MATERIAL METHODS:** The present study was done on 5000 healthy blood donors comprising 3261 replacement and 1739 voluntary donors in Amritsar to know Hepatitis C virus seropositivity in the region and compare it in relation to various etiologic factors. All blood samples were tested for anti-HCV antibody by 3rd generation ELISA. Results of study were analyzed statistically. **RESULTS:** Overall HCV seropositivity amongst 5000 donors was 0.98% which was significantly lower in voluntary than replacement donors (0.23 vs.1.37%). Maximum HCV seropositivity was seen in 30-39 years group in both voluntary and replacement donors. Rural blood donors had higher seropositivity than urban. The most prevalent risk factor was injection (I.V.) drug use present in 14/49 (28.57%) cases. The second most common route was tattooing which was present in a total of 12/49 anti HCV positive cases and in 6 out of these cases (i.e. 12.24% of total) no other history could be elicited than tattooing. Another important route was sharing of shaving kits or visit to roadside barber which solely was present in 5/49 (10.2%) cases. **CONCLUSION:** Percutaneous route with tattooing, sharing shaving kits or roadside barber visit is the important risk factor to consider for the transmission of Hepatitis C infection besides injection drug use.

KEYWORDS: Hepatitis C, percutaneous route, seropositivity, ELISA, healthy blood donors.

INTRODUCTION: Hepatitis B (HBV) and Hepatitis C (HCV) infections are 'silent' diseases that remain asymptomatic for decades. As for Hepatitis C, one out of every hundred persons in India may be chronically infected by the virus and most among these 12 million persons do not know they are infected.¹

Prevalence of Hepatitis C has been observed to be relatively higher in Punjab, Andhra Pradesh, Pondicherry, Arunachal Pradesh and Mizoram.² A study conducted in Arunachal Pradesh showed a prevalence of 7.89%.³ Injection drug users (IDUs) are at very high risk of acquiring HCV infection, with a study conducted in Mizoram showing that the prevalence of HCV was 71.2 % among the active injection drug users (IDUs) studied.⁴

In some studies conducted in Andhra Pradesh, cultural practices such as tattooing, traditional medicine (e.g. bloodletting), rituals among pilgrims (e.g. scarification) and body piercing have been observed to lead to a significantly higher rate of HCV transmission.⁵

Interestingly, several studies conducted in these states highlight different risk factors which are believed to have led to the relatively higher prevalence of the condition. Drug abuse, unsafe

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sexual practices, contaminated blood transfusions, use of unsafe therapeutic injections and health care related procedures like dental procedures have led to a steady rise in the number of people with HBV, HCV infections in Punjab. Unhygienic use of needles in acupuncture and tattooing has significant potential in spreading the infections.^{2,6} Educating communities about the importance of staying protected from Hepatitis is an essential first step towards prevention from the spread of the infections and controlling the disease prevalence.

This study was conducted to evaluate the prevalence of Hepatitis C infection in blood donors with percutaneous route and other possible etiological factors. The present study was taken up to evaluate and compare the prevalence of Hepatitis C virus among blood donors in relation to the various factors as per the proforma and to find out Correlation of etiological factors with HCV seropositivity.

MATERIAL AND METHODS: The present study included 5000 healthy blood donors coming for blood donation in the Department of Blood banking, Govt. Medical College and Hospital, Amritsar and in the voluntary blood donation camps organized by the department. Informed consent of each donor was taken identified by a donor registration number. A detailed clinical history physical examination focused on various risk factors for acquiring HCV infection done and findings were recorded in the proforma attached along with. Study was conducted on all ages and both sexes of donors.

All those donors were not considered for this study who were found to be either unsuitable for blood donation (Based upon physical finding of thickened veins, insufficient bleeding, prick failure cases etc.) or were not available for detailed history taking and physical examination (Odd hour donations).

Collection of blood Sample: Taking all aseptic precautions 4 ml of blood from each blood donor was collected and put in a sterile numbered glass tube. The serum was allowed to separate at room temperature and then centrifuged at 3000 rpm for 5 minutes. The serum was collected in a sterile disposable plastic vial. Then it was labeled and stored in the freezer compartment of refrigerator till the test was performed i.e. preferably within 48 hours.

Method of Testing: Every serum sample was tested for antibody to Hepatitis C virus by 3rd generation Microlisa kit supplied by J. Mitra and Co. Ltd. and all the seroreactive samples were further subjected to ELISA in duplicate to rule out any false positive or false negative test results.

ELISA (Enzyme Linked Immunosorbent Assay) for HCV: The 3rd generation HCV Microlisa is based on a highly sensitive technique, Enzyme Linked Immunosorbent Assay which detects antibodies against HCV in human serum and plasma. The 3rd generation HCV Microlisa utilizes a combination of antigen with the sequence of both HCV structural and non-structural antigens i.e. Core, E1, E2, NS3, NS4 and NS5. It has an obvious advantage over the available 2nd generation and 1st generation ELISA with improved sensitivity and specificity.

The combination of antigens for the structural HCV proteins is coated onto the microwells.

Diluted sample and controls are incubated in the test well. Antibodies to HCV, if present, bind to the immobilized HCV antigens on the microwell during this incubation period.

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The microwells are then thoroughly washed with the diluted wash buffer to remove excess of unbound anti-HCV or other human IgGs which may interfere with the test. An enzyme conjugate, antihuman IgG conjugated with HRPO is added. The excess of enzyme conjugate is again removed with diluted wash buffer. At this state the microwells hold only the bound antigen anti HCV-enzyme conjugate complex.

In the next step, the freshly prepared substrate solution is incubated with the complex in the micro wells. The enzyme substrate reaction leads to development of a yellow colour which is indicative of the Ag-Ab reaction which has occurred in the microwell. In the final step the stop solution is added and the optical density of the developed colour is read photometrically.

Calculation of Results and Validity of the Test:

Nc	-	Absorbance of the negative control
$NC\bar{x}$	-	Mean absorbance of negative control.
PC	-	Absorbance of the positive control
$PC\bar{x}$	-	Mean absorbance of positive control.

Test Validity:

Positive control acceptance Criteria:

PC or $PC\bar{x}$ must be >0.5. If it was not so, the run was invalid and was repeated.

$$PC\bar{x} = \frac{\text{Total Absorbance of positive controls}}{3}$$

Negative control acceptance Criteria:

NC or $NC\bar{x}$ must be <0.150.

Cut off value:

$$\text{Cut-off} = 0.1 \times PC\bar{x} + 0.1$$

INTERPRETATION OF RESULTS:

1. Test specimens with absorbance value less than the cut-off value were non-reactive for Anti-HCV.
2. Test specimens with absorbance value greater than or equal to the cut-off value were reactive for Anti-HCV.
3. Specimens with absorbance value equal to or greater than the cut-off value were considered initially reactive. Original specimen was retested in duplicate.
4. If any one of the duplicate retested sample was reactive, the specimen was considered repeatedly reactive.

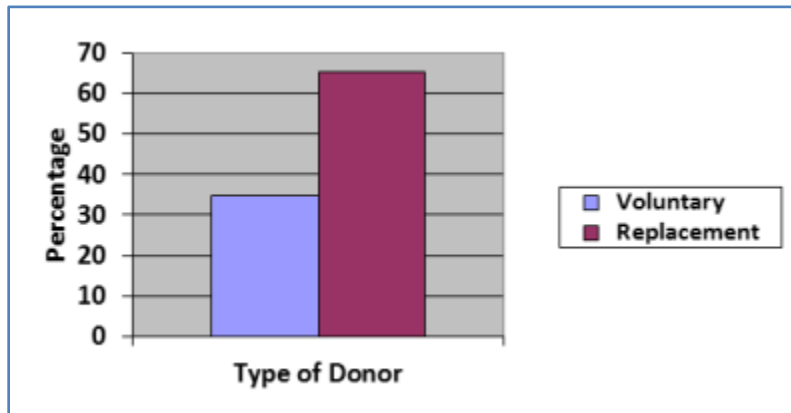
Statistical Analysis: Data generated from the study was analyzed according to statistical methods using chi square test.

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OBSERVATIONS: The present study was carried out on a healthy set of replacement as well as voluntary blood donors donating blood in the Blood bank and blood donation camps organized by the department. A total of 5000 blood donors were examined. A thorough questioning was done focusing on all the probable risk factors of Hepatitis C virus transmission with due importance to the minor percutaneous routes of transmission which often go undetected.

In the present study, out of the total 5000 blood donors, 3261 i.e. 65.22% were replacement blood donors and 1739 i.e. 34.78% were voluntary blood donors as shown in graph I.

GRAPH I: DISTRIBUTION OF BLOOD DONORS ACCORDING TO TYPE OF DONOR



Graph I

Age Group (yrs)	Voluntary		Replacement	
	No. of Donors	Percentage	No. of Donors	Percentage
<20	321	18.45	237	7.26
20-29	911	52.38	1838	56.36
30-39	381	21.90	857	26.28
40-49	75	4.31	289	8.86
≥50	51	2.93	40	1.22
Total	1739	100	3261	100

TABLE I: DISTRIBUTION OF BLOOD DONORS ACCORDING TO AGE OF DONOR

As shown in Table I the age distribution of blood donors the age group of 20-29 years contributed maximally i.e. more than half of the voluntary as well as replacement donors. Donors in the age group 30-39 years formed the second largest group. Thereafter the contribution towards blood donation decreased with increase in age in both voluntary as well replacement donors; the donors beyond 50 years contributing just 2.93% and 1.22% respectively.

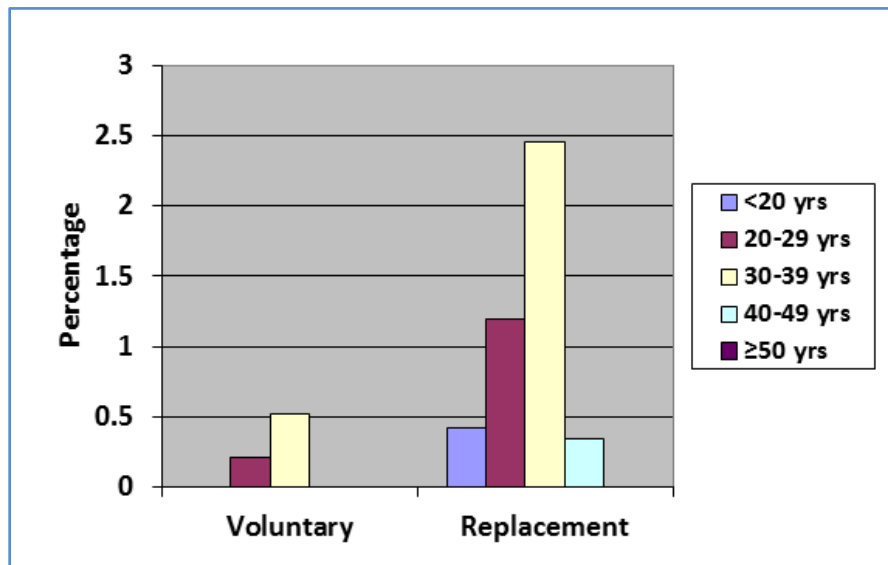
An important observation was that the youngest donor group of <20 years was not far behind the other groups in voluntary blood donation and its percentage was more than double in comparison to the replacement donors of same age.

In voluntary donors maximum HCV seropositivity was seen in 30-39 years age group i.e. 0.52% (2/381) followed by 0.21% (2/911) cases belonging to 20-29 years age group as shown in

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graph II. In replacement donors also, maximum HCV seropositivity was present in 30-39 years age group i.e. 2.45% (21/857) followed by 20-29 years age group i.e. 1.19% (22/1838) cases as shown in graph II. There was no anti HCV donor in <20, 40-49 and >50 yrs of age group In replacement donors also, maximum HCV seropositivity was present in 30-39 years age group i.e. 2.45% (21/857) followed by 20-29 years age group i.e. 1.19% (22/1838) cases as shown in graph II. The lowest HCV seropositivity was seen in 40-49 yrs of age group. (0.34%, 1/289) The HCV seropositivity in < 20yrs of age group was 0.42%. (1/237) There was no anti HCV reactive blood donor in > 50 yrs of age group.

GRAPH II: HCV SEROPOSITIVITY ACCORDING TO AGE DISTRIBUTION AMONG BLOOD DONORS



Graph II

When the HCV seropositivity difference between various age groups in replacement donors was compared, HCV seropositivity in 30-39 years group was found to be significantly higher than 20-29 years and 40-49 years group but this was not the case with voluntary donors as shown in graph II.

HCV seropositivity Replacement vs Voluntary	p value	Statistical significance
<20 yrs	-	-
20-29 yrs	<0.05	S
30-39 yrs	<0.05	S
40-49 yrs	-	-
≥50 yrs	-	-

Table II: HCV seropositivity in voluntary vs replacement group in different age groups

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Also there was a statistically significant difference between voluntary and replacement donors with respect to their HCV seropositivity in both the age groups 20-29 and 30-39 as shown in table II.

Replacement			Voluntary		
HCV seropositivity	p value	Statistical significance	HCV seropositivity	p value	Statistical significance
<20 vs 20-29	>0.05	NS	<20 vs 20-29	-	-
<20 vs 30-39	>0.05	NS	<20 vs 30-39	-	-
<20 vs 40-49	>0.05	NS	<20 vs 40-49	-	-
<20 vs ≥50	-	-	<20 vs ≥50	-	-
20-29 vs 30-39	<0.05	S	20-29 vs 30-39	>0.05	NS
20-29 vs 40-49	>0.05	NS	20-29 vs 40-49	-	-
20-29 vs ≥50	-	-	20-29 vs ≥50	-	-
30-39 vs 40-49	<0.05	S	30-39 vs 40-49	-	-
30-39 vs ≥50	-	-	30-39 vs ≥50	-	-
40-49 vs ≥50	-	-	40-49 vs ≥50	-	-

Table III: HCV seropositivity in replacement vs voluntary group according to different age groups

In voluntary donors maximum HCV seropositivity was seen in 30-39 years age group i.e. 0.52% (2/381) followed by 0.21% (2/911) cases belonging to 20-29 years age group. There was no anti HCV positive donor in <20, 40-49 and ≥50 year groups.

In replacement donors also, maximum HCV seropositivity was present in 30-39 years age group i.e. 2.45% (21/857) followed by 20-29 years age group i.e. 1.19% (22/1838) cases. The lowest HCV seropositivity of 0.34% (1/289) was seen in 40-49 years group. Also there was a statistically significant difference between voluntary and replacement donors with respect to their HCV seropositivity in both the age groups 20-29 and 30-39 as shown in table III.

The various probable risk factors for acquiring HCV infection were found in 38 out of 49 (77.55%) anti HCV positive blood donors. The most prevalent risk factor was injection (I.V.) drug use present in 14/49 (28.57%) cases. These donors gave a history of prolonged hospitalization or had received multiple injections from quacks.

The second most common route was tattooing which was present in a total of 12/49 anti HCV positive cases and in 6 out of these cases (i.e. 12.24% of total) no other history could be elicited than tattooing. In rest of 6 cases tattooing was present along with an associated risk factor such as multiple sex partners or another minor percutaneous route. No history of IV drug use or blood transfusion was present in any of these 12 cases.

Another important route was sharing of shaving kits or visit to roadside barber which solely was present in 5/49 (10.2%) cases. A history of ear piercing was present in 2 donors but these donors also gave history of tattooing or multiple sex partners.

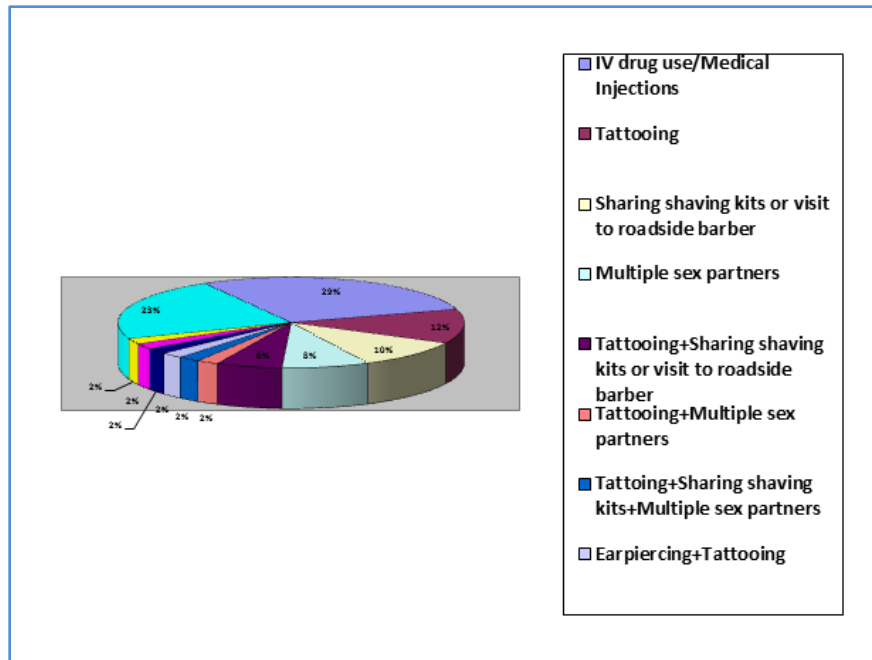
A history of multiple sex partners alone was present in 4 (8.16%) anti HCV positive cases.

Past history of blood transfusion or surgery was given by one case each (2.04%).

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A total of 7 cases gave a history of more than one route of infection and 4 out of these had a history of minor percutaneous routes (tattooing, sharing shaving kits or visit to roadside barber or ear piercing) only as shown in Graph III.

Graph III: PIE CHART SHOWING PAST HISTORY OF ANTI-HCV REACTIVE BLOOD DONORS:



Graph III

DISCUSSION: It is now a well-established fact that Hepatitis C virus is the most common cause of Transfusion transmitted hepatitis.⁷ The present average risk of post transfusion HCV infection per unit blood transfused is 1 in 100000.⁸ Thus, to ensure safe blood transfusion practice, not only is the mandatory screening of blood necessary, it is equally important to study the prevalence and demography of HCV infection in the blood donor population along with the risk factors causing it.

In this study 65.22% blood donors were replacement and 34.78% were voluntary blood donors. This was in well concordance with many of the studies which showed that replacement donors constitute the single largest group. In the retrospective study conducted by Pahuja and associates 99.48% donors were replacement donors.⁹ Similarly there were 83.6% replacement donors in the study of Singh et al.¹⁰

Better response towards blood donation was seen in the present study in the young (20-29 years) age group which was similar to that in the study of Thakral et al in which maximum voluntary donors were aged <30 years and to the study in Nepal.^{11,12} Donors in the age group 30-39 years formed the second largest group in present study.

The overall HCV seropositivity amongst 5000 blood donors was 0.98%. The reported HCV seropositivity from different regions of India correlates well with HCV seropositivity in the studies of Singh et al (0.9%) on North Indian blood donors,¹⁰ Pahuja et al (0.95%) on Delhi blood donors⁹ and Bagga et al (0.88%) at Patiala.¹³

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Maximum HCV seropositivity was seen in 30-39 years age group in both voluntary and replacement donors and HCV seropositivity in 30-39 years group was found to be significantly higher ($p < 0.05$) than 20-29 years and 40-49 years replacement donor group. Thakral et al also found this difference to be most apparent in the age group 18-30 yr.¹¹ Other studies with highest prevalence in age group 30-39 years were Fejza et al,¹⁴ Ayolabi et al¹⁵ and Bagga et al (replacement group).¹³

The various probable risk factors for acquiring HCV infection were found in 77.55% anti HCV positive blood donors similar to Thakral et al who found risk factors for acquiring infection 81% anti HCV positive blood donors.¹¹ The most prevalent risk factor in present study was injection (I.V.) drug use present in 28.57% cases who gave a history of prolonged hospitalization or receiving multiple injections from quacks. IV drug use was found to be most common risk factor also by Crawford et al,¹⁶ and Similarly Khattak et al¹⁷ and Akhtar et al¹⁸ revealed that positive donors had a history of past hospitalization or multiple injections.

In the current study second most common route was tattooing present in a total of 24.48% cases and in half of these cases (i.e. 12.24% of total) no other history could be elicited except for tattooing. No history of IV drug use or blood transfusion was present in any of the cases with history of tattooing. However a history of sexual route or other minor percutaneous route was present in half. Tattooing as an important route of transmission was also suggested by Patino-Sarcinelli et al,¹⁹ Luksamijarulkul et al,²⁰ Tohme et al.²¹

In 10.2% HCV seropositive cases of present study a solitary history of sharing shaving kits or visit to roadside barber was present. This high rate was in corroboration with the study of Thakral et al who documented 32% transmission via this route.¹¹

With only history of multiple sex partners present in 8.16% anti HCV positive cases sexual promiscuity was the other risk factor. A role for sexual transmission was suggested by studies of Crawford et al,¹⁶ Luksamijarulkul et al,²⁰ and Brandao et al.²²

The present study highlighted an important fact that exposure through minor percutaneous routes of transmission like tattooing and sharing of shaving kits or visit to road side barber along with multiple unsafe intravenous injections may have played an important role in HCV transmission in our blood donors. Therefore a need arises not only to routinely screen the blood donors for HCV but also to educate them about the modes of spread of this dangerous but preventable disease.

SUMMARY AND CONCLUSIONS: As Hepatitis C virus screening in blood donors reduces the chances of further transmission it should be a routine. A structured questionnaire should be used to inquire about modes of spread of all blood transmitted diseases including Hepatitis C. All donors giving a history of risk factors should be prevented from donating blood.

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