

ARTICLE INFO

Date Received:
07/11/2016;
Date Revised:
21/11/2017;
Date Published Online:
25/02/2018;

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How to Cite:

Akhtar H, Badshah Y, Akhtar S, Hasan F, Faisal M, Zaidi NSS *et al.*, (2018). Prevalence of hepatitis B and hepatitis C Virus infections among male to female (MFT) transgenders in Rawalpindi (Pakistan) . Adv. Life Sci. 5(2): 46-55.

Keywords:

HCV, HBV, Transgender, Pakistan, Unsafe sex

Open Access



Prevalence of hepatitis B and hepatitis C Virus infections among male to female (MFT) transgenders in Rawalpindi (Pakistan)

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Abstract

Background: Transgenders are the individuals, who due to certain psychological or physical requirement mismatch with their naturally received genders. Among transgender types, male to female (MTF) transgenders are highly prevalent in Indo-Pak and are feared to be at high risk for sexually transmitted viral (human immunodeficiency virus HIV, hepatitis B virus HBV, hepatitis C virus HCV and Epstein Barr virus EBV) and bacterial diseases (gonorrhoea, syphilis, campylobacter, chancroid, shigella, granuloma inguinale and chlamydia). This sensitive issue, therefore, requires more attention of researchers, policy-makers, and non-governmental organizations (NGOs) in Pakistan.

Methods: Prevalence of HCV and HBV infection was studied in 306 transgender males with a median age of 29 years (range 15-64years) living in Rawalpindi, Pakistan. Molecular techniques such as ELISA and Nested PCR were performed to confirm the presence of the both viruses in each sample of subjects. Furthermore, logistic regression and chi-square test were used for statistical analysis.

Results: This study showed that occurrence of HCV infection was predominant among the studied group; whereas presence of HBV was insignificant and was not included for statistical analysis. PCR confirmed that 25.5% of the test group was HCV positive, with the highest percentage (83.33 %) of HCV positive among the individuals who claimed safe sex practices and had knowledge about the medical condition of their partners. 34.61% of the transgender men who shave outside their homes, were found to be HCV positive.

Conclusion: In light of these statistics, ignoring this threat could be dangerous not only for the transgender community but also for the society, in general.



Introduction

Hepatitis B virus and Hepatitis C virus infections are threatening health problems with almost three to four hundred million carriers worldwide [1-3]. Sixty to eighty percent of the carriers progresses to chronic liver diseases [1-4]. Chronic infections caused by both of these viruses results in over 900,000 deaths annually and are the leading cause of hepatocellular carcinomas, fibrosis and liver cirrhosis [1,2,5].

HBV is endemic in South Asia, affecting about 10% of the population [5]. According to WHO, the prevalence of HBV in Pakistan is around 3% and it falls in the low endemic zone of the world (Fig. 1) [6-8]. Even though a universal HBV vaccination program has been implemented, there is still a need for an efficient and individualized therapy regimen for chronic hepatitis B infection to prevent its progression into end-stage liver diseases and hepatocellular carcinomas. Similarly, one out of every twelve individuals is at a risk of hepatitis C and it is also a wide spread viral diseases across the globe (Fig. 2) [9].

In some recent studies conducted in different cities of Pakistan, the prevalence of HCV was 6.5% in Hafizabad (Punjab) and 5.5% in Kech (Balochistan) (Fig. 3), whereas it has also been comparably found across the border with India [10-17].

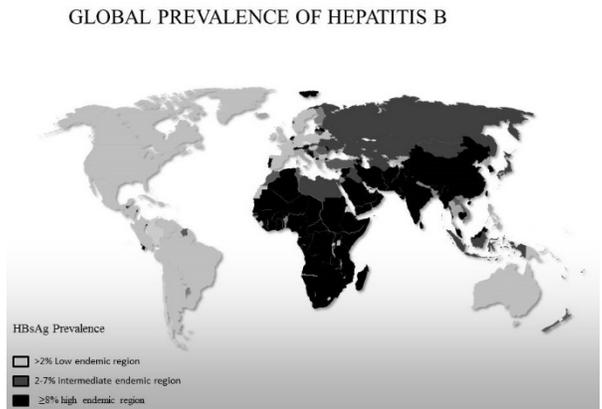


Figure 1: Global Prevalence of Chronic Hepatitis B Infection, 2013 [18-20].

Both HCV and HBV are blood-borne viruses that spread via contact with body fluids during unsafe sex practices, blood transfusions, child birth and sharing of blades or needles [21-23]. HBV/HCV co-infections are very common and are associated with life threatening complications like liver cirrhosis and hepatocellular carcinomas [24-27].

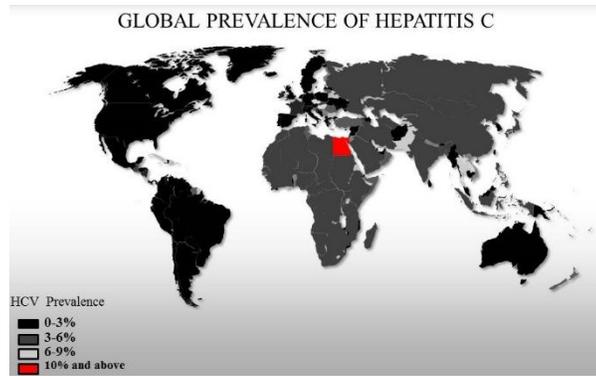


Figure 2: Global Prevalence of Chronic Hepatitis C Infection, 2013 [9, 15, 28-33].

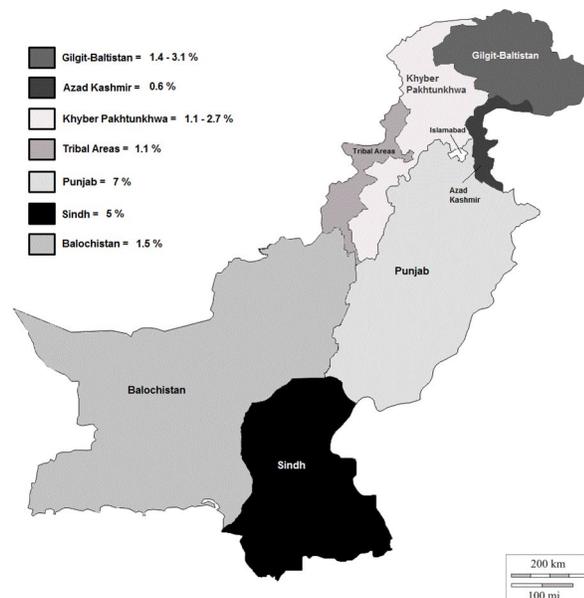


Figure 3: Prevalence of Chronic Hepatitis C Infection in Pakistan, 2012 [27, 48-57].

The aim of the present study is to draw a focus on HCV and HBV infections among transgender men in the city of Rawalpindi (Pakistan). Transgender is a broad term for individuals whose self-perceived gender identity is opposite to their biological gender. Transgenders are mostly under-privileged and constitute an economically and socially stigmatized class of our Indo-Pak society with a variety of gender identities such as: male to female, (MTF), female to male, (FTM) or just transvestites [34-41]. In Pakistani society MTFs or transgender men are known as Girya, Hijra, Chava, and Zanana [42-47]. Unemployment and social stigmas makes the MTFs become a part of the sex industry, thus, providing a cheaper alternative to other sex workers. Being under privileged, these MTFs are at a

high risk of various infections, including skin diseases, malaria, diarrhea, tuberculosis and especially sexually transmitted diseases (STD) such as acquired immunodeficiency syndrome (AIDS) and hepatitis [23,26].

Being transgender man in Indo-Pak society is a major social taboo which has been firmly discouraged by the majority resulting in their seclusion from the society [58]. Mostly these individuals spend their lives with their fellows and boss (guru) and drug dealing, sexual activities, dancing and begging are part of their livelihood [59-62]. Besides, they are also involved in selling unsafe sex due to lack of understanding of safe sex practices [63].” The main objective of this study is to find the relation of unsafe sexual activities practiced by MTFs due to lack of awareness or other business limitations with respect to the prevalence of sexually transmitted viral diseases (STVD) in this group of society. Customers of MTFs are mostly poor and illiterate, who seek for easy and cheap sex around their localities and according to a guru interviewed in this study, he said, “many of our customers run away if we ask them to use condom, because they then presume that there is something suspicious or we are having some disease. Many of us don’t even know about STDs other than AIDS”.

Methods

A cross sectional study for prevalence of HBV and HCV infections was conducted among transgender men located in Rawalpindi (Pakistan) from November 2009 to June 2010 [64]. The sampling procedure and data collection was done in the same way as previously described [64] but some additional information and a brief summary of other parameters is described as follows:

Sex Partner Test

Subjects were inquired that whether they or their partners were ever diagnosed for STVDs and whether they used a condom last time they had sexual intercourse. The two response options were yes = 1, no = 0.

Shaving Habits

Subjects were inquired about their shaving habits such as whether they shave their body and face at home or

they visit a barber shop. The response options were given numerical values as shaving at home is 1, shaving at a barber is 2 or for both is 3 [64].

Marital Status

This variable was measured by evaluating this question, ‘Are you married?’ and response has two options which were 1 for married and 0 for unmarried. Few of the subjects were married and had kids, which verified their bisexual activities [64].

Sexual Activity

Subjects were inquired about their normal sexual activities and the age of their first sexual experience and whether they ever have had anal intercourse (numerical criteria for answers: 1=yes, 0=no). Present study varies from already conducted studies related to STVD transmission, as the group understudy is always involved in homosexual activities [64].

Street Dentist

This variable was measured by evaluating the answers of the following questions; ‘Do you have any dental problem? Have you ever been to any dentist? Which dental hospital did you visit?’ As the use of paan and gutka among transgender men is very common, we found this variable to be an important determinant in exposing this mode of transmission of STVDs [65, 66]. The response options were yes = 1, no = 2 and we further divided yes into three categories; treatment from hospitals, treatment from street dentists (present on footpaths and mostly are without roofs) or no treatment [67, 68].

Education

The three grade levels were coded into a dummy variable (till 5th grade = 1, till 10th grade = 2, higher than 10th grade = 3) [64].

Blood Transfusion

This variable was measured by this question, ‘Have you ever undergone blood transfusion?’ and response options were same as previous criteria i.e. 1 for yes and 0 for no. Furthermore some of the responses were divided into donors or recipients of blood and their frequency was also recorded.

Sex Rate per Week

A variable was generated to measure the frequency of sex per week of the subjects under study and response was categorized as less than or equal to 20 and more than 20.

Sample Collection/Preparation

To study the the prevalence of HCV and HBV infection among 306 transgender men in the city of Rawalpindi (Pakistan). For molecular analysis, 15ml venous blood from each subject was collected and stored into EDTA anti-coagulating tubes. 1.5ml of the collected blood was centrifuged at 2400 rpm for 10 minutes which separated the serum from whole blood. Sera of all subjects were used for immunochromatographic method (Accurate, one step HBsAg Devices) to screen HBsAg and HCV-Ab in blood samples. Samples with positive antigen or antibody were further validated by PCR. HBV and HCV testing was carried out anonymously, however all of the subjects obtained their reports of the survey data only with lab serial numbers.

Detection of HBV-DNA by Nested PCR

HBV DNA was extracted from serum using the QIAamp Viral DNA kit (QIAGEN Inc., Hilden, Germany) and subjected to Polymerase chain reaction (PCR) by using Esco (96 well thermocycler). Twenty five cycles of DNA amplification were performed. The first round PCR was performed using sense and antisense primers followed by second round PCR (nested PCR), using the first round product with inner sense and antisense primers. PCRs were carried out using PCR Master Mix (Promega, Cat. # M7502) for 25 cycles. Nested PCR products (10 µl) were analyzed by electrophoresis on 2% agarose gels and visualized under UV fluorescence.

Detection of HCV-RNA by Nested PCR

HCV RNA was extracted from serum using the Roboscreen kit (Germany) and subjected to Polymerase chain reaction (PCR). Viral RNA was taken to reverse transcribe the 5' NCR of HCV using Moloney murine leukemia virus reverse transcriptase (M-MLV RTase, Fermentas) in a total reaction volume of 20 µl. The reaction mixture contained 4 µl MMLV (5X) buffer, 1 µl M-MLV reverse transcriptase (RT) enzyme, 1 µl dNTPs (10mM), 0.13 µl RNase inhibitor (Fermentas), 1.5 µl RNase free water, 1 µl specific anti-sense primer and 10 µl template (viral RNA). cDNA was synthesized using thermocycler. Cycle conditions for cDNA were as follows: 37°C for 55 minutes followed by 95°C for 5

minutes. cDNA product was used for qualitative analysis of HCV infection. The first round PCR was performed using sense and antisense primers followed by second round PCR (nested PCR), using the first round product with inner sense and antisense primers. PCRs were carried out using PCR Master Mix (Promega, Cat.# M7502) for 25 cycles. Finally, 10 µl of the second round PCR product was electrophoresed on 2% agarose gel. Specific bands of HCV detections were visualized under UV light.

Inclusion Criteria

All transgender men- *hijras* were included [64].

Exclusion Criteria

Female sex workers, FTMs (Female to Male), transgender females, gender queer and drag queen/kings were excluded from this study [64].

Results

Data Analysis

Investigation of statistically significant links between shaving, education, heterosexuality, number of sex partners per week and prevalence of HCV are described in this article. The data analysis was performed by means of SPSS (16.0 version) for Windows XP [69]. Detailed analyses were conducted by calculating the frequency distribution of HCV and HBV infections (prevalence of HBV infection is very low, as assumed because of its vaccination, so it is not shown in comparison with different parameters used with in the study) among transgender men in association with the eight parameters described previously. Multiple logistic regression model was applied to illustrate the association between the prevalence of HCV (HBV data not shown in results) among transgender men and its dependence on some other factors correlated to them. Seven predictor variables were considered for this purpose.

- Age of the respondent (transgender)
- Sex frequency in a week\ number of partners per week
- Education of transgender men
- Marital status or heterosexuality
- Body shaving behavior
- Blood transfusion history
- Dental problems and visits to street dentists

First two variables; Age and Sex frequency in a week were classified into categorical variables because the frequency of sex was showing a relation with the age, as it was higher among young age groups. Asking for safe sex habits of sex partner, visit to street dentists, heterosexuality behavior or marital status and blood transfusion history were split into binary variables; 0 and 1 [0= No and 1= yes]. Education of transgender men and shaving behavior were split into binary variables, as they were categorical variables; if a variable has 4 categories then it was transformed in 4-1 binary variables e.g. shave variable has 3 categories; shave at home, from barber and both so it was split into two binary variables i.e. home and barber. Frequency of sex partners per week was divided into 3 categories based upon number of sex partners per week ranging from one to twenty, more than twenty up to forty and more than forty as 1, 2 and 3 respectively. Hence the 3rd function cannot be introduced since an intercept is present in the model so the last category in each variable was a reference category. Hierarchical technique was used to select the model. Associations of these variables for HCV are performed by the Chi-Square test, as summarized in Table 1 (supplementary data).

Overall the prevalence of HCV was found to be 25.5% in a total of 306 transgender men. The highest percentage was found to be 83.33 % among those, who claimed to have knowledge about the safe sex habits of their partners (Fig. 4 in supplementary data). Statistical analysis shows that 34.61% of the individuals who went to barbers were infected with HCV against 28.08% of those who shaved at home (Fig. 5 in supplementary data). Body shaving was found to be common among this class of sex workers and they frequently share razors due to lack of awareness and shortage of assets (Fig. 5 in supplementary data). 90 out of 306 transgender men were found to have had blood transfusions and 19 out of these 90 were found to be HCV positive (Fig. 6 in supplementary data). 85 out of 306 transgender men included in this study had some dental problems and visited at least once in the past 3 months the nearest street dentist. 28 out of these 85 had HCV and it seems that the other patients of those street clinics are in jeopardy, as the sterilization facilities of these clinics are not satisfactory (Fig. 7 in supplementary data). Two categories were defined to measure the prevalence of

HCV with respect to the number of sexual partners per week; first one was with less than and equal to twenty different partners and the second one was with greater than twenty sexual partners per week. 34 out of 101 (33.66%) subjects with a high number of sexual partners per week were found to be HCV positive, as compared to 44 out of 205 (21.46%) subjects, who had fewer sexual partners per week. (Fig. 8 in supplementary data). Prevalence of HCV among homo and bisexual transgender men was found to be 28.4 % and 12.5 % respectively. Transgender men involved in horizontal transmission of STVDs can further spread these diseases to opposite genders, as few of the married transgender men were fathers of kids too. Vertical transmission of STVDs through female sex partners of heterosexual transgender men can also be a risk in raising the prevalence of HCV (Fig. 9 in supplementary data). Awareness comes through education and results shows the same as the educational background had a significant association (p-value = 0.012), likewise the prevalence of HCV dropped dramatically (i.e. 9.52% for transgender men, educated above 10th standard). However, education was found to be insignificant in the logistic regression model (Table 2 in supplementary data) (Fig. 10 in supplementary data).

Multivariate Analysis

Multiple logistic regression model was used to illustrate the relationship between the transmission of HCV among transgender men and its connection with related factors. The following five predictors were found to be important for this purpose (see Table 2).

- Asking for safe sex habits of the sex partner
- Body shaving behavior
- History of blood transfusion
- Visits to street dentists
- Number of sex partners per week

The relative odds with respect to their daily routine activities and behaviors are shown in table 2. During interviews, we observed that the level of education was linked with the knowledge about different diseases and their modes of transmissions. However, educational background of transgender men was insignificant by stepwise variable selection method in this Logistic regression equation (in supplementary data).

The analysis shows that all the predictors have significant predicting power i.e. the question asked about the knowledge related to the safe sex habits of their sex partners has shown highly significance in the studied group. Respondents were categorized into three groups according to their shaving behavior, i.e. shave at home, shave from barber shop, or both. The third category was used as a reference in this model. Results showed that the odds for shaving from barber were two times greater than the other group, whereas shaving at home was also quite high and reached almost two and half times more. The odds for having HCV in transgender men were two times higher when they were involved in blood transfusions. Visits to street dentists also increased the odds up to one time, as compared to the transgender men visiting a proper dental hospital. In addition to this, the odds were one and a half times higher, if the transgender men had more than 20 partners per week.

Discussion

Pakistan is a developing country with much trepidation like economic strains, illiteracy, poverty, social and ethical issues and much more. One out of these apprehensions is health related issues, which includes STDs and STVDs. Pakistan lacks proper data on the prevalence of HBV and HCV infections and particularly co-infections among sex workers of the transgender societies. Transgender men are one of the stigmatized and under privileged groups of Indo-Pak community with a lot of social, ethical and cultural issues at the national level. Illiteracy and lack of awareness leads them to poverty and a number of other factors add to their seclusion from society [58,59].

One out of many reasons behinds the swift spread of viral diseases in transgender populations can be the lack of attention paid by the government or discrimination in the private and public health related sectors. Unemployment and the economic strains of society have dragged many of them towards prostitution as source of employment [70,71]. Client based sex, unprotected anal sex, survival hurdles, physical abuse and drug abuse adds a lot in the increasing prevalence of these STDs and STVDs amongst these groups [72,73].

The present study focuses on the behavioral patterns of transgender men with reference to the prevalence of HBV and HCV among this group. A group of 306 transgender men were questioned about their weekly

sexual activities and their answers were confirmed by their gurus (boss), who were also part of this business. Results showed that HCV (25.5%) is more prevalent than HBV (2.941%) amongst the studied group, whereas, the prevalence of HCV in Pakistan is believed to be much lower than this [72]. The highest percentage was found to be 83.33% among those, who claimed to have knowledge about the safe sex habits of their sex partners (Fig. 4 in supplementary data).

Young respondents included in the study were mostly dancers and paid much attention to their appearance because they had to earn money through their personal presentations, but due to their good body maintenance, they are also more involved in the sex selling business. Results showed that, 12 out of 306 transgender men claimed to have sex with customers, whom they know very well including their health related issues. 10 out of those 12 were found to be HCV positive (83.33%), whereas the prevalence of HCV among the repudiating group of 294 transgender men was 23.13%. This might be interesting because either they are harmless for the society or they told untruth stories.

As education means knowledge or awareness towards many aspects of life, health care is also somehow related to this parameter. Results showed that transgender men, who only had the most basic education, i.e. they didn't even cleared their primary education, had a higher risk of being infected with HCV, as compared to those, who cleared their 10th grade. Educated transgender men were found to be more vigilant about avoiding unprotected sex and had lower prevalence of HCV i.e. about 9.82% as compared to 22.58% and 30.99% among subjects who were less educated below 10th and 5th standards respectively. Juvenile school drop outs or lack of education may end into unsafe sexual activities, as the data shows a high prevalence of HCV in that class. Lower literacy rate and lack of awareness were found to have a direct relation with the prevalence of HCV and HBV in transgender community.

The analysis highlighted bisexuality as another important factor involved in dispersion of STVDs, as 18.3 % of the respondents involved in this study were married and were involved in both hetero- or homosexual activities. Results showed that unmarried\homosexuals had a high prevalence of HCV (28.4%) as compared with married\heterosexuals (12.5%). Few married respondents do have kids, so had to give time to their married lives, which somehow

implicated more responsibilities on them and they had a reduced sexual activities.

Transgender males have many shaving behaviors and patterns. They were categorized into two groups i.e. those who shave at home and those who shave at barbers. Home shaving included razors, threading and clipping through tweezers or a pair of scissors. Subjects who shaved at barber's shop had a high prevalence of HCV (34.61%) in contrast to those who shaved at home (28.08%). Thus sterility at low budget barbers shop can be questioned because they may reuse the same instruments without sterilizing them or reuse sterilizing liquids for many days. Another key factor at low budget barber shop can be the use of potash alum, which is still used for facial cuts and thus comes in contact with the blood of many [74]. Transgender men living in clans also stated that they in routine share scissors and razors for body shaving. Sharing of blades due to lack of resources can be a vital factor in the dispersal of HCV at home.

Number of sex partners per week was found to be a significant factor after analysis. Transgender men with twenty plus sex partners per week had a very high prevalence of HCV (33.66%) as compared with those, who had few sex partners per week (21.46%). Sexual contact is the key factor in transmission of STVDs and our results showed the same. Spread of HCV through unsterilized dentistry equipment is also an important parameter and was included in the study, because the transgender community of Pakistan frequently use cheap brain stimulants or mouth fresheners like areca nut/supari, gutka and pan (with or without tobacco) [75].

The use of these products leads to many dental and buccal problems, including tumors [75]. Dental problems lead those to dental clinics and due to a lack of resources, they visit low budget dental clinic or even street dentists, which are without proper hygiene practices. Unsatisfactory sterilization of dental instruments is quite common in these clinics, which leads to increase the risk of STVD spread. Visits to street dentists were found to be an important parameter in this case too and the prevalence of HCV was 10.32 % higher in the group, which claimed to visit street dentist quite often. Transgender men belong to an economically stigmatized community of our society who cannot

afford to visit health care facilities. Poverty, unemployment and economic stress answer the fact that they cannot afford professional dentists. 85 transgender men claimed to visit street dentists and 28 out of them were diagnosed as HCV positive.

Blood transfusion is a sensitive issue with respect to the spread of STVDs, but still many of the respondents had a history of blood transfusion. Blood transfusion was not very common in this community, as those who answered 'yes' had hardly undergone this process thrice within the last 5 years. Though there was a contrasting trend observed in the analysis, with the decreased rate of prevalence in transgender men who had undergone blood transfusion (21.11%) against those who had never undergone this process in the last 5 years (27.32%). This study depicts a fact that few parameters like history of blood transfusion and visits to street dentist may have a significant role in the propagation of HCV so an extensive research should be conducted to increase our knowledge towards these concealed issues in this community.

Alcoholism, prostitution, promiscuity and drugs are widespread among dancers in the transgender community. Injectable drug users (IDUs) add a lot to the prevalence of STVDs besides sexual contact, however, this case study showed a negligible number of IDUs in the transgender community thereby eliminating this risk factor. Use of condom was added as the factor to study the significance of HCV and HBV spread in the community but it could not be strongly related in the analysis, as a negligible number of respondents claimed to use it regularly. According to the respondents, the reason behind not using condoms regularly was due to an apprehension on the customer's part.

Domestic violence, unemployment, lack of awareness, poverty, ethical issues, rape, early age sexual activities and illiteracy are some of the factors believed to be contributing to the isolation of transgender community. This study evidently characterizes some problems of this community and further highlight the social stigmas of cultural and religious origin in Pakistan, furthermore, enlightening the need towards STVDs prevention in ignorant transgender communities. The health care department needs to be more concerned about the conditions and living environment of the transgender communities and should implicate regular free check-

ups. They should be given rights to live their life as normal citizens of a country.

Pakistan has a rising prevalence of HCV and HBV infections. This study stresses the importance of health care among minority groups in Pakistan. A number of issues concerning STVDs control was highlighted i.e. illiteracy, ignorance and background status leads to this unusual behavior. Transgender men are a secluded class within society with no proper rights, such as access to the basic life necessities, health care facilities, quality education or employment and are constantly feeling depressed and never looked upon as normal citizens of our society. Education, awareness and health care services should be provided to this community.

The statistical analysis of the above mentioned parameters: age, education, knowledge about sex partners, number of sex partners per week, blood transfusion, shaving behavior, street dentist and marital status showed that they all contribute to the high prevalence of hepatitis C in the transgender community. The overall prevalence of HCV was 25.5% among the total 306 transgender subjects in study.

Implications of the Study

The findings and conclusions of this study have implications for health care practitioners, NGO's and health educators trying to better understand the rate of prostitution and risk behaviors among transgender men in Rawalpindi and perhaps other cities of Indo-Pak. The prevalence of HCV, the sexual behavior and the use of condom among transgender men in the current study suggests that current educational efforts, awareness programs and strategies are inadequate. The conclusion of this study suggests that transgender men should be provided with proper medical facilities and treatments after screening.

Declared Ethical Approval

All the samples were collected, with the approval of the Institutional Review Board, Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology (NUST), Islamabad, Pakistan. The candidates who gave permission were informed about the tests to be conducted from that blood samples and the interviews were conducted in front of the *Guru* (chief or supervisor) of that community.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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