

Original article**Risk factors for hepatitis C disease in Tando Allahyar, Pakistan: a case-control study**Channa NA¹, Khan H²**Abstract**

Objective: Present study was undertaken to explore the epidemiological and dietary risk factors for hepatitis C disease in Tando Allahyar during June to December 2007. **Materials and methods:** 140 Hepatitis C patients and 149 controls (age, sex and locality matched with no personal or family history of hepatitis C) were randomly selected from the Tando Allahyar population during June 2007 to December 2007. A standard questionnaire specially developed for that purpose, was distributed among patients and controls. Accurate rapid card test was performed to confirm Hepatitis C virus (HCV) positive or negative patients and controls. O.R with 95% confidence interval was computed and p values were calculated by chi square test. **Results:** Over all findings showed that among 140 HCV patients 64% were females and 36% males (mean age range was 20-48 years). Our results showed strong positive association of sedentary life style (OR=4, 95% CI, 1.48-10.6, p=0.005), marital status, +ve family history, B+ve blood group, piercing, syringe use (70%), multiple pregnancies (OR=4.72, 95% CI, 1.47-15.15, p=0.008), blood transfusion (OR=4.4, 95% CI, 1.2-14.95, p=0.017), take out food debris by office pins (NS), shave at barber's shop (NS), dental procedures (NS), medicines used in past (OR=3.08, 95% CI, 1.2-7.71, P=0.016), G.I.T diseases (OR =8.66, 95% CI, 1.28-55.58, p=0.023), stones of kidney or gallbladder, jaundice, Tuberculosis and psychological problems (OR =2.5, 95% CI, 1.04-6.13, P=0.04) with hepatitis C disease. We did not find any association of surgery, BMI, ENT, diseases, meat consumption and smoking with the disease. Vegetables OR =0.842, 95% CI, 0.361-1.964, P=0.694), pulses, tomatoes (OR =0.31, 95% CI, 0.041-2.53, P=0.31), and tea (OR =0.598, 95% CI, 0.251-1.431, P=0.25) were found to be the protective factors for Hepatitis C disease. **Conclusion:** Sedentary life style, multiple pregnancies, blood transfusion, medicines used in past, G.I.T diseases, and psychological problems are significantly positively associated with Hepatitis C disease.

Key words: Hepatitis C disease, HCV infection, epidemiological risk factors, Diet, blood transfusion, G.I.T diseases, and psychological problems.

Introduction

Hepatitis C disease is caused by hepatitis C virus (HCV) infection and it may become the leading cause of other diseases such as: chronic liver diseases [1 – 2], cirrhosis [2 – 3], and hepatocellular carcinoma [1 – 4]. Globally it affects approximately 3% of the world's population. 30% of those infected patients clear the virus spontaneously, whereas, remaining 70% infected chronically (30% of those 70% develop Cirrhosis) [5]. The highest HCV prevalence in the world occurs in Egypt, where the prevalence of HCV infection increases steadily with age [2]. According to a cohort study in Egypt the incidence rate was 0.08% in an area of Upper Egypt where the background prevalence was 9% and 0.68% in the Nile Delta where the background prevalence was 24% [2]. Shah et al. reported 0.5 – 14% seroprevalence in different population groups in Pakistan [6].

Hepatitis C disease is spread able disease and the most efficient transmission of HCV is through direct percutaneous exposure to blood [7], but it is less efficiently transferred by mucosal exposure to blood [8]. There is contradiction between certain modes of transmission such as cosmetic procedures (body piercing), religious and cultural practices (acupuncture) [2, 9]. It is difficult to determine the incidence of newly acquired HCV infection because most of acute infections are asymptomatic and available assays do not distinguish acute from chronic infection [2].

Some food items have been reported to have protective or toxic effects on the liver in animal models and humans [10]. The aim of present study was to evaluate the positively or inversely associated dietary and other epidemiological risk

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factors for Hepatitis disease progression and / or transmission.

Material and methods

Sample Population

An epidemiological case control study was conducted in Tando Allahyar (Sindh), Pakistan. Tando Allahyar is an area with peri urban community; it is lying between Hyderabad and Mirpurkhas. The subjects recruited in the study were 140 HCV patients and 149 controls (age, sex and locality matched) during the study period, June 2007-December 2007. Mean age range of male subjects was 21-36 years, and female subjects was 28-44 years. Institutional Ethical Committee approval was made prior to start study and informed consent form was also signed by all participants.

Laboratory Analysis

3ml of blood was drawn from each subject (cases and controls) by veinpuncture, using sterilized needles and syringes. The blood was collected in sterilized sample vials having screw cap. Serum was separated and allowed to store at -20°C prior to use. Accurate rapid test kits were used to analyze all the samples of patients as well as controls.

Questionnaire

Each subject was personally and carefully interviewed through a specifically structured questionnaire. Because of some cultural limitations and sensitivity of some questions males and females were interviewed by the same gender. The protocol was divided into 2 sections, the 1st comprised of questions about their demographic characteristics, environmental and social risk factors and 2nd section was comprised of questions about their dietary habits.

The respondents were also interviewed regarding vegetable, pulses, and meat consumption. Type of food, fat and tea consumption information was also collected from all the subjects.

Statistical Analysis

We performed descriptive analysis by calculating mean, and percentages. The associations were assessed by calculating logistic regression with Odds ratio and 95% confidence intervals was estimated on Statgraphics plus 5.1 software. The odds ratio equal to 1.0 was considered as reference, <1.0 as protective factor, and >1.0 as risk factor for hepatitis C disease [11]. p- value was calculated by

chi-squared test using Minitab software release 13.2. P<0.05 was considered as a level of significance.

Results

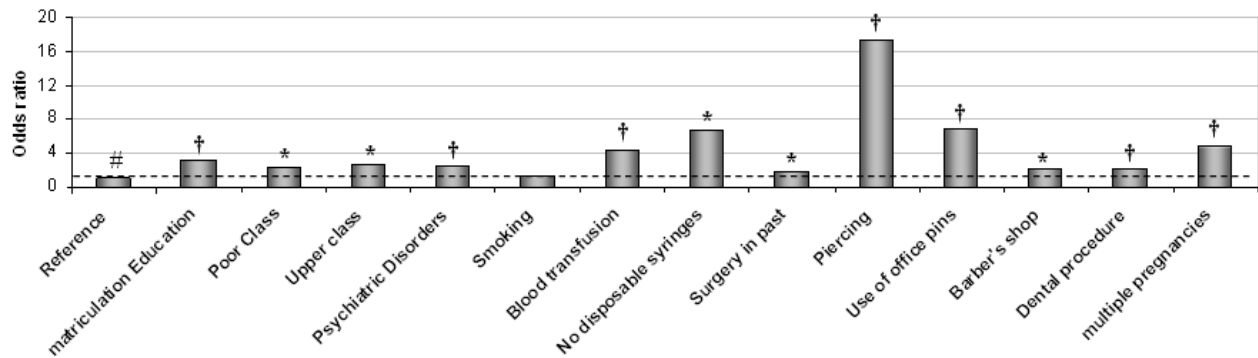
Table 1 shows the demographic characteristics of both groups. Out of 140 HCV patients 35.7% were males and 64.2% were females. The peak age range for those patients was 31-45 years had prevalence rate of 48.57%, followed by those aged 16-30 years, whereas, reverse was true for less than 15 years age group. The study comprised of 77% married HCV patients and remaining was unmarried.

Table 1: Demographic Characteristics of Cases and Controls.

Demographic Characteristics	Cases (%) (n=140)	Controls (%) (n=149)
Gender		
Male	35.7	36.9
Female	64.2	63.1
Age Wise Frequency (years)		
≤15	02.8	02.6
16-30	35.8	34.8
31-45	48.6	47.6
≥46	12.8	14.0
Ethnic Origin		
Mohajir	31.4	56.4
Sindhi	15.7	10.1
Punjabi	28.6	33.5
Balochi	22.8	0.00
Pathan	01.5	0.00
Marital Status		
Married	77.2	73.2
Single	22.8	26.8
Blood Group		
A+ve	25.7	16.7
B+ve	32.8	40.4
O+ve	21.4	39.5
O-ve	01.4	00.0
AB+ve	10.2	3.40
AB-ve	08.5	00.0
Family History		
Yes	62.8	00.0
No	37.2	149.0

The ethnic distribution of study population showed majority (31.4%) of HCV patients were belonged to Mohajir ethnic group. The prevalence of HCV disease was found high with B+ve blood group as compared to controls. Majority of HCV patients (62.8%) were found to be with positive family history of this disease.

Risk factors for HCV disease



Comparison group for each factor (>10 classes, middle class, no disorder, no smoking, no blood transfusion, use of disposable syringes, no surgery, no use, no dental procedure, three pregnancies)

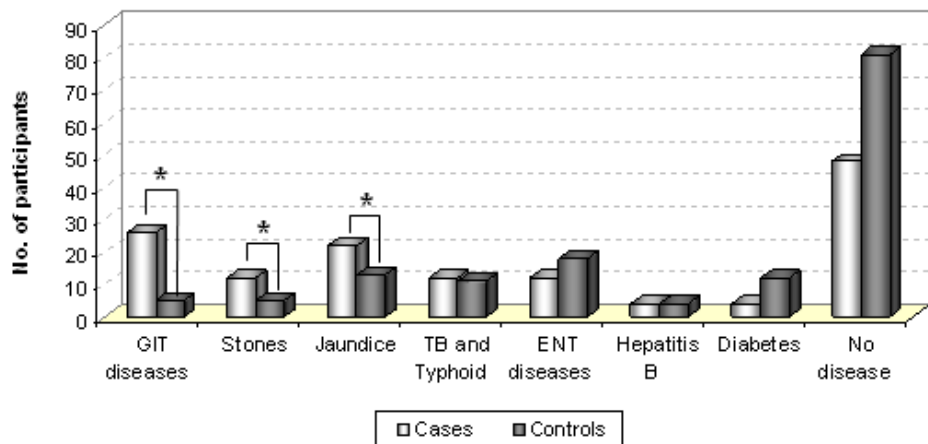
^Poor class, had earning of <8000 rupees/month, middle class had earning 8000-15000 rupees/month, upper class had earning of >15000 rupees/month.

In Logistic regression analysis * $P < 0.05$, † $p < 0.01$

Figure 1: Comparison of odds ratio among epidemiological factors for hepatitis C disease

Figure 3 compares the different types of food, vegetable, pulse, green chilies, meat, sugar, tomatoes and tea consumption between cases and controls. We found a significant negative association of green chilies (OR=0.36, 95% CI, 0.210-0.62, $p=0.000$), tomatoes (OR=0.127, 95% CI, 0.049-0.329, $p=0.000$) in any form (raw, fried, curry), 3 – 4 times/week consumption of pulses and taking more than two cups of tea per day (OR=0.59, 95% CI, 0.37-0.966, $p=0.035$) with hepatitis C disease. No consumption of vegetables was significantly positively associated (OR=3.1, 95%CI, 1.04-9.5, $p=0.04$) with hepatitis C disease, whereas, reverse was true for those consuming vegetables more than 6 times per week. Fried food consumers were found to be highly significantly positively (OR=25.2, 95% CI, 9.06-70.06, $p=0.000$) associated with hepatitis C disease as compared to all other dietary risk factors (Figure 3). No association was found for red and white meat and not taking tea with hepatitis C disease.

In Figure 1 epidemiological factors are addressed. The patients both with poor socioeconomic status (OR=2.3; 95% CI, 1.07-5.33, $p=0.033$) and upper



* $p < 0.05$ by Logistic regression analysis

Figure 2: Association of other diseases with Hepatitis C disease

class (OR=2.66, 95%CI, 1.3-5.2, $p=0.005$) and psychiatric disorders, mostly anxiety and depression ($p=0.000$), were found to be significantly positively associated with HCV disease. Concerning the educational status our results revealed that subjects studied less than 10 classes were positively associated with HCV disease, (OR=3.11, 95% CI, 1.92 – 5.04, $p= 0.00$). We found no association for smoking (OR=1.31, 95% CI, 0.64 – 2.69, $p=0.45$) with hepatitis C disease. When association of some risk factors of transfusion with hepatitis C disease were explored, it was revealed that blood transfusion ($p=0.000$), disposable therapeutic syringes ($p=0.046$), surgeries in past ($p=0.043$), ear and nose piercing ($p=0.000$), inserting contaminated needles and pins in mouth for taking out food debris ($p=0.000$), shave at barber's shop ($p=0.01$), dental

procedures ($p=0.001$), were found to have a significant positive association with hepatitis C disease. A significant positive association ($OR=4.8$; $95\% CI, 2.46-9.46$ and $p=0.000$) was also found for the HCV patients (females) having more than 3 children (multiple pregnancies).

Figure 2 revealed that gastrointestinal diseases ($p=0.000$), kidney and bladder stones ($p=0.009$), and jaundice ($p=0.007$) had a significant positive association with hepatitis C disease with a higher odds ratio as compared to other factors. Statistically non significant positive association was found for T.B, typhoid ($p=0.17$), and hepatitis B ($p=0.46$) with hepatitis C disease. Whereas, we found no association of ENT diseases ($p=0.77$), and diabetes ($p=0.56$) smoking ($p=0.45$) with hepatitis C disease. Ear and nose piercing ($OR=17.35$, $p=0.000$) were found to be the biggest risk factor for hepatitis C disease as compared to all the other risk factors (Figure 1).

Discussion

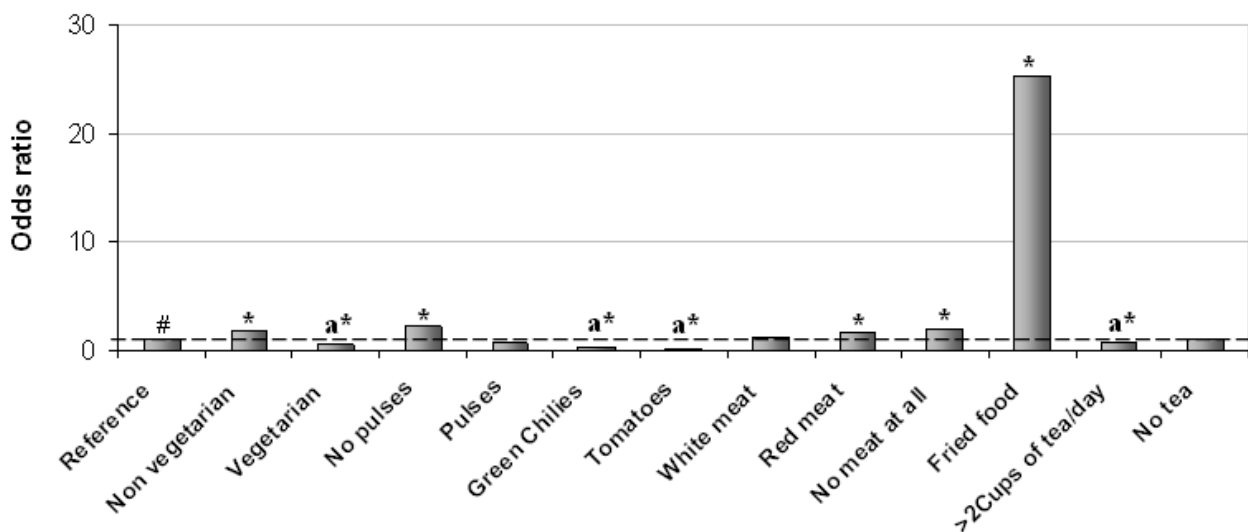
The distribution of HCV infection in males and females were found to be 37% and 64% respectively in present study (Table 1). The possibility of women are more prone to acquire hepatitis C infection in present study may be due to multiple pregnancies and ear and nose piercing (Figure 1) [9, 12, 13]. Unsterilized tools used during delivery of child may increase the risk of hepatitis C transmission. More exposure of unsterilized tools during each child delivery may

increase chance of getting HCV infection in women [14]. Surgery was also an established risk factor contributing to the transmission of HCV infection in present study as well as other study. Dental surgeries can cause contamination of Hepatitis C Virus after treatment of anti-Hepatitis C Virus patients and that if sterilization and disinfection are inadequate there is the possible risk of transmission to other individuals [15].

We found the 31 – 45 years as peak age range for hepatitis C patients having prevalence rate of 48.57% (Table 1), this is in agreement with other studies also [2, 16]. Mcquillan et al. reported highest HCV prevalence among persons aged 30 – 49 years [2]. Seropositivity for HCV infection increases until the age of 40 years and then decreases over time [17].

Concerning about the family history (Table 1) we have found that 62.8% of patients had positive family history for HCV infection. HCV virus can be detected from saliva of infected person; casual household contact and contact with saliva of infected person may cause transmission if healthy person has any cut or injury where saliva can come in contact, although it is very inefficient mode of transmission [18].

Majority of Pakistani population consists of middle to low income class, unemployment is over one-twelfth of labor force, over one third of the population lives in poverty and over half the population is illiterate [19].



Comparison group for each factor,
* $P \leq 0.05$, a* significant negative association

Figure 3: Comparison of odds ratio among dietary risk factors for hepatitis C disease

Present study showed that illiteracy and poverty were also the risk factors in acquiring HCV infection (Table 2 and figure 2). Uneducated persons with extremely less (poor class) or extremely excess (upper class) money can expel one to involve in unfair or wrong deeds (such as involvement in drugs, sexuality etc). Different psychiatric disorders (O.R=2.5, 95% C.I, 1.5 – 4.2, P=0.000) including anxiety and depression were the major risk factors for acquiring HCV infection in present study (Figure 1). But it is still not clear that either these psychiatric disorders lead to HCV disease or patient after acquiring HCV infection undergo psychiatric disorders. Direct involvement of central nervous system by hepatitis C virus leads to depression, dementia and other psychiatric manifestations [20].

HCV infection is mainly transmitted by blood transfusion which a well is known risk factor reported by many investigators, our study revealed comparable results [2, 7, 9, 14, 17 – 21]. HCV infection transmission risk factors found in present study were unsafe therapeutic syringes use; unsterilized needles/pins use; shave at barber's shop, dental procedures, gastrointestinal diseases, stones and jaundice (Figure 2). Many scientists have reported that use of unsterilized syringes and therapeutic injections are significantly positively associated with transmission of HCV virus which is also true for present study [2, 7, 9, 12, and 21]. Poverty and illiteracy are the major social problems in Pakistan; unsterilized used needles are being used by practitioners (especially quack doctors) who do not even know that HCV can be transmitted by injections [12]. Inadequate medical waste disposal system is also very much common in Pakistan. Medical practitioners in their private clinics reuse syringes until those get blunt for making their profit [12]. It is estimated that about half of all injections administered in Pakistan involve reused syringes [13]. Intentional or unintentional use of office pins in papers or between teeth spaces to take out food debris may cause the contamination of office pins by Hepatitis C positive people which may be transmitted to healthy persons, we found a significant positive association for that in present study. As we know that exposure to accidental needle sticks and sharp exposure may also transmit HCV as it is evident from present study also [7]. People who visit frequently for any kind of dental procedure had more prevalence rate of HCV than those who do not visit for any kind of dental procedure [21].

Another risk factor for HCV transmission was face and armpit shaving at barber's shop (Figure 1). The delicate skin of the face and armpit has a very fine skin which is susceptible to micro trauma. It may lead to possible exposure to HCV through a contaminated razor as their awareness about hepatitis and associated risks of transmission is very low. The spread of hepatitis C can promote due to their reused razor on multiple clients [14].

Other diseases showed significant positive association with HCV were G.I.T diseases, kidney and bladder stones and jaundice with $p < 0.05$ in present study (Figure 1). Gastric and enteric helicobacter species have been associated with pathogenesis of many gut problems as well as some extra gastric diseases like hepatocellular carcinoma. DNA of these bacterial species causes different liver diseases, and increases susceptibility towards HCV disease [22]. Jaundice was also associated with HCV positive status; however jaundice might be a marker for the disease. T.B and typhoid were positively associated ($p=0.17$) with HCV infection. However tuberculosis and HCV is also a blood born pathogen so injection drug use may be a risk factor for transmitting blood born pathogens such as: HCV virus, HBV virus and Mycobacterium tuberculosis [23].

We found a significant negative association for vegetables, tomatoes, green chilies and consumption of tea more than 2 cups with HCV disease ($p < 0.05$). An epidemiological study has shown that consumption of fruits and vegetables are negatively associated with several types of cancers, which may be due to antioxidant nutrients, (vitamin C, E and carotenoids) for preventing oxidative damage, which is thought to be involved in pathophysiology of many diseases [24]. The nutrients may inhibit viral RNAs and various proteins or other proteins required for HCV RNA replication [25]. Three known nutrients which are lipid in nature such as Beta carotene, vitamin D₂ and linoleic acid inhibit HCV RNA replication [25]. Beta carotene is present in many fruits, oil and vegetables (like carrots, green plants or green chilies, spinach, squash) [26]. Tomatoes were inversely associated in present study which contained Lycopene, a carotenoid having antioxidant and antiproliferative properties (Figure 3) [27]. It is also reported in an investigation that people who drink >2 cups/day tomatoes juice have less than half the rate of chronic liver disease as compared to those who drink <1 cup/day [28].

Our results showed that fried food was significantly positively associated with HCV disease with O.R, 25.2, 95% C.I, 9.06 – 70.06, P=0.000, whereas, reverse was true for meat consumption. Researchers demonstrate that high fat intake is associated with increased risk of progression of cirrhosis in those with chronic hepatitis C [6]. A diet restricted in total calories, fat, iron and protein intake reduces serum ALT levels in patients with long term hepatitis C virus infection [29].

Conclusion

High prevalence of HCV viral infection in Pakistan may be due to illiteracy, poverty, family history, and different psychological problems. The highest

risk factors involve in the transmission may be piercing and consumption of fried food followed by unscreened blood transfusion, unsafe therapeutic syringes, dental procedures, contaminated needles and sharp exposures, multiple pregnancies, GIT problems, stone and jaundice diseases. Various types of foods such as vegetables, pulses, green chilies, tomatoes and tea may act as protective factors for hepatitis C disease.

Government should make different strategies to aware people against HCV disease infection, and its transmission as there is no pre and post exposure vaccine against it.

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