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Prevalence of hepatitis C in selected patients in Parachinar, Kurrum Agency, Pakistan

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Abstract

A survey prevalence of Hepatitis C Virus (HCV) Patients in Parachinar, Kurrum Agency , Pakistan was performed from March 2010 to March 2011. 100 positive HCV cases were selected and interviewed. The highest prevalence of HCV was found in 36% males and 8% females with aged 16-30 years. HCV infection in married 55% males and 10% females. Population in rural area was more affected as in males 72% and females 10%. Socioeconomic condition of the cases belongs to business class people, in which 74% male and 7% females +ve. Risk factors of HCV patients from higher to lower range as share syringe were found in males 31% and females 7%, patients with surgical operations were in males 17% and females 12%. Health care workers, in 14% males and no female was affected. Sexually transmission of HCV, in males 3% and females 2%. Symptoms of HCV found in patients with descending order: abdominal pain in males 67% and females 12%; fatigue in males 65% and females 12%; fever in males 64% and females 6%; yellow skin color in males 53% and females 7%; dark color urine in males 35% and females 7%; rashes in males 35% and females 6%; vomiting in males 21% and females 4%; pale color stool in males 19% and females 5%. The HCV was diagnosed by strip, ELISA and PCR methods. Strip method was performed by males 9% and females 3%. The ELISA was performed by 74% males and 9% females while PCR was performed by only 2% males.

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Introduction

The word Hepatitis is derived from two Greek words, hepta meaning liver and itis meaning inflammation. They are named alphabetically for the order of their discovery A, B, C, D, E, F and G. In 1963, scientists found virus in the blood of hepatitis patients that was belong to neither hepatitis A nor hepatitis B and called that non A and B hepatitis (NA/NB). In 1973 Daniel Bradley and Chiron called NA/NB as HCV. There are many forms of hepatitis, including viral hepatitis, autoimmune hepatitis, fatty liver hepatitis, alcoholic hepatitis and toxin induced hepatitis caused by identified hepatitis viruses that attack and damage liver cells (Khan *et al.,* 2010; Mclnnis, 2003). HCV infection cases both acute and chronic hepatitis as shown in many other diseases. The mean incubation period of acute hepatitis is 6-7 weeks with symptoms like malaise, nausea, and pain in right upper abdominal region followed by dark urine and HCV RNA becomes undetectable. Hepatitis lasting more than six months is generally defined as chronic hepatitis. Many patients are asymptomatic, however, anorexia, yellow color of urine, rashes on skin, abdominal pain, weight loss, vomiting, nausea and fatigue are common. Some time with low grade fever and non specific upper abdominal discomfort jaundice is usually absent (Wang *et al.,* 2002).

Liver has an enormous job for maintaining the body's metabolic homeostasis. This includes the processing of dietary amino acids, carbohydrates, lipids and vitamins, synthesis of serum proteins, detoxification arid excretion into bile of endogenous waste products and pollutant. The liver is almost inevitably involved in blood borne infections. The foremost hepatic infections, however, are viral in origin. Risk factors for HCV were, lower education level, blood transfusion, smoking, and age greater than 50 years (Montalto *et al.,* 2004). Saharan Africa is of great interest because it is reported to have the highest HCV prevalence rate (5.3%), and a concurrent HIV epidemic (Desenclos *et al.,* 2000). The risk of infection from occupational exposure through needle sticks and is low and accounts for less than 4% of reported cases sexual transmission of HCV is uncommon, although sexually active people with HCV should practice safe sex to avoid the risk of blood to blood contact during intercourse. Higher rates have been reported when the mother is infected with HIV or if the mother has a high blood level of HCV. The virus has not been found in singles women therefore, transmission via this route is thought to be unlikely (Mclnnis, 2003).

The HCV is not transmitted via ordinary social contact such as hugging, shaking hands, or sharing of shower or toilet facilities, the sharing of razors, toothbrushes, and other personal grooming aids, however, discouraged because they may be contaminated with blood (Bhatti and Tariq, 1996). Many workers have studied HCV and HIV in healthy donors from both Armed Forces and civilian population, and collected cases from January 1996 to December 2000. They were tested by ELISA at Armed Forces Institute of Transfusion (Balogun *et al.,* 2002). In Pakistan different workers have conducted studies on different aspects of HCV infection but mostly these studies are confined to the central and northern parts of Pakistan (Khattak *et al.,* 2002). In some areas of the world, the prevalence of HCV remains very high suggesting that some yet unknown vector might be involved in its transmission in those areas. The incubation period for acute HCV infection following transfusion or accidental needle stick has been reported to average 6-7 weeks, but may range 2-26 weeks. Evidence of HCV infection has been found and constitutes massive medical threat, underscoring the urgent need for anti HCV vaccines and antiviral agents (Roy *et al.,* 2002).

Hepatitis C Virus has been classified on the basis of genomic organization as a new genus (Hepaivirus) in the Flaviviridae family. HCV is a single stranded RNA virus having a distinct genomic organization. The genome of HCV consists, about 9400 nucleotides that code for a poly protein of 3011 amino acids. The structural regions encode the core and envelope of the HCV while the nonstructural regions encode

enzymes and membrane binding sites. None coding as well as core regions is highly conserved while other regions of the HCV genome are variable. Presently interferon is the only approved therapy for chronic hepatitis occurring as a consequence of HCV infection. A number of factors may influence the progression of liver disease in patients with chronic HCV infection (Balogun *et al.,* 2002). In several studies, an association between disease severity and HCV genotype has been found. Patients with histological severe chronic hepatitis and cirrhosis are more likely to be infected with genotype than with patients with mild or moderate disease. One factor strongly correlated with severe disease is alcohol induced liver injury and other factors including older age at infection, male gender and immunodeficiency (Poynard *et al.,* 1998). Many advances have been made during last 10 years including characterization of HCV with its genetic diversity, development of third generation antibody diagnostic tests, better understanding of epidemiology and it's treatment with alpha interferon HCV as well as other members of Faviviridae family might enter the cells by binding to low density lipoproteins (LDL) receptors (Agnello *et al.,* 1999). The PCR can be used for the conformation of HCV infection in individuals with positive anti HCV antibody test, early diagnosis of acute HCV attack and follow up of antiviral drug treatment and screening of blood donors for safe transfusion (Taylor *et al.,* 1999). Subsequently, after more than 3 years of follow up, fibrosis change was -0.88 in the SVR group, +0.15 in the nonresponse group and +0.59 in the no treatment group. Clinically when collated with no treatment, interferon base monotherapy in content HCV related cirrhosis that are majorly without virol clearance has been analogous with a reduced rate of progression to decompensation or HCC nad reduced mortality (Nishiguchi, 1994) Objectives of the present paper include to find out the risk factors, responsible for transmitting of Hepatitis C virus and to identify the quantitative comparison of HCV patients.

Materials and methods

Area study

Kurram agency is one of the seven tribal agencies in Federally Administered Tribal Area (FATA). The head quarters of Kurram agency is Parachinar. It is mountainous area where the literacy rate is very low. Therefore, survey was conducted to find the exact data about HCV.

Study design

The present study is a small effort towards guidance of the people of Kurram agency. The data was collected through questionnaire, for the determination of HCV patients in Parachinar. The questionnaire consists of sixteen (16) questions related to patients and designed in such a way to get complete history of all participants. It contained the following sections, personal history, social, economic history, family history, signs, risk factors and symptoms of HCV patients. It were filled by 100 patients from different areas of Parachinar including Parachamkany, Malikheil, Sadara, Malikali, Shublan, Amalkot, Sultan, Pewar, Zeran and Shalozan. The basic information about patients were collected from some laboratories of Parachinar including Haidery blood bank and laboratory, Ali laboratory, Bangish laboratory, Hameed and sons clinical laboratory. The most of the people of area are illiterate, therefore, questionnaires were filled by local Dr. Rashid Hussain Turi, concern doctor of HCV in Agency Headquarter hospital Parachinar. First, the patients who were the local residents of Parachinar were informed about survey and its purposes. Then they were requested to co-operate in filling the questionnaires. In some cases even the patients have no basic idea about HCV. Figures of some patients are also added in the study to assist the work.

Strip method

Materials that used in the strip method were strip, disposable blood dropper, package insert, blood collecting vavil, timer, pipette and centrifuge

machine (for plasma only). Approximately 3 CC of blood has been taken from patient and collected in blood vavil. The blood was centrifuged for 5 minutes to get plasma, in centrifuge machine. Strip consists of three region, plasma sample region (S), control region (C) and test region (T). The obtained plasma in blood vavils was transferred to (S) region of strip by a pipette or a dropper. Strip was placed on a clean and level surface at room temperature for 10 minutes. Two distinct red lines appeared. One line was in the control region (C) and another line was in the test region (T), which indicated that result was positive. One red line appeared in the control region (C). No apparent red or pink line was appeared in the test region (T), which indicated that result was negative. Control line failed to appear, which indicated that result was invalid (Hussain, 2007).

Twelve patients did their blood test with strip method which was the most easily approach for testing HCV. Strip method was used just for finding whether result is positive or negative HCV.

ELISA method

The samples were screened using ELISA method to detect HCV. The materials that used in ELISA were strips, micro titer plate, enzyme, detergent, antibodies. The ELISA is an one step Enzyme Immunoassay Technique of the sandwich type for the detection of the surface antigen of the HCV in the human serum. The following laboratory practice was followed. Approximately 2 CC of blood has been taken from patient and collected in blood vavil. The blood was centrifuged for 2-3 minutes to get serum, in centrifuge machine. The kit used, was third generation ELISA (Table 1), the support frame of kit consists of necessary number of strips. Distributions of the strips were in the following order (advisable plate distribution) strip A_1, B_1, C_1 and D_1 each of 100 µl for negative control (R_3). Strip E_1: 100 µl for positive control (R_4). Five µl of serum was added in both strip of negative and positive control. The strips were incubated at 37 °C for 30 minutes. Conjugated solution (detergent, antibody and enzymes) of 50 µl were added in strip D_1 and E_1. The conjugate solution which was initially orange turned red after addition into strips containing samples. Strip D_1 and E_1 were incubated at 42 °C for 50 minutes. When the enzyme reaction was completed, the entire plate which consists of all strips was placed into a strip reader and the optical density (i.e., the amount of colored product) was determined for each strip. The amount of color produced was proportional to the amount of primary antibody bound to the proteins on the bottom of the strips (Hussain, 2007).

The ELISA method was performed by 86 patients as such facility was easily available in study area, however, the test results were obtained from Pakistan Institute of Medical Sciences (PIMS) Islamabad.

PCR method

Polymerase Chain Reaction is a technique in which a small fragment of Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA) can be rapidly cloned, or duplicated, to produce their multiple copies in molecular biology. It could be used to identify individuals from minute amounts of tissue or blood, to diagnose genetic diseases and to research evolution. It proceeded in a series of cycles, or rounds. Each successive round doubles the amount of RNA and thus more than 1 billion copies of a single RNA fragment could be made in just a few hours. Thirty PCR cycles could produce 1 billion RNA copies in less than three hours. In a real time PCR protocol, a fluorescent reporter molecule was used to monitor the PCR as it progresses. The fluorescence emitted by the reporter molecule manifolds as the PCR product accumulated with each cycle of amplification. Materials that used in the PCR method were primers, disposable blood dropper, agarose gel, blood collecting vavil, timer, phenol, chloroform, sigma's nuclei kit, distill water, enzymes, ethanol, pipette and centrifuge machine (for plasma only). Approximately 4 CC of blood has been taken from patient and collected in blood vavil. The blood was centrifuged for 5-6 minutes to get plasma, in centrifuge machine. In the PCR test, viral RNA was extracted from the patient's plasma by treated with

reverse transcriptase. The polymerase chain reaction process was then applied, using two primers (3′-5′ and 5′-3′) unique to the virus's genome. After PCR amplification was completed, the resulting RNA was denatured at temperature of 94-96 °C for 9 minutes. The second cycle started with annealing at 65 °C for 15 minutes in which both primers were separated. The separated primers were elongated at 72°C for 10 minutes. The specific primers bound to the vessel wall, and were then made visible with a probe bound to an enzyme (Brook *et al.,* 1989).

Table 1. Male patients effected with HCV; %: percentage of HCV male/female patients; 61 and above: patients with 60 years and above age.

S. No.	Age group	Male[1]	% of male[1]	Female	% of female[1]
1.	1-15	4	04	3	03
2.	16-30	36	36	8	08
3.	31-45	35	35	3	03
4.	46-60	5	05	4	04
5.	61 and above	2	02	0	0

Table 2. Marital status of male patients; %: percentage of HCV male/female patients with their marital status.

S. No.	Marital status	Male[1]	% of male[1]	Female	% of female[1]
1.	Married	55	55	10	10
2.	Un married	27	27	8	08

Table 3. Location of male patients; %: percentage of HCV male/female patients with their locality

S. No.	Location[1]	Male	% of male[1]	Female	% of female[1]
1.	Rural	72	72	10	10
2.	Urban	13	13	5	05

Table 4. Patients having monthly income; %: percentage of HCV male/female patients with their economic status.

S. No.	Economic status	Male	% of male[1]	Female	% of female[1]
1.	7000 and above	74	74	7	0 7
2.	3000-5000	14	14	5	0 5

The amount of virus in the sample could be quantified with sufficient accuracy to detect three fold changes. Special primers that bound to viral RNA and to certain primers bound to the wall of the vessel were added. In this way, viral RNA was fastened to the wall. Then new primers that bound at several locations to this RNA were added and other primers that bound at several locations to those primers. This was done to amplify the signal. Finally, primer that bound to the last set of primers and that were bound to an enzyme were added, the enzyme action causes a color reaction, which allowed quantification of the viral RNA in the original sample. The desired RNA band was resolved on a 0.8% low melting agarose gel. RNA from the gel slice was then purified using the conventional phenol chloroform protocol and RNA purification system and sigma's nuclei clean kit for different samples (Brook *et al.,* 1989).

To the molten gel slice, buffer sterile distill water was added to reduce the concentration of agarose 0.3%. Aqueous phase was separated after centrifugation at 14,000 rpm for 5 minutes. With equal volume of

phenol chloroform these steps were repeated 3-5 times. RNA was recovered by glycogen facilitated ethanol precipitation. RNA pellet was obtained by 15 minutes centrifugation at 14, 000. Washing step was repeated twice. After performing all the above steps correctly it was possible to find the quantitative analysis of HCV patient, the distance between defective and normal genes (Brook *et al.,* 1989).

Only two patients performed PCR test because in the study area there was no facilities of a costly machines and other modern laboratory equipments. It was impossible to send blood samples to other cities for having PCR due to long journey and less facilities of transportation. Real time PCR quantitative results of two patients were mentioned, the test results were obtained from Biotech Diagnostic Laboratory, Rawalpindi, Agha Khan University Hospital, Karachi. The overall procedure of PCR is demonstrated (Fig. 1).

Results

In this survey total 100 patients were studied during March 2010 to March 2011 at Parachinar. It was found that males 85% and females 15% were infected. When age of patients of HCV was considered, the subjects were divided into 5 groups. The minimum age was 1 year and the maximum age was 69 years. The highest prevalence of HCV found in males 36% and females 8% with the group aged 16-30 years, followed by in the group aged 31-45 years, males 35% and females 3%. In the group aged 46-60 years, males were 5% and females 4%, followed by in the group aged 1-15 years, males were 4% and females 3% while the lowest prevalence of HCV was found in group aged 61 years and above, males were 2% and no female in the same age group (Table 1).

The Prevalence of HCV infections according to marital status, married males were 55% and females 10%. Unmarried males were 27% and females 8% (Table 2). As for as demographic wise distribution of HCV infection was studied, the population in rural area was more affected, males were 72% and females 10%, the population in urban area, males were 13% and females 5% (Table 3).

The socioeconomic condition of the sasses belonging to high class people males 74% and females 7% were affected with HCV followed by causes having middle class status males 14% and females 5% (Table 4). The HCV prevalence was inversely related with education. The HCV prevalence was higher in those people who had on formal education males were 60% and females 10%, followed by those who had attended college males were 15% and female 1%. Patients with graduate and above educations males were 7% and no female. The last frequency of HCV prevalence was found in the illiterate males 4% and females 3% (Table 5).

The risk factors of HCV patients are given in descending order: share syringe males 31% and females 7%; surgical operation males 17% and females 12%; health care workers males 14% and no female; sewage workers males 9% and no female in same category; sexually transmission of HCV males 3% and females 2% (Table 6).

Patients with symptoms from higher to lower value: abdominal pain in males 67% and females 12%; fatigue in males 65% and females 12%; fever in males 64% and females 6%; yellow skin color in males 53% and females 7%; dark color urine in males 35% and females 7%; rashes in males 35% and females 6%; weight loss in males 23% and females 4%; vomiting in males 21% and females 4%; pale color stool in males 19% and females 5%; nausea in males 19% and females 3%; diarrhea in males 13% and females 7% (Table 7).

The Strip, ELISA and PCR tests were performed by patients in laboratories. The initial data about HCV patients were taken from local laboratories in the survey area, however, the test results were obtained from Pakistan Institute of Medical Sciences (PIMS) Islamabad, Biotech Diagnostic Laboratory Rawalpindi, Agha Khan University Hospital, Karachi. Twelve patients did their blood test with

strip method which was the most easily approach for testing HCV. Strip method was used just for finding whether result is positive or negative (Table 8).

Table 5. Male patients having different educational status; %: percentage of HCV male/female patients with their educational status; graduate and above: patients with graduate and above education.

S. No.	Educational status	Male[1]	% of male[1]	Female	% of female[1]
1.	Illiterates	4	04	3	03
2.	Primary	9	09	1	01
3.	Middle	30	30	5	05
4.	Secondary	21	21	4	04
5.	Intermediate	15	15	1	01
6.	Graduate and above	7	07	0	0

Table 6. Male patients having risk factors of HCV; %: percentage of HCV male/female patients with their risk factors.

S. No.	Risk factors	Male[1]	% of male[1]	Female	% of female[1]
1.	Shared syringe	31	31	7	07
2.	Tattooing	0	0	2	02
3.	Sexual	3	03	2	02
4.	Mother to infant	0	0	3	03
5.	Surgical operation	17	17	12	12
6.	Health workers	14	14	0	0
7.	Sewage workers	9	09	0	0

Table 7. Male patients having different symptoms of HCV; %: percentage of HCV male/female patients with their symptoms.

S. No.	Symptoms	Male[1]	% of Male[1]	Female	% of Female[1]
1.	Fever	64	64	6	06
2.	Nausea	19	19	3	03
3.	Yellow skin color	53	53	7	07
4.	Vomiting	21	21	4	04
5.	Rashes	35	35	6	06
6.	Myaligia	17	17	4	04
7.	Dark color urine	35	35	7	07
8.	Weight loss	23	23	4	04
9.	Fatigue	65	65	12	12
10.	Diarrhea	13	13	7	07
11.	Pale color stool	19	19	5	05
12.	Abdominal pain	67	67	12	12

Table 8. Tests performed by male patients; %: percentage of HCV male/female patients with their performed tests.

S. No.	Test name[1]	Male	% of male[1]	Female	% of female[1]
1.	Strip method	9	75.0	3	25.0

Eighty six patients performed ELISA as such facility was easily available in study area. Range of ELISA for detection of HCV is 0.28 µl, range of patient greater than 0.28 µl, the result was positive, range of patient less than 0.28 µl, the result was negative, where 1 µl=10^{-6} L. The minimum range of patient was 0.50 µl while the maximum range of patient was 3.50 µl (Table 19).

Table 9. Range of ELISA for HCV detection= 0.28 µl ; positive result: range of patient > 0.28 µl: negative result ; range of patient < 0.28 µl , 1µl=10^{-6}L.

S. No.	Range[1]	Male	% of male[1]	Female	% of female[1]
1.	0.50-1.50 µl	20	23.25	3	3.48
2.	1.51-2.50 µl	38	44.18	5	5.81
3.	2.51-3.50 µl	16	18.60	4	4.65

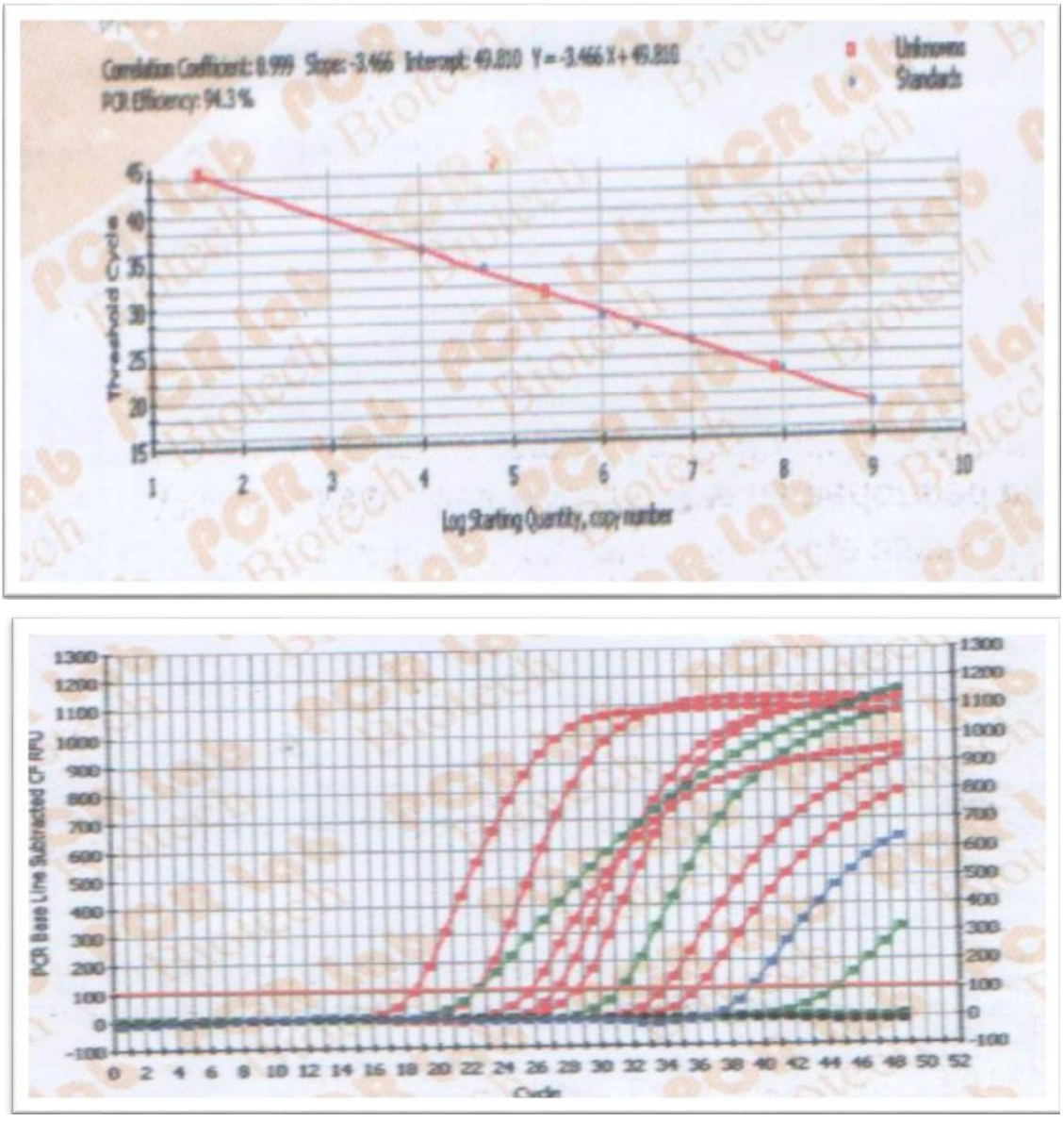

Fig. 1. Standard curved plot of patient (a): shows variation of effective RNA and thermal cycle (b); Range of PCR for HCV detection = 400 copies/ml, range of patient > 400 copies/ml, positive result, range of patient < 400 copies/ml, negative result, real time PCR positive result of patient 1 (222000 copies/ml) (b)

Only two patients performed PCR test. Real time PCR quantitative results of two patients mentioned, standard curved plot, shows variation of effective RNA and thermal cycle. Range of PCR for HCV detection is 400 copies/ml, range of patient greater than 400 copies/ml the, result was positive, range of patient less than 400 copies/ml, the result was negative, real time PCR positive result of patient 1 was 222000 copies/ml (Fig. 2).

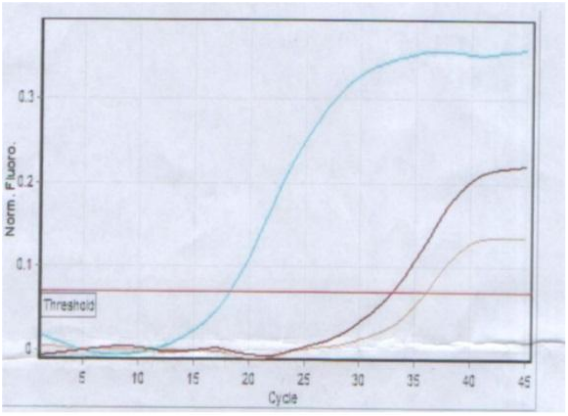

Fig. 2. Amplification plot quantatitive of patient 2, shows PCR cycle and RNA amplification. Range of PCR for HCV detection = 1.710 X 10^3 copies/ml, range of patients > 1.710 X 10^3 copies/ml, positive result, range of patients < 1.710 X 10^3 copies/ml, negative result. Real time PCR positive result of patient 2 (6.8 X 10^3 copies/ml).

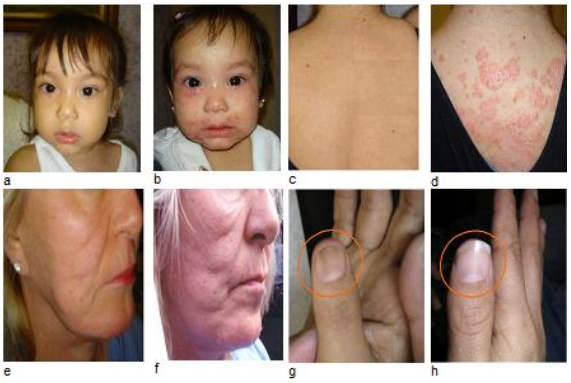

Fig. 3. Patient of HCV: Normal and healthy face skin of a child before suffering of HCV (a); rashes on face after affected with HCV (b); normal skin of back shoulder before suffering of HCV (c); pitches on the back of shoulder after affected with HCV (d); normal skin color before affecting with HCV (e); yellow and dull skin after affected with HCV (f); normal thumb and nail color before affected with HCV (g); yellow color of thumb and nail after affected with HCV (h).

Range of PCR for HCV detection is 1.710 X 10^3 copies/ml, range of patient greater than 1.710 X 10^3 copies/ml the result was positive, range of patient less than 1.710 X 10^3 copies/ml, the result was negative. Real time PCR positive result of patient 2 was 6.8 X 10^3 copies/ml (Fig. 3).

Photographs of some patients (Figure 5) were also added in the study to assist the present work. A child was found physically normal with healthy face skin before suffering of HCV (a); rashes were clearly found on face and other parts of the body, after affected with HCV. The most affected area of the body with rashes were hands, face and legs region (b); normal and clear skin of back shoulder of lady was found before suffering HCV (c); pitches with red spot and yellow color were found on the back of shoulder after affected with HCV, the abdominal region was found swollen with lose skin (d); aged woman had normal and white skin color and normal eyes colors were found before affecting with HCV (e); yellow colors of eyes and dull skin of the body was found after affected with HCV (f); a girl had normal thumb and nail of white colors before affected with HCV (g); yellow color of thumb and nail was found after affected with HCV (h).

Discussion

The survey of Hepatitis C Patients in Parachinar, Kurrum Agency, Pakistan was carried out from March 2010 to March 2011. During the survey total 100 patients were studied, in the whole population, 85% males and 15% females were affected. A structure questionnaire was used for collection of actual data of HCV patients, which were filled by the patients of the present study area.

The predominance of male cases in HCV infection has been observed all over the world (Mohammed *et al.,* 2000; Montalto *et al.,* 2002). The present findings also show the overall higher incidence of HCV in male as compared to female, but statistically sex was not a discriminatory factor. Ali *et al.,* (2004) reported that males are mostly affected with HCV

infection than females. This is because of activities of males barber contact, blood donation, tattooing, drug abuse which are thought to be major transmitting factors. Whereas females are mostly confined to their homes, as they have home orientated activities. However, transmission of HCV infection gender wise may not be followed equally well for the females population residing in different geographic region of the world.

The increasing age has high risk to acquire the disease probably due to greater exposure to multiple risk factors. However, the present findings reveal that the highest prevalence of HCV exists in the group aged 16-30 years males 36%, females 8%. Chang *et al.,* (2001) investigated that increasing age was a significant risk factor for HCV infection. It could be due to the fact that young people are more likely to experiment with drugs and sexual malpractices exposing them to high risk of being infected. Alter *et al.,* (1999) showed a same age related distribution. A low prevalence of HCV was found among older people and the highest prevalence was among young people in the United States. However, Tariq et al. (1998) reported that the high prevalence of HCV infection in 30-60 years age group in Pakistan. Balogun *et al.,* (2002) reported high frequency of HCV infection in 40-44 years age group in 1991 while in the 30-34 years age group in 1996. Although one can argue as the age group at the highest risks, these results nonetheless are indicative of age as an important factor in determining the risk of hepatitis infection.

In the present study marriage proves to be a risk for HCV infection, it does not necessarily have to be entirely due to sexual exposures since married couples have frequently common exposures, other than sexual, which could transmit HCV. In study married people appear to be at a higher level of risk for HCV infection than unmarried. Similarly Mohammed *et al.,* (2000) observed the trends for those who were farmed and married more likely to be HCV positive than those who did not meet these criteria. In the study area most of the people are unaware of HCV and sasses neglect the prevalence of HCV in initial stage, which is the main cause of spreading the disease in married people.

The present findings suggest the high prevalence of HCV infection in rural areas where males 72%, females 10%. Raffacle *et al.,* (2001) reported that the high prevalence of HCV infection among the general population in rural area of central Italy and suggested the in appropriate use of medical or surgery practices on the population for HCV transmission. One can argue that in rural area the standard of life is not so sound and unawareness of health care can promote prevalence of HCV.

The results based on the socioeconomic condition of the patients showed that patients belonging to middle class were more affected with HCV. Malik *et al.,* (1992) conducted a study in Pakistan where the majority of Hepatitis patients belonged to low socioeconomic group. HCV were the most prevalent among people who were below the poverty level this could be due to the fact that people of Parachinar work in middle east countries and bring HCV infection from these countries, some people work in different cities of Pakistan mostly truck drivers where rate of HCV infection is high and lastly higher prevalence may be due to settled Afghan refugees.

In the current study HCV infection is inversely proportional to education level which is consistent with those who showed the inverse correlation of HCV infection with education level. Wang *et al.,* (2002) reported that 73.9% of HCV prevalence in below junior high school and 39.7% in junior high school or above educated population in study from Taiwan. Chaudhry et al. (2003) founded the highest prevalence of HCV infection in lower educated population. One can argue that in study area the most of the people are less educated and suffering while performing their daily activities, because they are unaware of HCV.

In the present study, the most common symptoms associated with HCV infection were weight loss, fever and abdominal pain and occasionally accompanied by vomiting and yellow skin colors. However,

persons with acute HCV infection typically are either asymptomatic or have a mild clinical illness, 60-70% have no discernible symptoms, 20-30% have jaundice, and 10-20% have nonspecific symptoms (e.g., anorexia, malaise, or abdominal pain). Wang *et al.,* (2002) reported symptoms of HCV infection, prior to treatment and the most common symptoms that was fatigue 25%, jaundice 28%, and abdominal pain 24% of patients. In the present study for the general awareness and control strategies against this serious communicative disease, it is therefore important to scrutinize a potential risk group for the prevalence of HCV infection with a reliable screening method such as the blood screening.

From the present findings, it has been concluded that the high prevalence of HCV may be attributed to sharing syringes males 31% females 7%, surgical operations males 17%, and females 12%. McCormick *et al.,* (1999) observed that from different parts of world have found same risk factors. Experts found evidence to suggest otherwise when investigating a hepatitis outbreak in Karachi, Pakistan. They traced the infection of HCV outbreak at local clinic where multiple patients were injected with medications from syringes that were shared between patients. This incident carried 29% HCV. The existence of HCV infection among surgical patients or surgeons raises the possibility of its transmission in the surgical setting, from patient to surgeon, from surgeon to patient, or from patient to patient. Partly because of the low clinical attack rate with acute HCV infection, there have been few reported outbreaks or transmissions in this setting, but some evidence in some settings suggests surgery can be a risk for HCV transmission. Crofts *et al.,* (1999) reported that in Indonesia, HCV prevalence among 7,572 healthy volunteer blood donors from 21 of the 27 Indonesian provinces was 2.1% risk factors for HCV positivity included a history of surgery, blood transfusion, intravenous medication, and acupuncture, In Taiwan, among 126 family contacts of 42 HCV infected patients without histories of parental exposure, 21 (17%) were HCV positive, and histories of blood transfusion and surgery were the factors significantly associated with HCV infection, suggesting an independent route of infection rather than household contact in these people . In the present study it is clear that in the most hospitals uses of contaminated syringes and surgical instruments are common, which shows significance role in the prevalence of HCV.

In present study only 2 patients performed PCR tests because such costly facility is not available in the present study area. Most of HCV patients perform their tests by ELISA and strip methods. Raffacle *et al.,* (2001) observed that in the rural area of central Italy where patients performed their tests most commonly with strip methods. One can argue that these facilities are easily available with low cost as compare to PCR. In study area most of the laboratories use strip method because it is less time consumer with simple procedure for blood testing.

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