Seroprevalence of anti-HCV Ab associated with demography and consanguinity of Thalassemia patients in Rawalpindi/Islamabad region - A single centre study

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To my beloved parents for their love, support and prayers

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Ghufranud Din

Certificate

This thesis submitted by ***Ghufranud Din*** is accepted in its present form by the Department of Microbiology, Quaid-i-Azam University, and Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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List of Acronym/abbreviations (alphabetically)

BTI	Beta Thalassemia Intermedia
BTM	Beta Thalassemia Major
BD	Becton, Dickinson and Company
ELISA	Enzyme Linked Immunosorbent Assay
HBs Ag	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICT	Immuno-chromatography Test
KPK	Khyber Pakhtunkhwa
QAU	Quaid-i-Azam University
RNA	Ribonucleic Acid
TTI	Transfusion Transmitted Infections
USA	United States of America

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Abstract

In Pakistan consanguineous marriages are contributing an increase of diseases of genetic disorders including thalassemia. According to an estimate, each year approximately 5000 newborns are affected due to thalassemic genetic disorder. Whereas the management of thalassemia minor is being taken care of through medication but major and intermedia is primarily based on regular blood transfusions. However, mediocre transfusion services in developing countries result in iron overload and transfusion transmitted infectious diseases primarily hepatitis B and C. The aim of study was to elucidate current status of HCV seroprevalence in beta-thalassemic population from Rawalpindi/Islamabad region.

A total 95 subjects were observed; beta-thalassemia major (96%) and beta-thalassemia intermedia (4%). Among these, 47 (49%) were detected positive for anti-HCV antibodies. The patient data were divided in two groups, thalassemic only (n=48) and thalassemic plus hepatitis (n=47). Both groups were then observed for ferritin levels and no clear difference of ferritin level was seen in both groups. All recruited subjects were observed for chelation therapy/medication and behavioral complications; 83 (87%) patients were on chelation therapy, whereas 38 (38%) patients were found vaccinated for hepatitis B. Overall complications (Hepatomegaly, Splenomegaly and Splenectomy) were observed in (n=61) 64% individuals.In our study 51 (54%) subjects were males and 44 (46%) females (M: F ratio= 1.2:1). Seropositivity of anti-HCV was observed to be greater in males (53%) than the females (45%). The distribution of disease status (thalassemia and hepatitis) was not significantly associated with gender and age ($p>0.05$). Geographically the data showed highest representation of subjects from Punjab (n=76; 80%) and Khyber Pakhtunkhwa (n=11; 11.6%), while subjects from other regions of Pakistan have insignificant representation. Among the recruited subjects, the majority belonged to ‘low socio-economic status’ (45.3%), while there were 28.4% and 26.3% individuals belonging to ‘middle’ and ‘high’ socioeconomic status, respectively.

Thalassemic subjects were distributed with respect to their sporadic and familial presentations. Among the familial cases, a total of 93 subjects were found to be affected. Among the sporadic cases, the affected male to female ratio was 1.36:1, while in the familial cases there was equal number of affected males and females (χ^2

=0.7581; df.1; NS). Parity was scored for all the recruited subjects and parity orders from first to eights were observed. Majority of the subjects belonged to second parity (n=27, 29%), followed by first and third parity (n=24, 25% and 14, 15%, respectively). In each parity order, the sib ship size was increasing with increasing parity level. In order to assess TTIs in educated blood donors, 44 subjects were recruited at Quaid-i-Azam University. All of them were screened for TTI i.e. HBV, HCV and HIV. None of them was screened positive for any of the mentioned infections.

Despite the fact that in many countries standardized screening procedures for the blood related products are placed since 1990; higher prevalence rates of HCV among thalassemic patients requires greater attention in Pakistan.

INTRODUCTION

The thalassemias are a diverse group of hereditary anemias caused by decreased or lack of production of any type of globin chain—either the α or β globin chain. (Hilliard 1996). The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood). Beta-thalassemia includes three main forms: Thalassemia Major, which is also referred as "Cooley's Anemia" and "Mediterranean Anemia" Thalassemia Intermedia and Thalassemia Minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia". In thalassemia major affected subjects have two β -globin genes carrying a severe thalassemia mutation whereas thalassemia trait subjects have one β -globin gene carrying a thalassemia mutation. However in less common cases thalassemia mutation in two β -globin genes, at least one of which is mild; leads to beta-thalassemia intermedia. (Galanello and Origa, 2010; Rund and Rachmilewitz, 2005; Thein, 1992)

So far, more than 200 point mutations and few deletions have been reported in relation to beta-thalassemia. The point mutations mainly occur in functionally important regions of the beta globin gene (Huisman et al., 1997; Rund and Rachmilewitz, 2005). Modifier genes are the genetic variants that can lead to phenotypic differences in disease patterns e.g.in milder form of thalassemia i.e. thalassemia intermedia the primary genetic modifier include genetic variants that are able to reduce the globin chain imbalance. As a heredity disorder the beta-thalassemias are inherited in an autosomal recessive manner. Obligate heterozygotes parents carry a single copy of a disease-causing beta globin gene mutation that results in affected child. (Thein, 1992).

Approximately, 250 million peoples worldwide i.e. 1.5% of the global population is heterozygotes for beta-thalassemia and at least 2 million effected homozygotes are born annually, while some other reports have estimated that 3% to 10% of the world's population carries a gene related to thalassemia(Colah et al., 2010). Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%),

Sardinia (10.3%), and Southeast Asia (Flint et al., 1998). Migration and intermarriages between different ethnic groups are important factors for introducing thalassemia in different countries of the world. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta thalassemia that leads to the birth of 60,000 symptomatic off springs annually, majority falls in developing world. It was found that the total annual incidence of symptomatic individuals is 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union only (Vichinsky, 2005). Thalassemia International Federation states that only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world (Cappellini et al., 2008). Nonetheless, thalassemia is considered the most common genetic disorder worldwide. However, incidence rate is particularly higher in a broad belt extending from the Mediterranean basin through the Middle East, Indian subcontinent, Burma, Southeast Asia, Melanesia and the island of the Pacific.

Pakistan has a large population of more than 150 million people with an overall carrier frequency of approximately 5.6% for β-thalassemia. Punjab is the largest province of the country having more than 50% of the population (Baig et al., 2006). It has been estimated that about 5250 off springs are born with this disease annually in Pakistan.(Alwan and Modell, 1997)

Currently, allogeneic stem cell transplantation (SCT) has proven the only therapeutic approach that can cure thalassemia (Gaziev and Lucarelli, 2005). Several attempts have been taken to use hematopoietic stem cell transplantation (SCT) for curing thalassemia. Bone marrow transplantation under the age of 16 years offers a high probability complication free survival (Lucarelli et al., 1990).

Blood transfusion and blood conservation is playing a vital role in the current era of transfusion medicine (Goodnough et al., 1999). Transfusion therapy takes major role in the supportive therapy for the treatment of Cooley's anemia (Schorr and Radel, 1964; Wolman, 1964).Continuous transfusion therapy remains the major form of supportive treatment for patients with Cooley's anemia. The goal of transfusion therapy include treatment of anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption, which occurs in non-transfused patients as a

consequence of increased, although ineffective, erythropoiesis (Borgna-Pignatti and Galanello, 2004; Cappellini et al., 2008). However, blood transfusion is associated with several cumulative risks. All over the world Transfusion Transmitted Infection (TTI) is a major challenge to the transfusion services. The problem of TTI greatly relies on the prevalence of the infection in the blood donor community (Vidja et al., 2011). Safe blood transfusion and blood products still remain a major challenge throughout the world. Pakistan is also facing the same situation where Transfusion-Transmitted Infections (TTIs) continue to threaten the blood safety. The transmission of these infectious agents is of major concern, as transfusion therapy forms an integral part of modern health practices (Waheed, 2012).

The most important consequence of life-saving transfusions in thalassemia is the unavoidable accumulation of iron within tissues, which leads to progressive organ dysfunction and can be fatal without administering a chelating therapy (Cohen, 1987). Iron is an essential cofactor used in basic metabolic pathways and it also plays a pivotal role for innate immunity by generating toxic oxygen and nitrogen intermediates species. However pathogenic microorganism also have the shared requirements of iron for their metabolic pathways and thus increased iron due to multiple transfusion can lead to different infections(Schaible and Kaufmann, 2004).

Transfusion-transmitted infections (primarily hepatitis B and C) are still leading cause of death in developing countries where proper testing is not available (Rund and Rachmilewitz, 2005). Hepatitis C virus (HCV) is a positive strand RNA virus which belongs to flavi and pestiviruses family. It was first identified in 1989 in the USA as a major causative agent of post transfusion non-A, non-B hepatitis (Choo et al., 1989). According to WHO estimates, approximately 3% of the world population, or about 170 million people, may be infected with hepatitis C virus (Mast et al., 1999). HCV is a blood-borne pathogen and its primary rout of transmission is exposure to infected blood. However, in up to 50% of cases no recognizable transmission factor/route could be identified. About20% cases of HCV infections may become acute hepatitis and 50% chronic hepatitis, out of these 20% can progress to cirrhosis(di Bisceglie et al., 1991; Van der Poel, 1994).

The incidence of acquiring post-transfusion HCV infection is directly related to the number and amount of blood products received (Fink et al., 1993; Lai et al., 1993). In children receiving multiple blood transfusions for thalassemia, the incidence of HCV infection among thalassemic patients varies from 55% to 83% (Lai et al., 1993; Resti et al., 1991). Seroprevalence of anti- HCV among thalassemic patients was estimated to be 13.1%-65% in various regions of Pakistan (Ansari et al., 2012; Qurat-ul-Ain et al., 2011).Several studies have been conducted in different areas of Pakistan since 1995 including (Bhatti et al., 1995), (Younus et al., 2004), (Hussain et al., 2004), (Riaz et al., 2011), (Qurat-ul-Ain et al., 2011) and (Ansari et al., 2012).

HCV after its discovery in 1989, was quickly established as the major cause of non-A, non-B hepatitis. However, development of Hepatitis C screening test before blood transfusion in 1990 has greatly reduced the risk of HCV transmission via blood transfusion practices in developed countries of the world. In Pakistan, although Pakistan Medical Research Council has published a detail report on prevalence of HCV and HBV across the country, lack of public knowledge regarding HCV prevalence, risk factors and absence of a central surveillance system for safe blood transfusion make the situation worse in patients with regular blood transfusions like thalassemia. Several periodic studies have been conducted so far. Our study is also in continuity to these studies in order to bring the latest information about prevalence of HCV in thalassemic population of Islamabad region.

Aims and Objectives

The aims and objectives of this study are

1) To assess the prevalence of Hepatitis C amongst multiple blood transfused beta-thalassemic patients via serological procedure.
2) To elucidate information of demography, clinical and family history of blood transfusion dependent beta-thalassemic patients.
3) To evaluate ferritin levels and iron overload in multi-transfused beta-thalassemic patients.

REVIEW OF LITERATURE

2.1 Thalassemia

2.1.1 Global prevalence of thalassemia

Thalassemia was reported for the first time by Cooley and Lee in 1925 while they were working on a severe type of anemia in Italian, Greek and Syrian children in North America. Initially it was called as Cooley's anemia however it was observed that this disease was prevalent among the people of northern and eastern shores of the Mediterranean Sea and thus was called thalassa (sea) and haima (blood) i.e. thalassemia (Whipple and Bradford 1936). From 1925 to 1950 significant incidence of thalassemia was detected in Italy, Greek, Cyrus and America stated by Chernoff 1959. In 1992, Thein revealed that beta thalassemia is a heredity disorder of beta globin chains and it transferred to the next generation in auto recessive manner. A defected child belongs to obligate heterozygotes parents that carry a single copy of a disease- causing beta globin gene mutation. Huisman *et al.,* 1997 elaborated that several studies have suggested that thalassemia results due to a point mutation in the functionally active regions of beta globin chain and more that 200 point mutations have been detected so far.

Flint in his study in 1998 mentioned that due to changing pattern of the disease, beta-thalassemia is now prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. Later on in 2005 Vishinsky *et al.,* stated initially thalassemia was restricted to a particular part of the world but due to population migration and intermarriages between different ethnic groups had gradual impact on the changing epidemiology of this heredity disordered thalassemia is now introduced in almost every country of the world.

In the previous decade several studies suggested that thalassemia is an emerging global health burden, for instance Vichinsky in 2005 stated that 1.5% population of the world carries defected genes responsible for thalassemia with about 60,000 new individuals born every year. However it was clearly observed that majority of thalassemia subjects and incidence of new cases falls in developing countries. The

study revealed that in annual incidence of new cases is 1 in 100000 throughout the world and 1 in 10000 in European Union only.

In 9th symposium on Cooley's anemia 2010, Weatherall in lighted that previously under developed countries were not able to control and manage thalassemia properly due to the lake of knowledge. However there are still several ways to make the future of thalassemic children safe in those countries. On the other hand in the last 20 years the developed countries have made a great improvement in controlling thalassemia incidence. Unfortunately developing countries are still struggling in the long run of controlling thalassemia and its proper management. Several improvements have been made by these countries but still many improvements are needed to be done to reduce the global health burden.

2.1.2 Thalassemia in South-Asia and Pakistan

South Asia is among the over populated region of the world. Its population is approximately 1.4 billion and its annual infant birth rate 176 million that is one third of the world stated by D.J. Weatherall in 2010. According to (Modell and Kuliev 1993), the major problem faced by thalassemic population in these countries is the health burden on poor economy due to which proper management and control is not successful. Agarwal *et al.,* in 2003 reported approximately 45 million beta thalassemic carriers in Pakistan, India and Sri Lanka the developing countries of South Asian region. Sengupta 2007 in his study revealed that being heredity disorder, situation of thalassemia becoming more complex due to high practices of consanguineous marriages among the Indian population.

In one of the prevalence based study by Khattak *et al.,* 1992 it was found that 5% Pakistani population is carrier of beta thalassemic gene. The status of consanguineous marriages in Pakistan is not much different than India. Baig *et al.,* in 2006 stated that Pakistan has a high consanguineous marriage rate of 81%. High birth rate, increased practices of cousin marriages and the significant number of beta thalassemic population in Pakistan are the major hurdles in controlling thalassemia and situation is becoming worst day by day.

2.2 Therapy and complications

2.2.1- Transfusion therapy

Thalassemia is almost a non-curable heredity disorder. Currently the only possible therapy available is bone marrow transplantation as stated by Gaziev *et al.,* 2005 which is also linked with the age factor i.e. more promising results were seen in patients below 16 year age. Wolman 1964 suggested that thalassemia which is also referred as Cooley's anemia leads to a severe type of anemia unless regular blood transfusion is started as a supportive therapy. According to Borgna- Pignatti *et al.*, 2004, anemia, suppression of erythropoiesis and inhibition of gastrointestinal iron absorption are the consequence in non-transfused beta thalassemic patients and to overcome these consequences regular blood transfusion is indispensible. However, continuous blood transfusion therapy is associated with cumulative risk factors.

2.2.2 Iron overload condition

According to Chaston and Richardson 2003, ineffective erythropoiesis such as in case of thalassemia triggers inappropriate uptake of iron from intestine and eventually leads to severe iron overload.

Iron overload has life threatening consequences as described by Papanikolaou and Pantopoulos in 2005. Iron overload encounters growth hormone and thyroid dysfunction in its first decade. Later on impaired glucose tolerance along with hypoparathyroidism and hypogonadism can lead to delayed puberty due to iron toxicity. In the third and fourth decade of life, thalassemic patients with iron overload faces bone and cardiac diseases. Osteoporosis is seen in patients with hypogonadism and increased iron is deposited in their bones. Cardiac failure due to iron deposition is still leading death cause even in developed counties.

Although iron overload is a serious issue in multiple transfused patients as the body is not able to properly regulate that excess of iron, several chelating agents are used for iron chelation therapy. Franchini and Veneri in 2013 stated that introduction of iron chelating therapy has drastically increased the average life expectancy which was previously reduced due to iron overload. However, the demanding nature of the

previously known therapy results in poor patient compliance and alternative therapies should be implemented to facilitate patients.

2.2.3 Transfusion Transmitted Infections

Transfusion transmitted infections (TTI) are associated with transfusion therapy since the therapy was started in early 1940s. Prati 2002, in his study revealed that for a long time TTI remained as a major risk factor of transfusion therapy but now developed countries and some resource rich countries have greatly limited this risk of transmission. In one of his study 2006, Prati stated that introduction of anti-HCV screening has sharply decreased the risk of post transfusion hepatitis in developed countries. Unfortunately, the situation is still the same in developing countries where unscreened blood transfusion is still common.

Choudhury 2010 stated that in the first 30 years of transfusion therapy scientist were greatly concerned about Syphilis and Serum Hepatitis by Australian Antigen. However, discovery of HIV and Hepatitis C virus has greatly threaten the blood donors' societies all over the world. It was also observed that the magnitude of TTI is directly related to the degree of prevalence of the particular infection in the general population.

Vidja *et al.,* conducted a study in 2001 to estimate the prevalence of TTI in multi transfused beta thalassemic patients in India. In their study they found 7% patients were infected with TTI of which HBs Ag was 2%, HCV 2% and HIV 3%. Their study reflects that blood born viral infections are still a major issue related to multiple blood transfusions.

As a developing country, Pakistan is still facing the problem of TTI like other developing countries. Khattak *et al.,* reported a study 2002, based on the seroprevalence of Hepatitis B, C and HIV in healthy blood donors during 1996-2000 in Northern Pakistan. The study showed that HCV was most prevalent (4%) followed by HBV (3.9%) and HIV (0.007). Although donors screening has greatly reduced the risk of transmission but still the prevalence of TTI specially HCV is increasing by the time. In 2012, a study by Waheed stated that 8.34% healthy blood donors were found positive for anti-HCV antibodies.

2.3.1 Hepatitis C Virus

The hepatitis C virus is an RNA virus that belongs to the family flaviviridae. HCV was initially isolated from the serum of a person with non-A, non-B hepatitis in 1989 by Choo *et al.,* In 2002, Memon and Memon stated that Hepatitis C infection is a global issue. Although HCV was detected in the America for the first time but its epidemiology varies in different parts of the world. The incidence of HCV is higher in Eastern part of the world as compared to Western world.

HCV is blood-born pathogen and its primary rout of transmission is via infected blood and blood products. Prati in 2006 stated that blood transfusion now a day is very safe in developed countries and the clinicians are only facing HCV from the previous epidemics. However, the picture in developing countries is totally different where blood transfusion is still major cause of HCV infection. Other routs include unsafe surgical procedure, injectable drug abuses and unsafe sexual behaviors.

Lauer and Walker 2001 were talking about the pathogenicity of HCV. They described that course of HCV infection varies among different individuals. Several factors like male sex, older age, alcohol intake and co-infection increase the risk of progression. Whereas female sex and younger age infection are factors that decrease the progression risk.

Chen and Morgan 2006 revealed that 70%-80% infected individuals remain asymptomatic which makes it difficult to diagnose acute infection. It has been estimated that 75% to 80% HCV infections lead to chronic hepatitis out of which 10%-20% progress to cirrhosis, the end stage liver disease. However, by understanding the various routs of transmission HCV infections can be efficiently controlled.

Fassio 2010 was talking about one of the important complication of Hepatitis C i.e. Hepatocellular Carcinoma (HCC). He stated that progression of 10%-20% liver cirrhosis is a major risk factor for development of HCC. Annual incidence of HCC in patients with hepatitis and liver cirrhosis is ranging from 1%-8% in different countries of the world. Thus HCC due to HCV is of great concern and it has been suggested the leading cause of death in chronic HCV infected patients leading to liver cirrhosis.

2.3.2 Epidemiology of HCV

Since its discovery in 1989, HCV is a leading cause of liver diseases. Perz 2004, in his study found that approximately 2% (123 million) people are infected with HCV worldwide. The screening procedure has greatly reduced the burden of HCV in developed countries. Shepard *et al.,* 2005, stated that Germany (0·6%), Canada (0·8%), France (1·1%), and Australia (1·1%) are countries with lowest prevalence of HCV. The national prevalence rate of HCV antibody positivity has been estimated to be between 10-13% stated by Mohamed MK. 2004. Armstrong *et al.,* 2006, in a study revealed that prevalence of anti-HCV in USA was 1.6% and the major risk factor was history of intravenous injection.

Pakistan is one of the overpopulated countries of the world. As a developing country HCV is still prevalent in Pakistan. Several studies have been conducted in Pakistan including Luby *et al.,* 1997, Mujeeb *et al.,* 2000 and Khattak *et al.,* 2002 estimated that HCV is prevalent in various regions of Pakistan ranging between 2.4% to 6.5%. Jafri and Subhan 2010 illustrated that HCV prevalence in Pakistan is much higher than other countries in the region like India where HCV prevalence is only 0.9%. Transfusion of unsafe blood, therapeutic use of contaminated syringes and re-use of shaving razors are the major risk factors that spread of HCV in general population in Pakistan.

A study conducted by Attaullah *et al.,* 2011, describes that HCV genotype 3a is most prevalent in all provinces of Pakistan, followed by genotype1 in Punjab and untypeable genotype in Sindh, Baluchistan and Khyber Pakhtunkhwa. Ilyas *et al.,* 2014 published a study which illustrates that HCV is prevalent slightly higher in female (50.5%) as compared to male (49.5%) population of Pakistan. This shows that HCV is still a leading health issue in Pakistan and yet lots of efforts are needed to control HCV in Pakistan.

2.4 Hepatitis C in Multi Transfused Thalassemic Patients

2.4.1 Developed Countries

Since its discovery, HCV remained a major health issue for thalassemia patients due to multiple blood transfusions. Although after following the established blood screening protocols for HCV before transfusions has dramatically reduced the incidence of HCV in thalassemic population of developed countries, many developing

countries are still facing this problem as stated by Prati 2006. Dwyre *et al.,* 2010 illustrated that although HCV incidence is markedly decreased, yet due to false negative results the goal of Zero Risk is not achieved even in developed countries and newer technologies like pathogen inactivation (PI) should be adopted.

2.4.2 Developing Countries

Prevalence of HCV is no doubt a major challenge in developing countries unless safe blood transfusion is insured. By the time different studies have been conducted to depict the changing patterns of HCV prevalence in thalassemic population. Such studies include a study conducted by Jamal *et al.,* 1998, in Malaysia which suggests multiple blood transfusions a definite risk for transmission. In their study they found 22.4% anti-HCV positive thalassemic patients with regular blood transfusion. Seroprevalence of anti-HCV in thalassemic patients was studies in Bangladesh by Uddin *et al.,* 2009, and 16.4% of patients were recorded positive. A similar study was conducted in Egypt by Ragab *et al.,* 2012, which stated that large number i.e. 69% of Egyptian thalassemic population is positive for anti-HCV.

2.4.3 Iran

So far, a lot of work has been done on prevalence of HCV in thalassemic patients in Iran. Ansar and Kooloobandi stated in their study on prevalence of HCV in dialysis and thalassemic patients 2001, 63.8% thalassemia patients seropositive for anti-HCV antibodies. In 2006, and epidemiological survey was published by Mirmomen *et al.,* which illustrate 19.3% patients positive for HCV antibodies. Rezvan *et al.,* 2007, in a review article demonstrated that HCV is the most prevalent TTI in Iranian multi transfused patients. The estimated number of positive thalassemic patients was 22.3% from different geographical regions of Iran. Another study in Southwest Iran conducted by Boroujerdnia *et al.,* 2009 revealed that 28.1% of thalassemia patients were found positive for anti-HCV antibodies of which, HCV RNA was detected in 79.3% patients. Azarkeivan *et al.,* 2012, in retrospective revealed that incidence of HCV is on decline. Their study was based on the 14 years (1996-2009) patients file history. 27.5% patients were positive for anti-HCV of which only 19.2% were new cases detected after 1996.

2.4.4 India

Like other countries of the world India is also facing the problem of transfusion transmitted infections and specially HCV. However in comparison to other developing countries the situation in India is far better. Vidja *et al.,* 2011 found 2% thalassemia patients positive for HCV in Gujrat, India. Another study in 2011 by Bhavsar *et al.,* in Gujrat, India stated that 18% thalassemia patients were found positive for anti-HCV. Oza *et al.,* 2012 conducted a study in Ahmadabad, India based on prevalence of different blood born viruses in multi transfused thalassemic population. The findings illustrate that 7.8% of thalassemia patients were seropositive for anti-HCV antibodies. In 2012, Madhusudhan and Thyagarajan reported that in Chennai, India 32% thalassemia patients are positive for anti-HCV antibodies and 0% in healthy matched controls. This showed a significant increase of HCV infections in relation to the number of transfusion received.

2.4.5 Pakistan

Thalassemia in Pakistan exists as a major health issue. Different government and private sector thalassemia centers are working for the betterment of thalassemic population. These thalassemia centers ensure regular blood transfusion to thalassemia patients but unfortunately the unsafe blood transfusion in Pakistan leads to transmission of HCV in multi transfused patients. Thus HCV in multi transfused patients is of major concern besides thalassemia.

The very first study in Pakistan, after Hepatitis C virus was identified, was conducted by Bhatti *et al.,* 1995. None of these patients were transfused with anti-HCV screened blood and according to their study 60% thalassemia patients were screened positive for anti-HCV antibodies. In 2004, Younus *et al.,* conducted a study for prevalence of HCV in thalassemia patients in Rawalpindi/Islamabad region. Patients who had received minimum of 10 blood transfusions were included to the study subjects and all the patients were screened for anti-HCV by using third generation ELISA. Their findings revealed that out of 75 thalassemia patients, 42% patients were seropositive for anti-HCV antibodies. Akhtar and Moatter 2004, published a “letter to editor” about anti-HCV prevalence in thalassemia patients in Karachi. According to their statement 34.8% patients were positive for anti-HCV antibodies. HCV RNA was also detected in these patients. A study was published by Shah *et al.,* 2005, from North

West Frontier Provence (now KPK) revealed that 56.8% subjects were found positive for anti-HCV antibodies. It was also found that females (78.6%) were more affected than male (48.3%) patients.

Kapoor *et al.,* 2007 conducted a related study in Quetta. They found that 30% of thalassemia patients were anti-HCV antibodies positive. However, prevalence of HCV in healthy blood donors was only 2%. Hussain *et al.,* published a study in 2008 on prevalence of Hepatitis C in thalassemia patients from Peshawar and Islamabad. Their study illustrate that 41.7% thalassemia multi-transfused patients were positive for anti-HCV antibodies. In 2010, a short report was published by Riaz *et al.,* in Karachi related to prevalence of HCV. According to their report 43% thalassemic patients were tested positive for hepatitis C and majority (80%) of HCV positive patients were aged 10 years or above. This states that greater exposure of blood transfusion increases the probability of HCV transmission with increasing age.

In the same year 2011 Qurat-ul-Ain *et al.,* conducted a study regarding HCV prevalence in beta thalassemic population of Faisalabad. Their study reported 65% HCV prevalence in thalassemia patients which is the highest prevalence of HCV in thalassemic throughout the country. In 2012, Ansari *et al.,* published a short communication from Karachi related to prevalence of blood born viruses including HCV in thalassemic children. Thalassemia subjects along with volunteer blood donors were screened for HCV and it was found that 13.1% thalassemic children were screened positive for anti-HCV antibodies. However, only 1.9% volunteer donors were found positive for HCV.

Despite all these reports there exist a scarcity of the data related to the risk factors for the transmission of TTI in these patients mainly because there is no national surveillance program that can be pronounced responsible for the collection of such data in Pakistan. Thus, periodic studies are therefore one of the few options to elucidate the prevailing situation.

MATERIALS AND METHODS

3.1.1 Sampling site

Blood samples for present study were collected at Pakistan Thalassemia Welfare Society (PTWS), Rawalpindi, during July to September 2013. High number of multi-ethnic thalassemic patients from the adjoining districts came for blood transfusion or routine medical checkup to PTWS.

A blood donation camp was arranged at Quaid-i-Azam University on October 10, 2013. Their blood samples were also screened.

3.1.2 Inclusion criteria

Known cases of beta-thalassemia major and intermedia, who were transfused at least ten units of blood, irrespective of their demographic or clinical category, were included in this study. Complete clinical history and family history of patient was also needed. Those individuals who participated in the blood donation camp, enrolled in any department of QAU, were included for TTI screening as educated blood donors.

3.1.3 Sampling procedure

For the clinical assessment, 4.0 ml of venous whole blood was collected in a sterile gel vacationer by venipuncture with sterile disposable syringe. The blood samples were subsequently allowed to clot and centrifuged at 5000 rpm for 10 minutes for serum separation. Serum was stored under freezing temperature.

3.1.4 Socio-demographic data

A formal consent was obtained from the patients or their guardians/parents. The enrolled patients and their guardians were explained about the purpose of the study and its likely outcomes. A proforma was designed to collect demographic information on demography (age, gender, origin, ethnicity etc.), clinical history (type of thalassemia, age of diagnosis, number of blood transfusion, medication and therapy, and medical complications), and family history (i.e., consanguinity of parents, morbidity and mortality of sib ships).

A total of 105 patients were interviewed out of which 95 fulfilled the inclusion criteria. Each subject, based on family history and thalassemic condition was defined as sporadic or familial. If in the same family, at least two siblings were affected by thalassemia the case was considered as familial. Detailed pedigrees were constructed for the familial cases. Parity of the affected subjects and the numbers of normal sibs was also recorded. For screening of educated volunteer donors 44 subjects were selected and detailed information was collected by questionnaire.

3.1.5 Serological analysis

Serum was tested for anti-HCV by commercial ELISA kit according to the manufacturer's instruction (Anti-HCV ELISA version-1, made in China). The tests were performed in batches of 32 samples, running each time with two negative and two positive controls. Results were analyzed using micro plate reader (KHB ST-360). Sensitivity of this method was found to be 100% with specificity of 99.5%. The samples were considered positive when the sample absorbance/cut-off (SA/C) ratio was higher to 0.185 and negative when the cut-off ratio was <0.185. Serological analysis was performed at Biopath Lab and Diagnostic Center Rawalpindi.

Healthy blood donors in this study were screened using an Immuno-chromatography test (ICT) to identify the antibody to HCV, HIV and surface antigen for HBV using "Instatest" with very high sensitivity (99.9%) at Pakistan Thalassemia Welfare Society, Rawalpindi.

3.1.6 Results analysis

Results of ELISA and ICT screening were documented and further analyzed at Department of Microbiology, Quaid-i-Azam University Islamabad.

3.1.7 Statistical analysis

The data were recorded in MS-Excel and detailed descriptive summaries were generated for the vital attributes of the recruited subjects. Statistical calculations were performed through GraphPad Prism, ver. 5. The distribution of various disease parameters was checked across the demographic variables. Co-morbidity, clinical symptoms, therapy and medical complications in the thalassemic patients were also

explored in the socio-demographic attributes. Statistical significance was checked through Chi-test and t-test at a cut-off value of $p<0.05$.

RESULTS

A total of 105 random subjects were recruited in the current study, of which 95 subjects fulfilled the inclusion criteria. 91 subjects were observed to have beta thalassemia major (βTM) and 4 had beta thalassemia intermedia (βTI). Among these, 47 (49%) were detected positive for anti-HCV antibodies (Fig. 01).

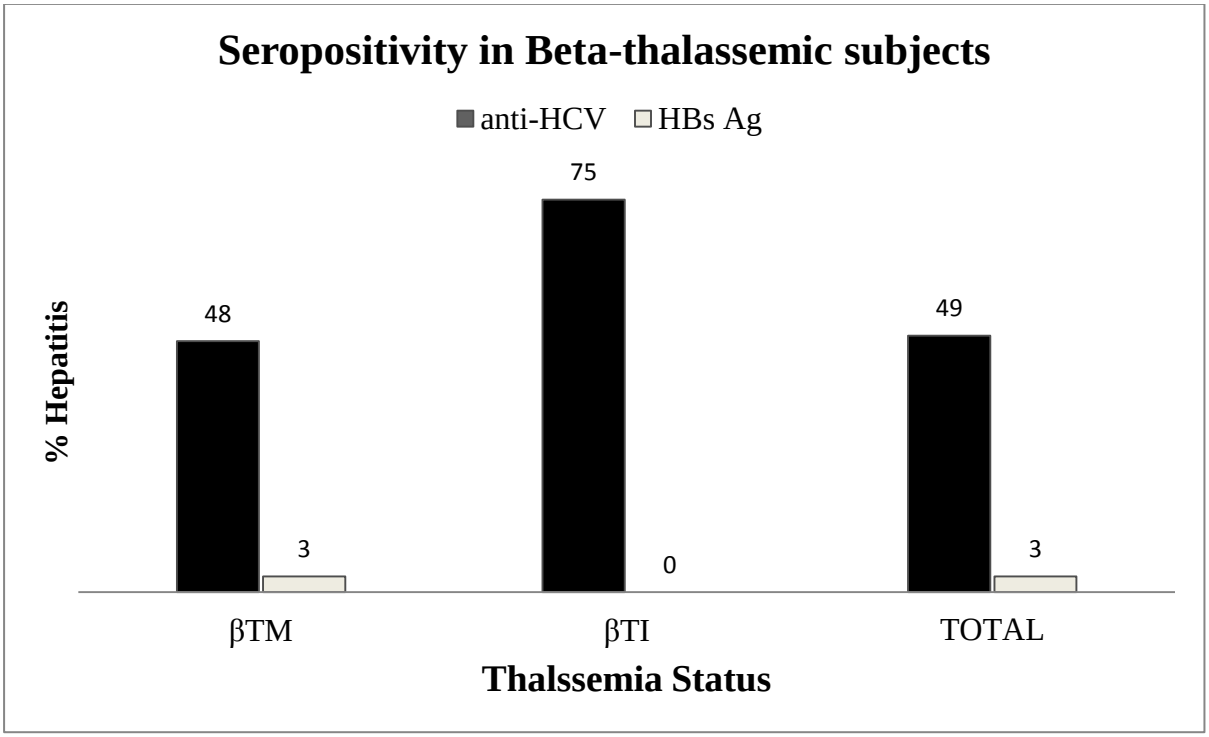

Fig. 01- Seropositivity in beta-thalassemic subjects

The patient data was divided in two groups, thalassemic only (n=48) and thalassemic plus hepatitis (n=47). Both groups were then observed for ferritin levels after dividing in male and female categories. Mean ferritin level with SD was calculated for all categories in both groups; however random distribution of ferritin level was seen with no clear difference in both groups (Fig. 02).

4.2.2 Comparison of clinical presentation among the thalassemia and co-morbid subjects:

All recruited subjects were observed for therapy/medication and complication behavior. 40 and 43 patients were on chelation therapy, whereas 19 and 17 patients were vaccinated for hepatitis B in thalassemic only (n=48) and thalassemic plus hepatitis (n=47) respectively. An overall complication (Hepatomegaly, Splenomegaly

and Splenectomy) was observed in 32(66.67%) and 29 (61.70%) individuals in thalassemic only and thalassemic plus hepatitis respectively (Table 2b).

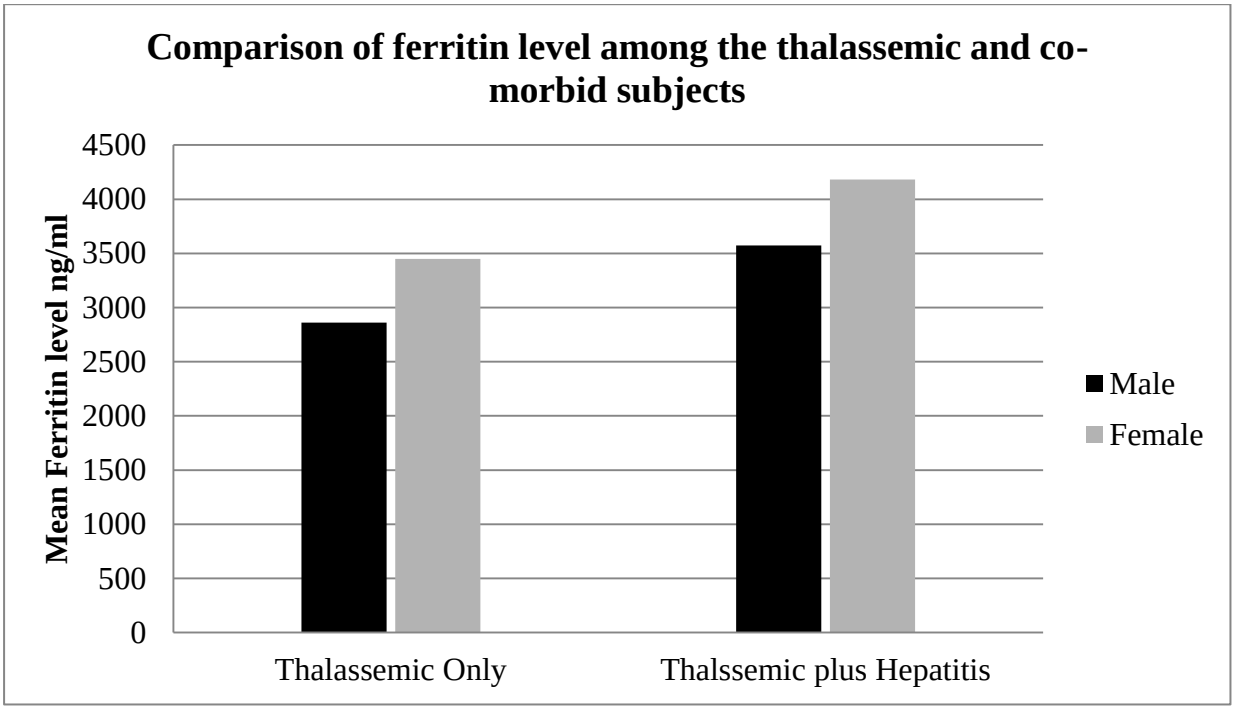

Fig. 02 Comparison of ferritin level among the thalassemic and co-morbid subjects

Table 01 Comparison of clinical presentation among the thalassemia and co-morbid subjects

Patients	**Total**	**Therapy/medication**		**Complications**			
		Chelation Therapy	**HBV Vaccine**	**Hepatomegaly**	**Splenomegaly**	**Splenectomy**	**All with HSS**
Thalassemic only	48	40 (83.33%)	19 (39.58%)	10 (20.83%)	20 (41.66%)	2 (4.16%)	32 (66.67%)
Thalassemic plus hepatitis	47	43(91.48%)	17 (36.17%)	16 (34.04%)	25 (53.19%)	4 (8.51%)	29 (61.70%)
All	**95**	**83 (87.36%)**	**36 (37.89%)**	**26 (27.36%)**	**45 (47.36%)**	**6 (6.31%)**	**61 (64.21%)**

4.2 Socio-Demographic distributions of b-thalassemic subjects and seroprevalence of hepatitis

In this cohort, 51 (54%) subjects were males and 44 (46%) females (M:F ratio= 1.2:1). The prevalence of anti-HCV was observed to be greater in males (53%) than the females (45%) (Table 02). Patients were ranging in age from 6 months to 27 years and hence, were divided into six age categories. There was highest representation of subjects of age 2.1-7 years followed by subjects with age 7.1-12 years. It was observed that degree of HCV positive individuals was increasing with increasing age (Table 02). The distribution of disease status (thalassemia and hepatitis) was not significantly associated with gender and age ($p>0.05$).

Geographically the data showed highest representation of subjects from Punjab (n=76; 80%) and Khyber Pakhtunkhwa (n=11; 11.6%), while subjects from other regions of Pakistan were in minor representation. There were 64.2% subjects belonging to rural background while there were 35.8% subjects originating from urban areas. Accordingly, there was highest representation of subjects speaking Punjabi language followed by Pashto speaking individuals (Table 02). However, the distribution of disease status was observed to be independent of the origin and language of the subjects ($p>0.05$).

Among the recruited subjects, the majority belonged to 'low socio-economic status' (45.3%), while there were 28.4% and 26.3% individuals belonging to 'middle' and 'high' socioeconomic status, respectively. Hepatitis seropositivity was observed to be highest in subjects belonging to 'high socio-economic status' (Table 02). The differences in the morbidity status were not statistically significant ($p>0.05$).

In reference to parental consanguinity, first cousin marriage was most common (66.31%) followed by out of family (26.31%) and marriage with in biradiri (7.36%). HCV was prevalent in biradiri and first cousin marriages i.e. 71% and 62% respectively. Least prevalent in out of family marriages was 28%.

With respect to the ABO blood groups, majority belonged to 'O' blood type (34.7%), and there were 92.6% subjects with Rh-positive group. However, highest prevalence of hepatitis seropositive was observed in 'A' blood type (63%), followed by subjects in 'B' type (55.6%) (Table 02)

Table 02: Demographic distribution of b-thalassemic subjects and seroprevalence of HBs-Ag and anti-HCV

Demographic variable	Subjects		Thalassemia		Hepatitis			χ^2 test statistics
	No	%	BTM	BTI	HBs Ag	Anti HCV Ab	%age HCV	
Gender								chi=0.708; df=1; p=0.7901; NS
Male	51	53.68	49	2	1	27	53	
Female	44	46.32	42	2	2	20	45	
Total	**95**	**100.00**	**91**	**4**	**3**	**47**	**49**	
Age								
<2	2	2.11	2	0	0	0	0	chi=1.553; df=4; p=0.8173; NS
2.1-7	36	37.89	36	2	2	14	39	
7.1-12	26	27.37	26	0	0	15	58	
12.1-17	21	22.11	21	0	1	11	52	
17.1-22	6	6.32	6	0	0	3	50	
22.1-27	4	4.21	2	2	0	4	100	
Geography								
Punjab	76	80.00	72	4	2	38	50	chi=1.051; df=5; p=0.9584; NS
Khyber Pakhtunkhwa	11	11.58	11	0	0	4	36	
Azad Jammu Kashmir	4	4.21	4	0	0	3	75	
Islamabad	2	2.11	2	0	0	1	50	
FATA	1	1.05	1	0	1	0	0	
Sindh	1	1.05	1	0	0	1	100	
Origin								chi=0.4908; df=1; p=0.4836; NS
Ruler	61	64.21	60	1	3	32	52	
Urban	34	35.79	31	3	0	15	44	
Language								
Punjabi	63	66.32	59	4	2	35	56	chi=1.854; df=6; p=0.9326; NS
Pashto	16	16.84	16	0	1	4	25	
Urdu	5	5.26	5	0	0	2	40	
Kashmiri	5	5.26	5	0	0	3	60	
Potohari	3	3.16	3	0	0	1	33	
Saraiki	2	2.11	2	0	0	1	50	
Sindhi	1	1.05	1	0	0	1	100	
Socioeconomic status								

Low	43	45.26	41	2	2	21	49	chi=0.8936; df=2; p=0.6397;NS
Middle	27	28.42	27	0	0	11	41	
High	25	26.32	23	2	1	15	60	
Parental consanguinity								
First cousins	63	66.32	63	0	3	29	62	chi=0.3022; df=2; p=0.8598;NS
Biradari marriage	7	7.37	7	0	0	5	71	
Out of family	25	26.32	21	4	0	13	28	
ABO Blood Groups								
A	27	28.42	26	1	1	17	63	chi=1.430; df=3; p=0.6986; NS
B	27	28.42	26	1	0	15	56	
AB	8	8.42	7	1	1	3	38	
O	33	34.73	32	1	1	12	36	
Rh blood types								chi=0.0955; df=1; p=0.7573; NS
Rh Positive	88	92.63	85	3	3	44	94	
Rh Negative	7	7.36	6	1	0	3	6	
Total	**95**		**91**	**4**	**3**	**47**	**49**	

4.3.1 Distribution of ferritin levels in various demographic categories of thalassemic patients

The distributions of ferritin levels, various therapies for thalassemia, and medical complications of thalassemia, were also checked across various socio-demographic variables (Table 03). On the basis of gender, female patients were observed to have greater ferritin levels as compared to males (3811±2558 vs. 3298±2085, respectively). However, the differences were not significant (t-test=1.203; p=0.2319) (Table 03). Among the six age categories, highest ferritin level was observed in 12.1-17 years with average of 5007±2643. With respect to geographic origin, the ferritin level was highest in patients from KPK, i.e. 4500±2592. Ferritin level was also highest in subject belonging to urban areas, speaking Potohari language, and having 'high' socio-economic status. With respect to parental marriage type, ferritin was higher in subjects whose parents had non-consanguineous marriages. With respect to blood groups, the ferritin was higher in subjects with blood type O and Rh positive (Table 03).

4.3.2 Therapeutic behavior and medical complications:

Among the recruited subjects, 83 (87.37%) were undergoing chelation therapy while only 36 (37.89%) had HBV vaccination (Table 4). Different complications were also observed in the subjects. For instance, splenomegaly (n=45), hepatomegaly (n=26), and Splenectomy (n=6) (Table 03). The detailed distributions of therapeutic behavior and medical complications are depicted in Table 03.

Table 03: Distribution of ferritin levels, chelation therapy, HBV vaccination and clinical complications in various demographic categories of thalassemic patients

Geography	Total	Ferritin level (ng/ml)10^3						Therapy/medication		Complications		
		<1	1-3	3-6	6-9	> 9	AVG/STD	Chelation Therapy	HBV Vaccine	Hepato megaly	Spleno megaly	Splenec tomy
Gender												
Male	51	8	18	18	7	0	3298±2085	43	17	15	23	3
Female	44	4	14	21	2	3	3811±2558	40	19	11	22	3
Age												
<2	6	6	0	0	0	0	700±2190	0	1	1	0	0
2.1-7	32	4	16	11	1	0	2737±1560	28	9	6	12	0
7.1-12	26	1	5	17	2	1	4232±2156	25	9	4	11	2
12.1-17	21	0	5	9	5	2	5007±2643	21	12	10	16	3
17.1-22	6	0	4	1	1	0	2952±2428	6	3	4	4	0
22.1-27	4	1	2	1	0	0	2923±1594	3	2	1	2	1
Number	**95**	**12**	**32**	**39**	**9**	**3**		**83**	**36**	**26**	**45**	**6**
Average		602 ±249	2059 ±558	4142 ±842	7494 ±868	10227 ±264	3503±2321					
Geography												
Punjab	76	9	28	30	8	1	3407±2155	67	30	21	36	6
Khyber Pukhtunkhwah	11	1	1	7	1	1	4500±2592	10	4	3	6	0
Azad Jammu	4	1	2	0	0	1	3442±4529	3	2	1	2	0

Kashmir												
Islamabad	2	1	1	0	0	0	839±6200	1	0	0	1	0
FATA	1	0	0	1	0	0		1	0	0	0	0
Sindh	1	0	0	1	0	0		1	0	0	0	0
Origin												
Ruler	61	8	23	25	5	0	3064±1828	53	22	19	31	2
Urban	34	4	9	14	4	3	4291±2876	30	14	7	14	4
Language												
Punjabi	63	10	25	21	7	0	3044±2095	53	22	19	32	4
Pashto	16	1	2	11	1	1	4300±2197	15	6	3	7	0
Urdu	5	0	2	1	1	1	5274±2313	5	3	1	3	2
Kashmiri	5	1	3	0	0	1	3140±3980	4	2	1	2	0
Potohari	3	0	0	3	0	0	5500±4560	3	2	1	1	0
Saraiki	2	0	0	2	0	0	4196±1067	2	1	0	0	0
Sindhi	1	0	0	1	0	0		1	0	1	0	0
Socio-economic status												
Low	43	7	18	14	4	0	2943±1912	36	12	10	15	0
Middle	27	4	8	9	5	1	3822±2697	23	12	7	17	4
High	25	1	6	16	0	2	4124±2398	24	12	9	13	2
Parental consanguinity												
First cousins	63	10	22	26	4	1	3118±2007	53	23	14	29	4
Biradari	7	0	1	6	0	0	3746±1184	7	3	2	5	0
Out of family	25	2	9	7	5	2	4408±3015	23	10	10	11	2
Blood groups												

A	27	3	8	13	2	1	3518±2399	24	11	5	17	3
B	27	6	6	12	3	0	3351±2138	21	7	9	11	1
AB	8	1	2	5	0	0	2869±1557	6	5	2	4	0
O	33	2	16	9	4	2	3730±2591	32	13	10	13	2
Rh												
Rh Positive	88	12	29	35	9	3	3506±2317	76	32	25	42	6
Rh Negative	7	0	3	4	0	0	3466±1700	7	4	1	3	0
Total	95	12	32	39	9	3	3503±2321	83	36	26	45	6

4.4 Distribution of hepatitis in various clinical presentations of thalassemia

Poly-transfusion in thalassemia patients is considered one of the major risk factor to acquire Hepatitis. Hence, the distribution of hepatitis was observed in various relevant clinical parameters of thalassemic patients (i.e., ferritin level, transfusion history, frequency of blood transfusion, iron overload, chelation therapy, and blood groups) (Table 04). HCV was observed to be increasing with increasing ferritin level. Blood transfusion frequency has direct relation with seorpositivity for hepatitis C, highest percentage (60%) was observed in patients with blood transfusion frequency of ≤10 days and the number of hepatitis C positive individuals was decrease with decrease in frequency (Table 5). Considerable number of individual was Hepatitis C positive with iron overload and chelation therapy (52%) as compared with individuals with no iron overload or chelation therapy (33%).Maximum number of positive individuals (63%) belongs to blood group A , group B (56%) and group AB (38%) falls at number 3 and 4. Whereas least positive cases (36%) was seen in group O. No clear difference was seen in Rh Positive (50%) and Rh Negative (43%) blood types.

Table 4: Distribution of hepatitis in various clinical presentations of Thalassemia

Ferritin level ng/ml x 10^3	**Subjects**		**Thalassemia**		**Hepatitis**		**HCV+**
	No	**%**	**BTM**	**BTI**	**HBs Ag**	**Anti HCV Ab**	**%**
<1	12	13	10	2	0	4	33
1.1-3	32	34	30	2	2	15	47
3.1-6	39	41	39	0	1	20	51
6.1-9	9	10	9	0	0	6	67
>9	3	3	3	0	0	2	67
Transfusion started at age (months, years)							
1-2 m	3	3	3	0	0	2	67
3-4 m	23	24	23	0	2	10	43
5-6 m	27	28	27	0	1	15	56
7-8 m	11	12	11	0	0	3	27
9-10 m	6	63	6	0	0	2	33
11-12 m	2	2	2	0	0	2	100
1-2 yrs	13	14	13	0	0	8	62

3-4 yrs	8	8	6	2	0	3	38
>4 yrs	2	2	0	2	0	2	100
Frequency in days							
≤10	10	11	10	0	1	6	60
15	47	49	46	1	1	26	55
20	6	63	6	0	0	3	50
25	6	63	6	0	0	1	17
30	24	25	23	1	1	9	38
>30	2	2	0	2	0	2	100
Iron overload							
Yes	83	87	81	2	3	43	52
No	12	13	10	2	0	4	33
Chelation Therapy							
Yes	83	87	81	2	3	43	52
No	12	13	10	2	0	4	33
Blood Groups							
A	27	28	26	1	1	17	63
B	27	28	26	1	0	15	56
AB	8	8	7	1	1	3	38
O	33	35	32	1	1	12	36
Rh							
Rh Positive	88	93	85	3	3	44	50
Rh Negative	7	7	6	1	0	3	43
Total	95	100	91	4	3	47	

4.5 Distribution of thalassemic subjects with respect sporadic and familial presentations, number of affected per family and other familial attributes

Thalassemic subjects were distributed with respect to their sporadic and familial presentations (Table 6). Familial cases were defined by the presence of more than one affected subject in the family. There were 60 subjects who were sporadic while 35 subjects had familial conditions. Among the familial cases, a total of 93 subjects were found to be affected. Among the sporadic cases, the affected male to female ratio was 1.36:1, while in the familial cases there was equal number of affected males and females (χ^2 =0.7581; df.1; NS). Among the familial cases, a total of 36 subjects had been deceased. The average number of normal sibs was observed to be higher for the familial cases compared with the sporadic (Table 5). The detailed distribution of number of deceases subject, total number of normal sibs, average number of normal sibs, and the prevalence of hepatitis among the sporadic and familial cases is depicted. in Table 5

Table No 05: Distribution of thalassemic subjects with respect sporadic and familial presentations other familial attributes

Familial nature	Affected family members	No. of recruited subjects	BTM (Affected in all families)		BTI (Affected in all families)		Total deceased in all families	Normal sibs in all families	Normal sibs (average/family)	Hepatitis	
			M	F	M	F				HBs Ag	HCV Ab
Sporadic (n=60)	1	60	34	25	1	0	2	36	1.70	2	31
Familial (all)		93	36	36	12	9	36	36	1.87	1	16
Familial (n=35)	2	44	19	17	4	4	16	17	1.5	1	13
	3	18	5	11	2	0	7	9	1.8	0	1
	4	16	6	5	1	4	9	3	-	0	1
	5	15	6	3	5	1	4	7	2.3	0	1
Total	**95**	**153**	**70**	**61**	**13**	**9**	**38**	**72**	**1.8**	**3**	**47**

4.6 Parity of thalassemic subjects with mean sib ship size

Parity was scored for all the recruited subjects and parity orders from first to eights were observed (Table 06). Majority of the subjects belonged to second parity (n=27, 29%), followed by first and third parity (n=24, 25% and 14, 15%, respectively). In each parity order, the mean sibship size was also estimated and generally, the sib ship size was increasing with increasing parity level (Table 06).

Table 06: Parity of thalassemic subjects with mean sib ship size

Parity Number	**Individuals**	**Mean sibship**
1	24	2.40±1.19
2	27	2.78±1.01
3	14	3.78±0.69
4	12	4.75±1.14
5	7	5.57±0.79
6	4	6.25±0.50
7	4	7.25±0.50
8	3	8.33±0.58

4.7 Assessment of highly educated volunteer blood donors and their screening for TTI

Total 44 subjects were recruited in this study. All of them were screened for TTI i.e. HBV, HCV and HIV. None of them was screened positive for any of the mentioned infections. The data was distributed in different categories such as gender, faculty, and origin. Level of knowledge about transfusion transmitted infections and thalassemia was also analyzed in these individuals. All donors were well aware about TTI and its route of transmission with few exceptions. Knowledge about thalassemia was also according to their level of education standard.

Subjects were also asked about their HBV vaccination. Surprisingly, HBV vaccination practice was found poor in these highly educated individuals. Out of 44

subjects 29% male and 44% female were vaccinated against HBV. Whether the donor was from faculty of biological sciences (Those who have strong knowledge about Hepatitis B) or from other faculties, the percentage of HBV vaccination was almost same i.e. 32% and 33% respectively. Urban individuals were more vaccinated 39% as compared to ruler subjects 27% (Table No. 07)

Table 07: Assessment of highly educated volunteer blood donors and their screening for TTI

Variables	Total	Screening for TTI Positive			Knowledge about		Hepatitis B Vaccination in %
		HBV	HCV	HIV	TTI	Thalassemia	
Gender							
Male	35	0	0	0	34	33	29
Female	9	0	0	0	9	9	44
Faculty							
Bio-Sciences	34	0	0	0	34	34	32
Others	10	0	0	0	9	8	33
Origin							
Ruler	26	0	0	0	26	25	27
Urban	18	0	0	0	17	17	39

DISCUSSION

Thalassemia is a well-recognized genetic blood disorder. Among its different types, beta thalassemia is the most common autosomal single gene disorder. It is prevalent in more than 60 countries of the world with up to 150 million carrier population (Weatherall and Clegg 2001). The overall prevalence of beta thalassemia is ranging from 4.3% in Gaza Strip (Jabour 1998) and 25% in district of Isparta (Tunc *et al.*, 2001).

To reduce the complication of severe anemia in beta thalassemic patients, early and regular blood transfusion therapy is indispensable. If safe blood transfusion in not ensured, patients are confronted to new health issues, particularly transfusion transmitted infections specially HBV, HCV and HIV (Mollah *et al.*, 2003). HCV infection is a serious threat in multi transfused patients as a major complication of transfusion therapy. HCV remains a major problem in countries where HCV is more prevalent in general population and therefore also in blood donors. The seroprevalnce rate of HCV in multi-transfused patients is directly related to the number of blood transfusions. High prevalence rate and high risk of chronic liver disease makes HCV more important than other transfusion transmitted infections (Williams *et al.*, 1992). However, for successful transfusion therapy proper chelation therapy is also necessary in such patients to prevent organ damage due to secondary iron overload (Prati 2000)

The current study focused on prevalence of anti-HCV antibodies in multi transfused beta thalassemic patients from Pakistan Thalassemia Welfare Society, Rawalpindi. PTWS receives a high influx of thalassemic patients from the adjoining districts which come for blood transfusion or routine medical checkup. Thus a true demographic picture of beta thalassemic population is depicted from different areas near by Rawalpindi. The study also focuses on secondary iron overload, its consequences and iron chelation therapy as the iron overload is second major complication of transfusion therapy besides HCV.

In our study among 95 subjects, beta thalassemia major and intermedia patients were 96% and 4% respectively from Rawalpindi and its surrounding regions comparable to that of 93% and 7% in Faisalabad (Qurat-ul-Ain., 2011), 86% and 14% in North

Americans patients (Pearson *et al.,* 1996) and 64% and 36% in Lebanon (Inati *et al.,* 2006).

According to our study HCV seroprevalence was 49% in beta thalassemic patients which coincide with the study of (Younus *et al.,* 2004) from Rawalpindi which shows prevalence rate of 42% and study of (Hussain *et al.,* 2004) with prevalence rate of 43% from Islamabad/Peshawar region. In contrast Qurat-ul-Ain *et al.,* 2011 reported a higher prevalence rate of 65% from Faisalabad and 57% reported by Shah *et al.,* 2005 from Peshawar. This may be due to the fact that prevalence of HCV in general population is higher in Faisalabad and Peshawar as compared to our area of study.

In current study the number of affected male was higher (53%) as compared to female (45%) but this difference was statistically not significant (P>0.05). Our results were in similarity to (Gurbak *et al.,* 2006) who reported no appreciable difference in male (55.5%) and female (44.5%) thalassemic patients from Gaziantep. A statistically non-significant difference between male and female thalassemic patients was also reported by (Asadi-Pooya and Doroudchi 2004). Our results are also in agreement with Riaz *et al.,* 2010 reported 45.4% female and 41.3% male patients, and Hemeed *et al.,* 2008 reported 52% female and 48% male thalassemic subjects with no statistical significance.

In our study we found statistically non-significant (p=0.817) seroprevalence of HCV with increasing age. Our results were in agreement to Ansar and Koolobandi 2002, reported a non-significant difference of HCV prevalence in different age groups (P=0.181). Bhavsar *et al.,* 2011, Riaz *et al.,* 2011 also reported a random distribution of HCV prevalence in different age groups. Thus there was no apparent age-specific distribution of HCV.

Demographic distribution of thalassemic patients in reference to geography, origin, language socio-economic status and blood group showed random distribution of HCV with no statistical significance (P>0.005). Further work is needed regarding socio-demography to bring a clear picture of demographic data in beta thalassemic population across the country.

In our study, random distribution of ferritin was found in various socio-demographic variables. Female patients had greater mean ferritin level as compared to male but the

difference was statistically not significant (P=0.231). Therapeutic behavior showed that 87.37% patients were on chelation therapy. In contrast (Ragab *et al.,* 2012) reported that only 46% Egyptian thalassemic patients were on chelation therapy. Such high number of iron overload (87.37% chelation therapy) in our study is due to poor compliance of patients towards chelation therapy. In our study 37.89% patients were found vaccinated for HBV which coincide with 50% vaccinated patients reported by Ansari *et al.,* 2012. Splenomegaly was observed in 47% patients whereas 27% patients had hepatomegaly. Our results were in agreement with Ragab *et al.,* 2012 that reported 49% splenomegaly in Egypt.

In the current study distribution of hepatitis C in different clinical parameters of thalassemia was studied. We found that increasing ferritin level, high frequency of blood transfusion and early age transfusion therapy leads high seroprevalence of HCV. Highest percentage (60%) was observed in patients with blood transfusion frequency < 10 days. Our results were in agreement with (Ansar and Kooloobandi 2002) and (Uddin *et al.,* 2009) as both reported that HCV prevalence in multi transfused patients is directly related to the number of blood units transfused. A significant positive correlation was reported by Ragab *et al.,* 2012 between HCV seropositive state and ferritin level (P=0.007) from Egypt. Thus further work is needed in this relevance. HCV was randomly distributed and no correlation was found between particular blood group and TTIs. Similar results were also reported by Riaz *et al.,* 2010 and Vidja *et al.,* 2011.

In our study data of family history was summarized to elucidate distribution of thalassemia and HCV with respect to sporadic and familial representation and other familial attributes. Familial to sporadic ratio of thalassemia was 1:1.7. The average number of normal sibs was observed to be higher for the familial cases compared with the sporadic. This may be due to the reason that families with their first affected child are reluctant about their next babies. HCV prevalence was significantly higher in sporadic cases (51%) as compared to familial cases (17%). With exception to study of shah *et al.,* 2005, no specific studies are available and more detailed picture can be depicted as a future perspective.

We calculated parity of thalassemic subjects with mean sib ship size. It was found that majority of the subjects belonged to second parity (28.42%), followed by first and

third parity (25.25% and 14.73% respectively). Our results were in agreement with Qurat-ul-.Ain *et al.,* 2011 that reported two children per family 51.85%, one child and three children per family 26.26% and 16.85% respectively. In contrast Shah *et al.,* 2005 reported that majority patients belonged to first parity (56.7%) followed by third and second parity (38.3% and 5.0%).

Our study revealed that none of the donor was screened positive for HBV, HCV or HIV out of 44 educated blood donors at university level. Our results were in similarity with the study of (Mujeeb *et al.,* 2000) who reported 2.1% HBV, 0.4% HCV and 0% HIV in college going volunteer donors which is very low to the prevalence of TTIs in general population volunteer donors. This may be due to the fact the educated population has better understanding and knowledge about risk factors and transmission routs of TTIs. (Ansar and Kooloobandi 2002) reported 0.5% HCV in general volunteer donors. In contrast to his study Ansari *et al.,* 2012 reported HCV 13.1%, HBV 1.25% and HIV 0% in healthy blood donors in agreement with study of (Waheed 2012) who reported HCV 8.34%, HBV 3.91% and HIV 0.89% in replacement donors (94%) and volunteer donors (6%). HBV vaccination was found only 36.5% in university level volunteer blood donors. Subjects were distributed according to gender, origin and study field but no significant difference was seen in these groups.

The indispensible blood transfusion therapy is playing major role in supportive therapy for thalassemia. However, besides poor quality of life, transfusion associated risks make life expectancy of multi transfused patients even shorter. Proper screening of blood before transfusion and screening of thalassemic patients on regular basis is necessary. Seroprevalnece of HBV, HCV and HIV are particularly low in college and university students in comparison to general population donors. Thus, it is needed to educate general population about TTIs, motivate and recruit educated donors to ensure relatively safe blood supply for transfusion services.

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