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VIRAL INFECTION PREVALENCE IN B-THALASSEMIA MAJOR PATIENTS RECEIVING FREQUENT BLOOD TRANSFUSIONS

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ABSTRACT

Background: β-thalassemia is a hereditary blood related disorder characterized by partial or complete loss of production of hemoglobin's β globin chain, which leads to severe anemia. HCV infection is commonconcern in β-thalassemia patients, particularly due to multiple transfusion, with increased risk of acquiring blood born infections. The prevalence of HCV varies across different region and populations depending on screening method, transfusion practice and geographical factor. HBV andHIV infection also occur in β-thalassemia patients, primarily through blood transfusion and other mode of transmission like vertical transmission.

Objectives : The purpose of study was to find out the prevalence of HCV, HIV and HBV in β-thalassemia majorpatients who were receiving regular blood transfusions.

Methodology: A descriptive study was conducted in Pakistan Institute of Medical Sciences, Islamabad. This study included127 patients of β-thalassemia major who were refer to tertiary care hospital in PIMS (Pakistan Institute of Medical Sciences). Patients of all age who were confirmed for β-thalassemia major were included in current study. Blood samples were collected from 127 β-thalassemia patients, and after serum separation screening for HCV, HBV and HIV were performed by ICT kit method.

Results: Out of 127 β-thalassemia patients, 78 (61.4%) were male and 49 (38.6) were female. Most affected age group was 0-10 years containing 54.3% patient population with mean age 11.3±5.8 years. Mostcommon blood group among β-thalassemia patients was B 46 (36.2%) following O 39 (30.7%), A 32 (25.2%) and AB 10 (7.9%). 61 (48%) were HCV positive, and 1 (0.8%) was HBV positive. Antibodies against HIV were not detected in any samples. Statistical difference of HCV and HBV was found significant among different age groups (p-value <0.05) and non-significant in gender and blood groups (p-value >0.05).

Conclusion: The prevalence of HCV infection in patients with β-thalassemia is substantially higher than that of HBV and HIV. Older age patients are more vulnerable to HCV infection as compared to younger ones. This situation should be managed and monitored through the administration of antiviral DAAsfor the treatment of HCV in patients received more blood transfusion.

Key Words: Hemoglobin, Thalassemia major, Thalassemia intermediate, Hepatitis B Virus, Hepatitis C Virus, Hepatitis E Virus, Deoxyribonucleic Acid.

INTRODUCTION

Thalassemia

Thalassemia, is a very common blood disorder among wide range of genetic disorders with autosomal recessive inheritance, that decrease hemoglobin production, the frequency of synthesisof the alpha or beta chain of adult hemoglobin (Hb A) is decreased [1]. The thalassemia-causing genetic defects are located in the human globulin gene, which encodes the alpha and beta hemoglobin polypeptide chains on chromosomes 16 and 11, respectively, and results in thalassemia when both the alpha and beta chains are occupied [2]. Red blood cells in thalassemia aredestroyed because of an imbalance in the synthesis of globin chains, which leads to red blood celldestruction, hinders erythropoiesis, and results in hemolysis in the peripheral circulation[3].

In 1925, Rietti-Greppi-Micheli illness, which is now known as thalassemia major (TM), and Cooley's anemia, which is now known as thalassemia intermedia (TI) [4 5], respectively, were both recognized as a separate disease in Italy and the US. Throughout the following 20 years, it becameevident that thalassemia was classified as homozygous or composite heterozygous conditions for thalassemia minor [6], a congenital minor recessive microcytic anemia. Thalassemia, which is caused by errors in the formation of the alpha and beta globin chains of hemoglobin, was later discovered to be the most prevalent monogenetic disease affecting humans. Along with the Mediterranean region, Africa, the Middle East, Southeast Asia, and the western Pacific all have ahigh prevalence of it[7].

Thalassemia subtypes

Alpha-thalassemia

It is a dangerous condition where there is no production of alpha globin, which results in anemia developing even before birth. The expecting woman must deliver the child herself, putting her own health at serious risk. Typically, this form is unfair to life [8].

Clinical manifestations

Fetal hemoglobin edema Bart syndrome (Hb-Bart), which is caused by the removal or inactivationof four globin genes, and hemoglobin-H disease (HbH), which is typically brought on by the removal or inactivation of three -globin genes, are the two clinically significant events occur in alpha thalassemia (α-thalassemia) [9].

Beta-thalassemia

A deletion of the beta globin or a combination of mutations (about 200 harmful variants have been found) are the two main causes of the genetic condition beta thalassemia located on chromosome11, the (HbB) gene. The majority of these mutations affect the transcription system as point mutations in HbB gene and affect its product's regulation, translation, and assembly. Thalassemiamajor, sometimes known as "Cooley's anemia," is a serious and potentially fatal anemia. Jaundice,pale skin, hunger, common illnesses, enlarged organs, and common infections are other symptoms.This is a severe variant that necessitates frequent blood transfusions. There are no blood transfusions necessary for the mild form of indirect thalassemia [10].

Clinical characteristics

Low hemoglobin levels, nucleated red blood cells on an abnormal peripheral blood smear, and hypochromic microcytic anemia are common symptoms of β-thalassemia, which is distinguishedby

decreased production of the β subunit of hemoglobin[11].

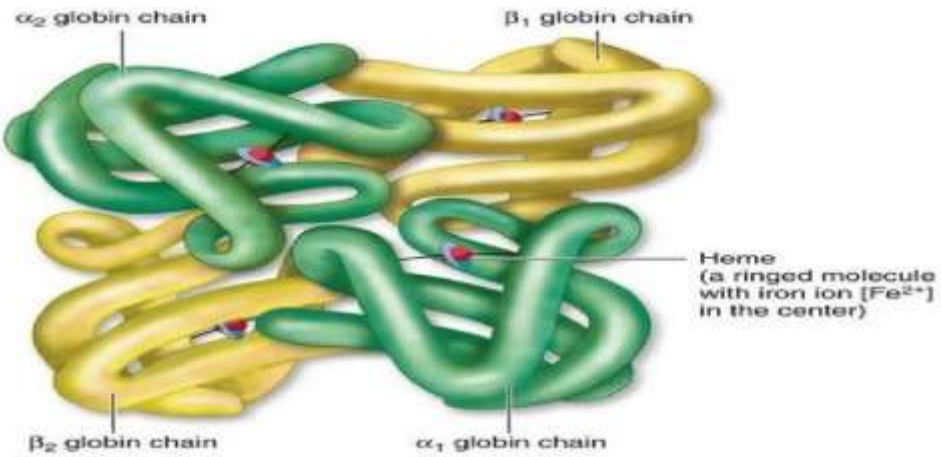


Figure 1.1: Alpha and beta globin chain

Delta-thalassemia

Delta thalassemia is brought on by a mutation in the genes in charge of synthesizing the delta chain. This mutation reduces the gene's capacity to generate delta chains, much as beta- thalassemia. A mutation is referred to as delta 0 if it avoids the creation of a delta chain, and delta+ if a delta chain does form. The absence of the delta chain and inability to detect HbA2 in the blood (normal 3.5%) are the results of two delta0 mutations. But when two Delta+ mutations are inherited, HbA2 levels fall. All patients with delta thalassemia have normal hematological sequences, despite the fact that the presence of a delta mutation can make it difficult to diagnose beta thalassemia. HbA2 is increased in beta thalassemia in the interim, although the presence of delta can lower HbA2, masking the beta-thalassemia diagnosis. An uncommon hemoglobinopathy known as delta-beta thalassemia is characterized by the absence or reduced manufacturing of β and δ globin. The compensatory mechanism is an increase in gamma chain synthesis, which leadsto a significant quantity of fetal hemoglobin (HbF) in the blood, which is evenly distributed amongred blood cells [13].

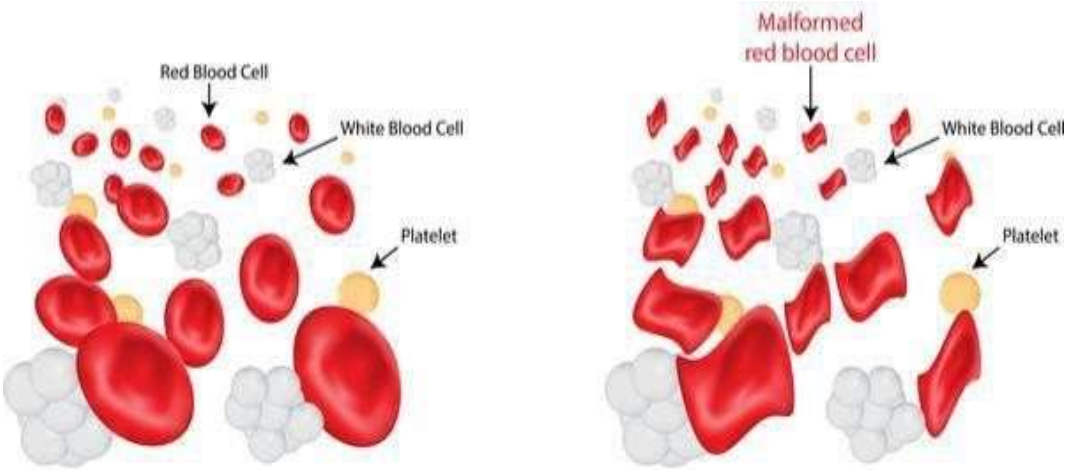


Figure 1.2: Red blood cell abnormalities in thalassemia

Combination of hemoglobin disorders

Thalassemia may coexist with other hemoglobinopathies; clinically, Beta-thalassemia major and thalassemia intermedia are similar to hemoglobin E/thalassemia. Clinically comparable to sickle-cell anemia, hemoglobin S/thalassemia is characterized by spleen enlargement. It is common in Cambodia, Thailand, and some regions of India throughout nations throughout Africa and the

Mediterranean, hemoglobin C/thalassemia is a common condition that results in moderate to severe anemia and splenomegaly. The northwest parts of Pakistan and India are prone to hemoglobin D/thalassemia [15].

Epidemiology

The thalassemia is a broad-spectrum disease which has spread from the Mediterranean countries, via the Middle East & Africa, to the South & Southeast Asian nations. Due to the ongoing emigration of people from these areas to the West, thalassemia is currently spreading across the globe. In a nation, thalassemia affects both sexes at a rate of roughly 4.4% per 10,000 live births per year [16, 17]. For the first two years of life, these patients need regular blood transfusions to prevent severe anemia and accompanying medical implications by keeping hemoglobin levels above 10 g/dL. Total, consistent blood transfusions and chelation therapy lengthen the lives of thalassemia patients, but these benefits come at the cost of lethal infections such iron overload andtransfusion-related illnesses (ITT), like hepatitis B and C virus (HBV and HCV) [18]. The WHO estimates that 240 million people have chronic HBV infection and 170 million people have HCVinfection. Between 0.3% and 5% of thalassemia patients worldwide have hepatitis.7% for the HBV(hepatitis B surface antigen) [19].

Signs and Symptoms

The intensity and degree of the inherited abnormality determine the thalassemia symptoms. The production of hemoglobin is reduced and anemia is mild to more severe in accordance with genetic defects. Minor thalassemia is frequently asymptomatic and symptomless. Mild anemia is the most common sign in this situation. Severe hemolytic anemia frequently coexists with major thalassemia. Fatigue, failure to thrive, jaundice, an enlarged spleen and liver, and joint problems, particularly in the face bones, are some of the symptoms of thalassemia major [20].

Diagnosis

Many individuals suffering from thalassemia are mistakenly identified when moderate microcytic anemia appears in their blood tests. Iron deficiency, thalassemia, lead toxicity, sideroblastic anemia, and chronic disease anemia are all potential causes of microcytic anemia. Measures like the mean corpuscular volume (MCV), the red blood cell distribution width (RDW), and medical history of patient may be helpful ruling out some of these causes. Up until the hematocrit drops below 30%, MCV is commonly less than 75fl in thalassemia and seldom <80fl in iron deficiencyanemia. The Mentzer Index (MCV/erythrocyte count) can discriminate between thalassemia and iron deficiency in children. In iron deficiency anemia, the ratio is frequently >13, whereas in thalassemia, it is <13.13 is thought to be an unclear ratio of [21].

Treatment

Blood transfusions, folic acid, iron chelating drugs, and hematopoietic cell transplants are all common forms of treatment. The patient's clinical profile determines the treatment option.

Blood transfusion

Regular blood transfusions and iron chelation therapy are the pillars of thalassemia treatment. Timely and consistent blood transfusions reduce the problems of severe anemia and increase the likelihood of survival. However, transfusions carry the possibility of complications. Understandingthe various adverse effects of blood transfusion is a crucial aspect of management for thalassemiapatients. The prevalent term for adverse events associated with the transfusion of blood products is transfusion reactions[22]. Blood transfusion is the primary required treatment but it is associated with the risks of iron overload resulting in dysfunctioning of endocrine system and the possibility of contracting a transfusion-transmitted infection such as hepatitis [23].

Iron chelation

Current clinical iron chelators include subcutaneous or intravenous deferiprone, oral deferasirox, and

oral deferiprone. In general clinical practices, ample control of tissue iron levels isn't attainedin a substantial proportion of patients, despite clinical trials demonstrating that this is possible [24].

Hepatitis virus

Hepatitis virus are group of viruses that cause inflammation of liver. The inflammation of liver iscause by many infectious and noninfectious agents leading to range of health problem. There arefive main strains of hepatitis: A, B, C, D and E. Each type spread differently and can cause varioussymptoms and complications. [25]

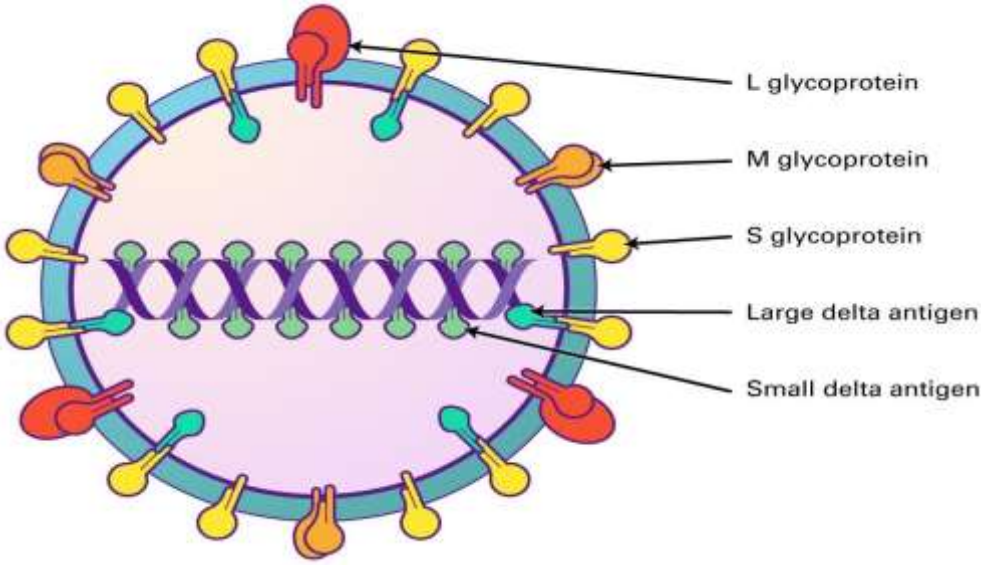


Figure 1.3: Hepatitis virus.

Hepatitis A virus: it spread by contaminated water, food and close contact with the diseased individual. It usually causes a mild short-term illness, but in rare cause can cause liver failure.[27]
Hepatitis B virus: Hepatitis B is likely lethal liver infection that is caused by the HepatitisB virus which is spread through the blood and bodily fluids, such as from unprotected sex or sharing needle. HBV also cause both chronic and acute infection, and can lead to serious liver damage, liver cancer and other complications. [28]
Hepatitis C virus: also spread through the blood often from sharing needles and receivingblood transfusion, HCV also cause both acute & chronic infection, can also cause severe liver damage, liver cancer and other complications. [29]
Hepatitis D virus: can only infect people who are already infected with HBV as it requires the HBV to replicate. It can cause more serious liver damage then HBV. [30]
Hepatitis E virus: cause inflammation of liver which spread via polluted water and eatable stuff, and it is very commonly found in developing countries. It usually responsible for the short-term, mild illness, but can more severe in pregnant women and people with pre-exitingliver disease. [31]
Hepatitis is serious viral disease and cause by virus which mainly target the liver of patient. Liver is essential organ that process the absorbed nutrients, cleans the blood from pathogens and toxic agents and fight the infection. When this vital organ gets infected, its functional abilityalso deteriorates. There could be other sources which cause hepatitis like drugs, excessive alcohol consumption, toxin and some other medical situations but main reason still remains hepatitis viruses. [32]

Hepatitis B Virus (HBV)
Hepatitis B virus is blood-borne virus that primarily affect the liver. It is major public health problem worldwide, with approximately 257 million persons living with chronic HBV infection.HBV can cause both serious acute & chronic hepatitis which can initiate serious liver damage, liver cancer and other serious disorders. [33]

HBV transmitted through blood or bodily fluid and unprotected sex, sharing needles and infected equipment, and from mother to child during birth. [34] The pathogen can live without a host for minimum seven days and can be transmitted through contact with contaminated objects. [35]

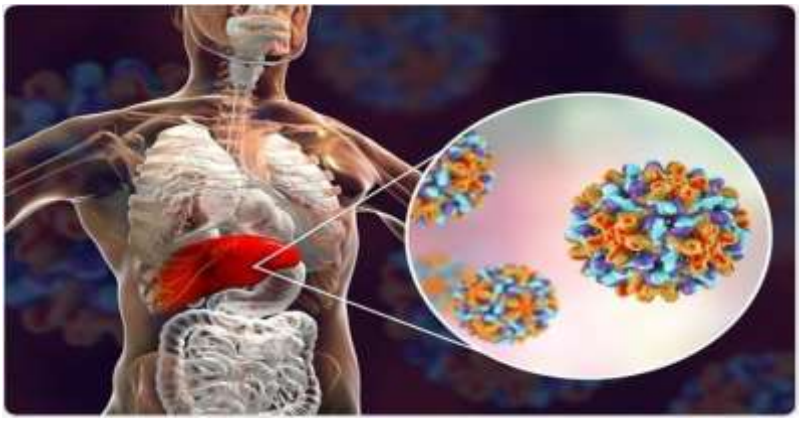


Figure 1.5: Structure of HBV virus. Capsid flexibility and function [36]

Epidemiology of HBV

Hepatitis B virus is most significant communal well-being problem in Pakistan. HBV is highly endemic in Pakistan, with an estimated 5-7% of population being infected with virus. [37] The prevention of HBV infection varies by region in Pakistan, with highest rate of infection report in northwest and southwest regions of country. [38] The risk of chronic HBV infection is highestin infants, young, and children, with up to 90% of these infected at birth developing chronic infection. Chronic HBV can lead to serious liver infection, cirrhosis and cancer in Pakistan. HBV is highly endemic in numerous regions of world. [39] Particularly in Sub-Saharan Africa &Asia. In these regions up to 10% population may be chronically infected by HBV. In the United States there are upto 2.2 million individuals which are chronically infected with HBV. The rate is seen among the foreign-born individuals from countries with high rate of HBV transmission.

Structure of Hepatitis B virus

The structure of Hepatitis B virus is very complex, which is made up of a lipid bilayer derived from the host cell membranes, and an inner nucleocapsid core. The nucleocapsid core containsthe viral genome, which are partially double-standard circular DNA molecule of about 3.2kb inlength.[41]

The core is composed of core antigen, which forms a spherical shell around the genome, and the e antigen (HBeAg) which is secreted form of core antigen that is important in replication ofvirus. The envelope of HBV virion contains three surface proteins: small (S), medium (M) andlarge (L) antigen. [42] The S protein form the outermost layer of envelope and is the main target of neutralizing antibodies. The M and L protein are derived from a common precursor protein and are involved in virion assembly and infectivity. [43] HBV are classified in nine genotypes (A-I) which differ in their nucleotide sequence by at least 8%. The genotypes have distinct geographic distributions and are associated with difference in disease progression and treatmentresponse. [44]

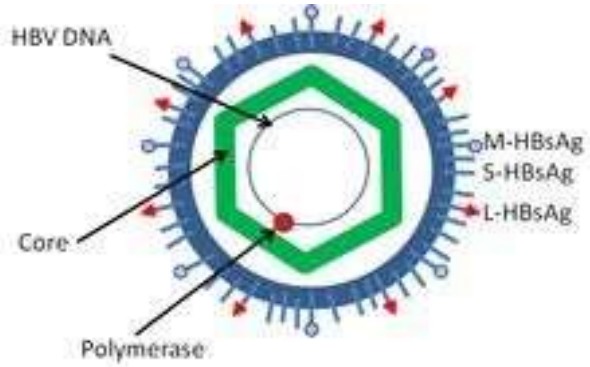


Figure 1.4: Hepatitis B Virus. [45]

Liver with HBV infection highlight inside human body and close up inside view of HBV virus [46].

Replication of HBV

Replication of HBV is complex process that involved several steps, including entry, uncoating, transcription, reverse transcription, encapsidation and budding. HBV enters host cell via attachment to the specific receptor on the cell surface, follow by endocytosis and uncoating ofviral nucleocapsid. [47] The transportation of viral DNA genome to the nucleus of cell, where it is transfer into a covalently closed circular DNA (cccDNA) molecular that is serving as template for viral transcription. HBV transcription is regulated by the viral-X protein (HBx) which activates cellular signaling pathway and enhances transcriptional activity of viralenhancer/premotor regions. [48] The viral pregenomic RNA (pgRNA) is transcribed and serves as template for reverse transcription of viral DNA genome by viral polymerase (p protein). The newly synthesized viral DNA is then encapsidated into nucleocapsid and transported to the endoplasmic reticulum (ER), where they are enveloped by viral surface proteins to form maturevirion. Mature virion is then secreted from the infected cell by budding from the ER and Golgimembrane. [49]

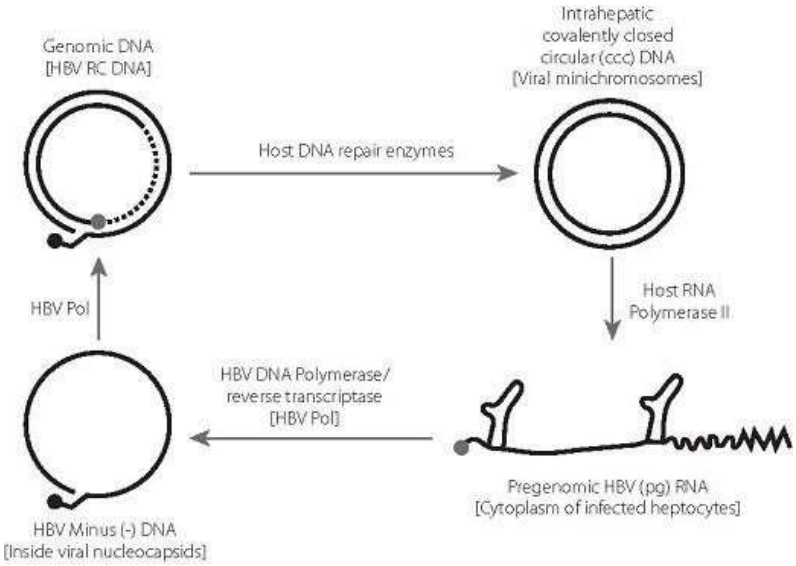


Figure 1.6: The lifecycle of HBV focuses viral genome replication. HBV polymerase play important role in replication of virus. Polymerase inhibitor block the reverse transcription pathway, RNA minus strand DNA synthesis, and also block DNA plus strand DNA synthesis andthere are suppressing the step of cccDNA step. [50]

Transmission of HBV

HBV virus is particularly transmitted by blood and bodily fluid. Perinatal transmission is HBVcan be

transfer from mother to child birth or through breast milk. This is the most common way of transmission in area where HBV is highly endemic, such as Asia and Africa. [51] Sexual transmissions, HBV can be transferred through the sexual contact with an infectious partner. Especially most common among men who have sex with men and heterosexual with multiple partners. Injection drug use HBV can be transmitted through sharing of needle and other equipment. [52] Occupational exposure, healthcare workers and other individuals who are exposedto blood and other bodily fluids are at increased risk of HBV transmission. House hold contact,HBV also transmitted through the house hold contact with infected person, such as allocation of personal items like razor and toothbrush. [53]

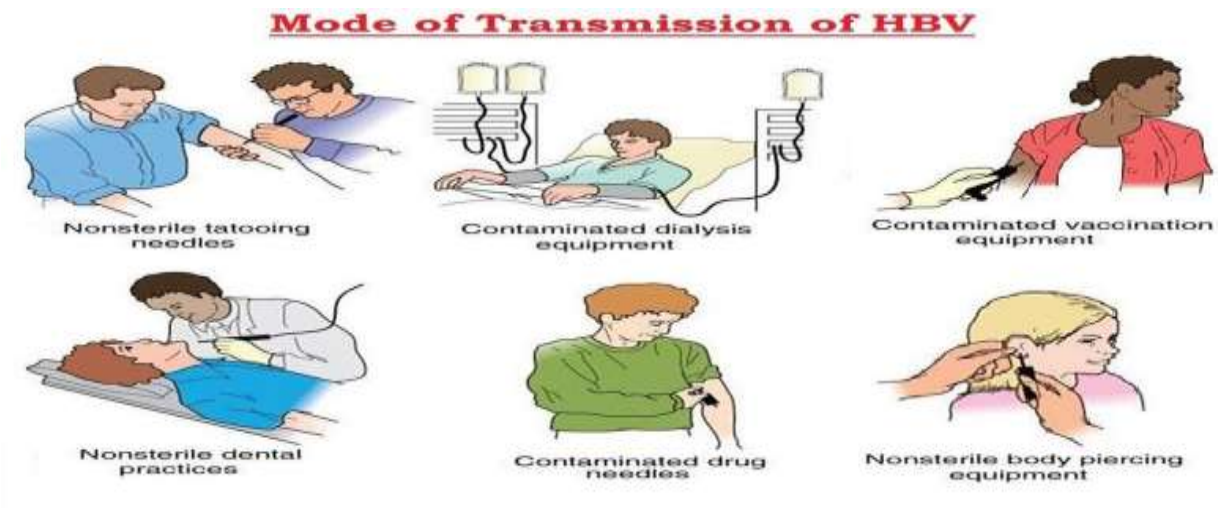


Figure 1.7: Mode of transmission of HBV [54]

Sign and Symptoms of HBV

Hepatitis B virus (HBV) is an infectious particle can cause a broad range of symptoms, rangingfrom mild to severe. Some people may not show any symptoms at all, while others may developa chronic infection. Here are some of the most common signs and symptoms of HBV: Fatigue:Feeling tired or weak is a common symptom of HBV infection. Loss of appetite: HBV infectioncan cause a loss of appetite and weight loss. [55] Nausea and vomiting: Some people with HBV infection may experience nausea and vomiting. Abdominal pain: Pain in abdomen is also a common symptom of HBV infection.

Jaundice: Skin color become yellow and color of eyes become white is a common sign of liver dysfunction and is a common symptom of HBV infection. Dark urine: HBV infection can causethe urine to become dark in color. [56] Clay-colored stools: Pale or clay- colored stools are a common symptom of HBV infection. Joint pain: HBV infection can cause joint pain and swelling. Fever: A low-grade fever is a common symptom of HBV infection. Rash: Some peoplewith HBV infection may develop a rash. It is the most important point to be noted that many people with HBV infection do not show any symptoms, and the symptoms may not appear untilseveral weeks after the initial infection. [57]

Hepatitis C virus

Hepatitis C virus causes inflammation of liver. It is a blood borne virus which is major reason of liver related ailments worldwide, including liver cancer & liver cirrhosis. HCV was discovered in 1989, and since then significant progress has been made in understanding the virus and developing treatment. [58] Hepatitis C viral infection can cause inflammation and damage of liver. Inflammation is swelling that happen when body tissue become injured or infected. Inflammation can also damage the body organ. Hepatitis C virus cause both chronic and acute infection. [59]

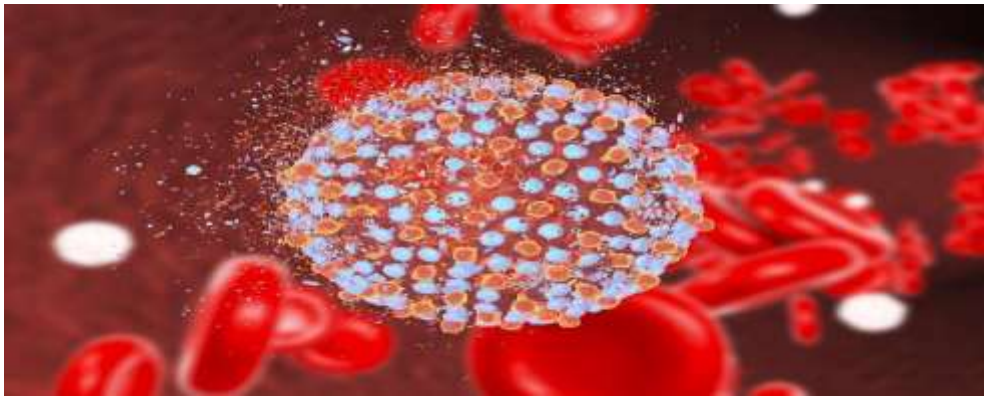


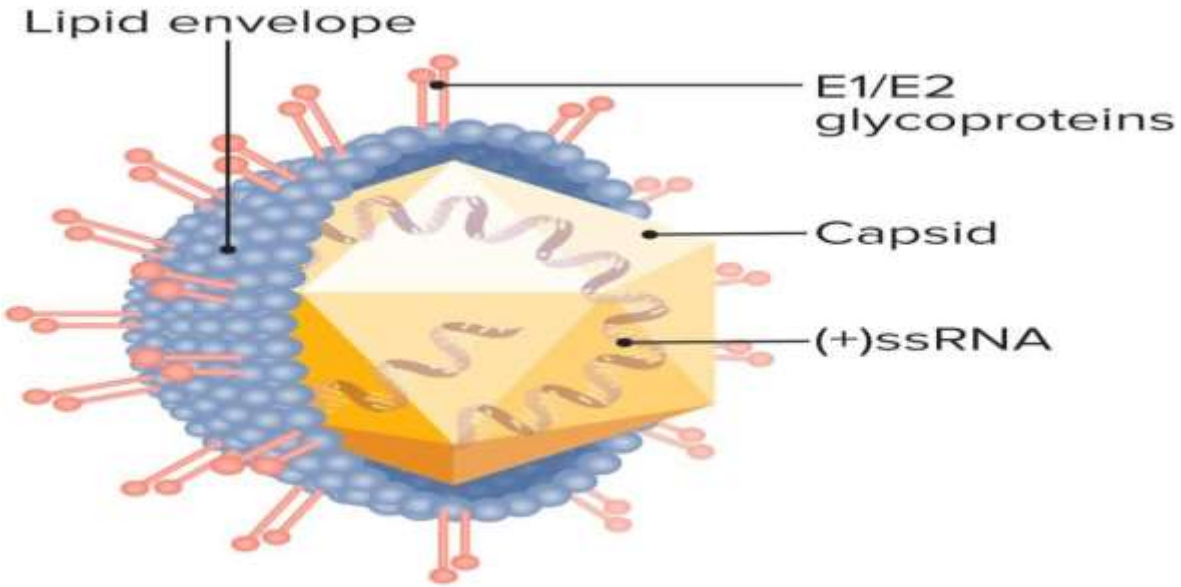
Figure 1.8: Hepatitis C virus. [60]

Acute infection: acute infection is new infection which lasts for a shorter period of time, symptomscan last up to 6 months. Sometime body's immune system manages to ward off the virus and patient heals itself.

Chronic infection: chronic hepatitis C virus cause infection which lasts for longer period of time because the immune system of patient is not sufficient to drive away the virus. Approximately 70 to 85% patients with acute disease becomes chronically infected due to this reason and ultimately end up with more serious liver conditions such as liver carcinoma, liver cirrhosis or liver failure.[61]

Structure of HCV

HCV is a small enveloped virus, which belongs to the Flaviviridae family. The virus particles contain nucleocapsid, which is consists of viral RNA genome and viral core proteins. The HCV is a single stranded, positive sense RNA virus of somewhere around 9.6 kilobases length. The genome encodes a polyprotein that split by host and viral protease to produce structural and non-structural proteins. [62] The HCV nucleocapsid is complex of viral RNA and viral core protein. The core protein form inner shell of nucleocapsid, which is essentials for viral particleassembly and release. The HCV envelope protein E1 and E2 are glycoprotein that form heterodimer and are in control of viral bond and entry into the host cells. [63] The envelope proteinis target for neutralizing antibody and are highly variable, allow the virus to dodge host immunesystem. The HCV lipid envelope which is derived from the host cell membrane and contain viral protein envelope. The lipid envelope are essentials for viral particle assembly and releaseand is also target for host immune response. [64]



Replication of Hepatitis C virus

The replication of HCV is very complex process that involved multiple steps and contact betweenviral and host factors. The entry of HCV in host cell happens through receptor-mediated endocytosis. [66] The viral envelope proteins E1 & E2 bind to the specific receptor on the cell surface, such as CD81. After entry of RNA it is translated into a polyprotein by host cell ribosomes. The polyprotein is cleaved by viral and host protease into individual viral proteins. Then replication of viral genome occurs, the RNA serve as template for replication. [67] The RNA-dependent RNA polymerase (RdRp) encoded by virus catalyzed the synthesis of new RNA strand using genome as template. The new synthesis of viral RNA and protein assembly into viral particles in the endoplasmic reticulum (ER) membrane. The core protein of HCV plays important role in assembly process. Then the assembled viral particles are release in the host cell through secretory pathway, and virion can infect new cells and start new replication cycle again. [68]

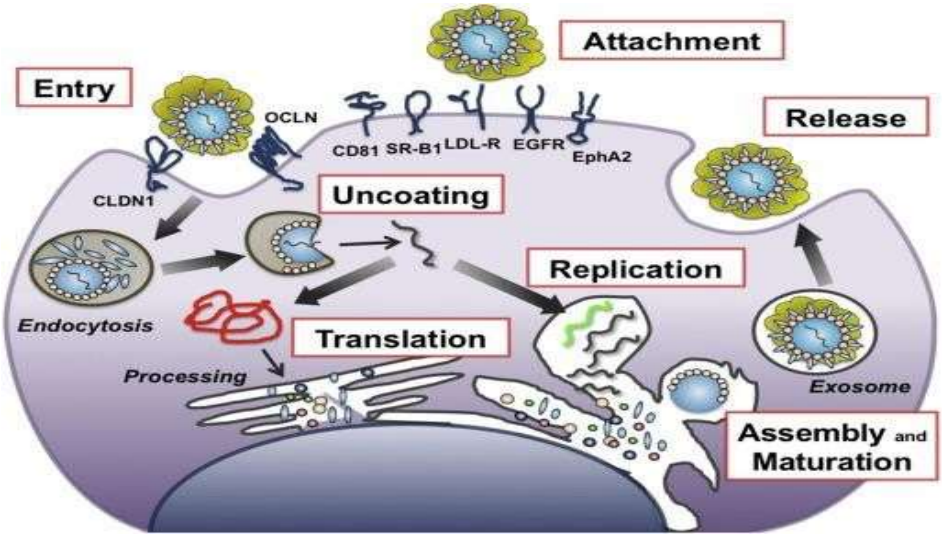


Figure 1.10: Entry, attachment, release, uncoating replication, translation and assembly and maturation of HCV. [69]

Transmission of HCV

HCV transmitted through infected blood, the sharing of contaminated needles and injection equipment among the drug user most common mode of HCV transmission. [70] HCV can transmit through blood transfusion and organ transplant, although this is rare in many countries due to improved screening of blood and organs. Occupation exposure are very common pathway to spread of HCV among people, Health care workers and first responder may risk of HCV transmission through accidental needlestick or exposure to the infected blood. [71] Vertical transmission is very common pathway for the spread of HCV, it can be transmitted from infected mother to their child during childbirth, although occur in very rare cases. HCV can alsotransmit through sexually, risk is very low as compare to mode of transmission. Tattooing and body piercing are also cause of HCV transmission. [72]

Signs and Symptoms of HCV

Hepatitis C can produce a wide range of symptoms, from mild to severe. However, many peoplewith HCV may not experience any symptoms for years, even decades after becoming infected.Here are some common signs and symptoms of HCV: Fatigue: Feeling tired or lacking energy isa common symptom of HCV infection. Jaundice: The skin and eye color become yellow due tothe extra production of bilirubin in the blood is a possible symptom of HCV infection. [73] Abdominal pain: Some people with HCV may experience abdominal pain or discomfort, especially in the area of the liver. Joint pain: HCV infection can cause joint pain or muscle aches. Itchy skin: Some people with HCV may experience itching or a rash on their skin. Loss of appetite: HCV infection can cause a loss of appetite or nausea.[74] Dark urine: HCV infection can cause dark urine due to the buildup of bilirubin in the blood. Pale stools: HCV infection can cause pale or clay-colored stools. It is important to note that many of these symptoms are not specific to HCV infection and can be caused by other conditions as well. [75]

OBJECTIVES

The purpose of study was to find out the prevalence of HCV, HBV and HIV in β-thalassemia major patients who were receiving regular blood transfusions.

MATERIALS AND METHODS

The study was a descriptive study conducted at the Pakistan Institute of Medical Sciences, Islamabad, from December 2023 to May 2024. It involved 127 β-thalassemia major patients aged 1-32 years who were referred to a tertiary care hospital. Inclusion criteria encompassed patients of all ages confirmed

to have β-thalassemia major, while exclusion criteria ruled out non-thalassemia patients, patients with β-thalassemia minor, β-thalassemia intermedia, α-thalassemia, and other anemic conditions. Blood samples (approximately 3 ml) were collected before transfusion and placed in serum separating vials. These samples were then analyzed for various viral markers using commercially available ICT kits, all processed in the same laboratory by the same individual using consistent reagent quantities. Statistical analysis was performed using SPSS Version 25. The materials and reagents used included gloves, HCV, HBV, and HIV buffers, a clock, a centrifuge, HCV, HBV, and HIV kits, and yellow top vials.

HCV, HBV and HIV Detection:
For the detection of HCV, HIV and HBV we used the Kit method.

HCV Detection:
For HCV detection, a drop of the plasma or serum sample (around 25 µl) is placed into the sample well, followed by the addition of 2 drops of buffer (around 80 µl). After 10 minutes, results are read based on the appearance of colored lines. The principle involves specific antibodies in the kit binding to the HCV antigen if present in the patient's blood. A secondary antibody with a detectable marker binds to the HCV antigen-antibody complex, creating a visible signal. The procedure requires adding the sample to the test well, allowing it to react with HCV-specific reagents, and observing for indicators like color changes or line formation. Interpretation involves checking for colored lines, with specific line patterns indicating a positive result, as per the kit's guidelines.

Figure 4.1: HCV kit

HBV Detection:
For HBV detection, a kit method is used to identify the presence of Hepatitis B surface antigen (HBsAg) in the patient's blood. The kit contains antibodies specific to HBsAg, which bind to the antigen if it is present in the sample. A secondary antibody, labeled with a detectable marker like an enzyme or fluorescent dye, binds to the HBsAg-antibody complex, creating a visible signal. The procedure involves adding the processed serum or plasma sample to the test well, allowing it to react with the reagents, and observing for indicators such as color changes or line formation. Interpretation is based on the presence or absence of specific markers, with their presence indicating a positive result for HBV infection.

Figure 4.2: HBV kit with positive result.

HIV DETECTION

HIV detection using kit methods is essential for diagnosing and monitoring HIV infection, employing techniques such as immunoassays or molecular methods to detect viral antigens or antibodies. These kits are used for screening at-risk individuals, routine testing, and monitoring disease progression and treatment response in HIV-positive individuals, offering rapid, convenient, and highly sensitive results. The principle involves detecting specific HIV markers in biological samples. The procedure includes transferring the blood sample to the test device, allowing the test to develop, and waiting for the recommended time. Results are indicated by lines, colors, or symbols, with a negative result suggesting no HIV antibodies or antigens were detected and a positive result indicating their presence.

Figure 4.3: Showing HIV positive Result

RESULTS

Out of 127 β Thalassemia patients 78 (61.4%) were male and 49 (38.6%) were female as shown in figure 5.1. The most affected blood group among enrolled patients was found B, which was detected in 46 patients (36.2%) whereas 39 (30.7%) patients had O blood group, 32(25.2%) had Aand 10 (7.9%) patients had AB blood group, as shown in figure 5.2.

In the present study all 127 patients were screened for HCV, HBV and HIV. Among 127 patients,the HCV positive patients were 61 (48%) and 66(52%) were negative, while 126 (99.2%) patientswere negative and 1 (0.8%) patient was positive for HBV. None of the patient was found to be positive for HIV as shown in Table 5.1.

In the present study enrolled β Thalassemia patient's age was 1 to 32 years with mean age 11.3±5.8as shown table 5.2. The patients were distributed into 4 age groups, with first group range 0 to 10years containing 69 (54.3%) patients in this group, 25 (36.23%) patients were HCV positive and 44

(63.77%) were negative and all 69 patients belonged to 1st group were HBV negative. Second group cover 11 to 20 years consisting 49 (38.6%) patients in this group 32 (65.31%) patients wereHCV positive and 17 (34.69%) patients were HCV negative and 49 (100%) patients were HBV negative, 3rd group comprise 21 to 30 years consisting 8 (6.3%) patients in this group 3 (37.5%) patients were HCV positive and 5 (62.5%) were HCV negative, and 1(12.5%) patient was HBV positive and 7 (87.5) patients were HBV negative. Last age group ranged between 31 to 40 years consisting 1 (0.8%) patient this single patient was HCV positive and HBV negative. There was significant difference of HCV and HBV infection among the age groups of the patients, ($p<0.05$).Higher age patients were more affected with HCV and HBV as shown in table 5.3 and 5.4 respectively.

There was no significant difference of HBV and HCV infection between male and female, ($p>0.05$)as shown in table 5.4. Similarly there was no significant difference of HCV and HBV infection among blood groups, ($p>0.05$) as shown in table 5.5 and 5.6 respectively.

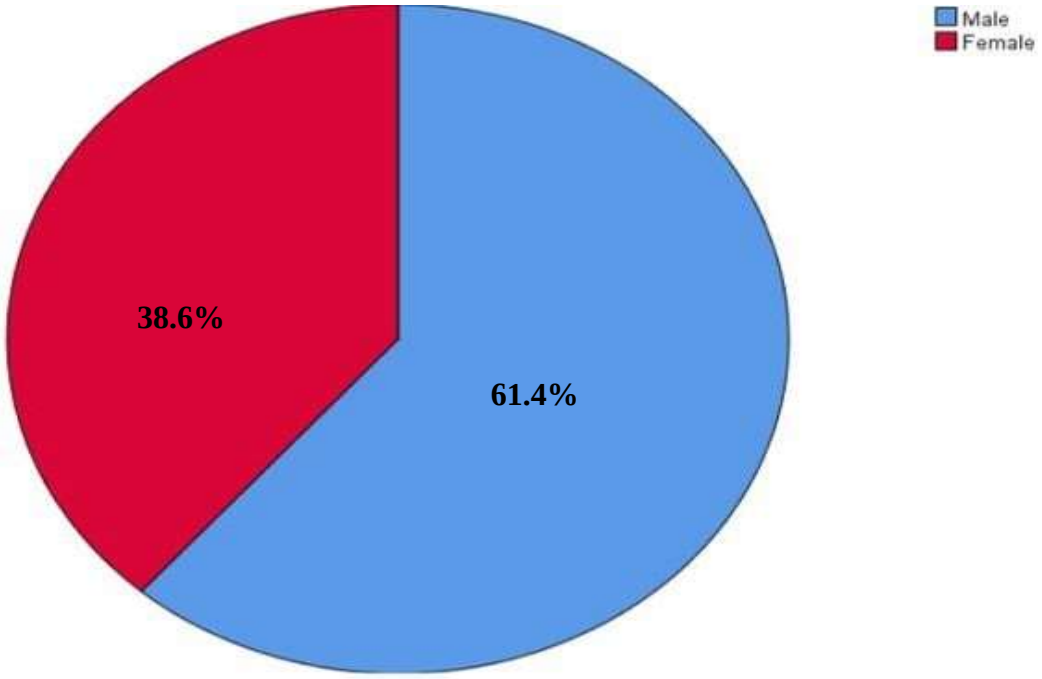


Figure 5.1: Frequency Distribution of Gender in Patients

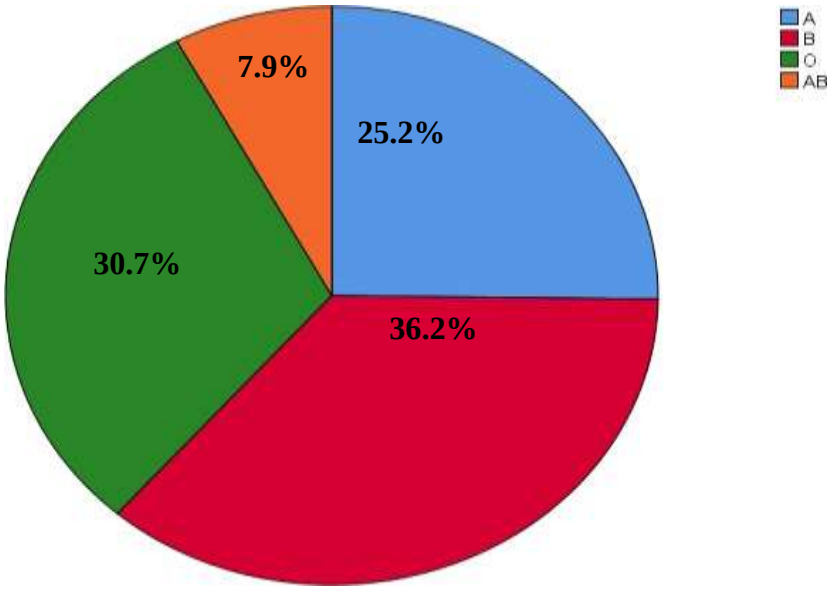


Figure5.2: Frequency Distribution of Blood Group among Patients

Table no 5.1: Comparison of infection prevalence among patients

Infection Status	HCV (%)	HBV (%)	HIV (%)
Positive	61 (48)	1 (0.8)	0 (0)
Negative	66 (52)	126 (99.2)	127 (100)

Table no 5.2: Frequency distribution of patients in different age groups

Age Groups (Years)	Total	Percentage (%)	Mean age ± standard deviation
0-10	69	54.3	11.3±5.8
11-20	49	38.6	
21-30	8	6.3	
31-40	1	0.8	

Table no 5.3: Comparison of HCV prevalence in age groups

Age Groups(Years)	Positive (%)	Negative (%)	Total	p-value
0-10	25 (36.3)	44 (63.7)	69	0.01
11-20	32 (65.3)	17 (34.7)	49	
21-30	3 (37.5)	5 (62.5)	8	
31-40	1 (100)	0 (0)	1	

Table no 5.4: Comparison of HBV prevalence in age groups

Age Groups(Years)	Positive	Negative	Total	p-value
0-10	0	69	69	0.002
11-20	0	49	49	
21-30	1	7	8	
31-40	0	1	1	

Table no 5.5: Comparison of HCV & HBV prevalence in gender

	Gender	Positive (%)	p-Value
HCV	Male	35 (57.3)	
	Female	26 (42.7)	0.369
HBV	Male	1 (100)	
	Female	0 (0)	0.426

Table no 5.6: Comparison of HCV prevalence in blood groups

Blood groups	Positive	Negative	Total	p-value
A	12	20	32	0.471
B	24	22	46	
O	21	18	39	
AB	4	6	10	

Table no 5.7: Comparison of HBV prevalence in blood groups

Blood groups	Positive	Negative	Total	p-value
A	1	31	32	0.393
B	0	46	46	
O	0	39	39	

AB	0	10	10

Discussion:

Blood transfusion is inevitable for survival of thalassemia patients as it improves their life span. Blood transfusion increase the overall survival of β-thalassemia patients. Transfusion transmittedinfections have been a major threat to the health of such people as blood transfusion is most common way for the transmission of disease-causing agents like HBV, HCV and HIV. The presentstudy aim was to evaluate the prevalence of HBV, HCV and HIV in β thalassemia patients.[100]

According to present study it was found that the prevalence of β thalassemia is higher in males (61.4%) as compared to females (38.6%). These results are similar to the previous study conductedby Bhavsar et al. males were 65% and females were 35%. this study showed that the prevalence of β-thalassemia was high in male as compare to females. Somehow these results were also similarwith other previous study conducted by Mubashira Murtaza et al. males were in majority 55.2% and females were 44.8%.[101]

The present study showed that the enrolled β-thalassemia patient's age was 1 to 32 years with meanage 11.38±8.26. The patients were divided into four groups, with first group (0 to 10 years) comprising the highest frequency of patients, and fourth group containing least no of patients (0.8%). These results were similar to the previous study conducted by Hassan Raji Jallab et al. agewas ranging from 2 to 39 years, with mean age 13.38 ± 8.26 years. Regarding age distribution in previous study 83.5% patients were less than 20 years which is somehow consistent with present study where 72.4 % patients were in less than 20 years categories. It could be due to high fatality rate of thalassemic patients comparing with general population.[102] Somehow these results were also similar with other previous study conducted by Saeed Nazir Puno et al. In this study age of 100% patients were included less than 20 years. The mean age of these patients was found to be 6.13± 2.37. Regarding age distribution in previous study 42.1% patients were less than 20 years which is somehow consistent with present study where 72.4 % patients were in less than 20 yearscategories.[103] The risk of virus was present with the passage of time the risk of HCV, HBV and HIV were increase due to multiple transfusion of blood, but the thalassemia patient's survival rate was very low due to multiple transfusion effects the other organs of body.

The current study showed that the blood group B was most common in β-thalassemia patients, (36.2%) while AB was least common (7.9%), with 30.7% had O and 25.2% patients had A blood group. These results were consistent with previous study conducted by Muhammad Hanif et al., the most affected blood group was B which was detected in 101 patients (45.1%), while (28.6%) had O blood group, while (19%) had A, and (7.14%) had AB blood group. Blood group B could be most common in β-thalassemia patients, due to the fact that in the individual with blood groupB, the expression of H antigen, a precursor to A and B antigen, is higher as compare to other bloodgroups.[104] The increase expression of H antigen on red blood cells may result in more available site for abnormal beta-globulin chain to bind, leading to increase level of abnormal hemoglobin and create the symptoms of β-thalassemia.

Among 127 patients 126 (99.2) HBV were negative and 1 (0.8%) patient was HBV positive, whileHCV positive patients were 61 (48%) and 66 % (52%) were negative. Positive patients for HIV were not found in present study. These results were similar to the previous study conducted by Sumaira Khalil et al. it was ranging in 35% patients were positive HCV and 5% patients were positive for HBV. The difference in percentage of previous and current study due to sample size of previous study was less as compare to current study. The sample size of previous study was 80patients. The current study percentage of positive HCV results was highest rate as compare to the HBV and HIV.[105] So, thalassemia patients were highly infected with the HCV and HBV infected person moderate and HIV infected patients were very low in general population of Lahore.

Somehow these results also similar with other previous study conducted by Maryam Jafroodi et al. among 1113 thalassemia cases 152 were found HCV positive, this study showed that prevalenceof HCV showed that 12% in age group less than 20 years and 68% in age group of 20-30 years. The

present study shows highest rate of HCV because lack of awareness about Hepatitis C virus among the general population.[106] Many people may not be aware of mode of transmission, prevention method and importance of early diagnosis and treatment. The lack of awareness contributes the spread of virus. Another most common way of HCV highest rate was blood transfusion. Blood transfusion prior to the implementation of stricter blood screening measure, therisk of HCV transmission through blood transfusions were relatively high in Pakistan. Although situation have improved with the introduction of more stringent screening protocols, past case has contributed to the prevalence of HCV. Inadequate health care infrastructure and no vaccine available for the HCV were also affect the highest rate of HCV present in the population.

The current study showed that there were according to p value no significant difference of HCV and HBV infection between male and female. These results were similar to the previous study conducted by Chandani C et al. there were no statical difference among male and female patients.HCV infection was found to be highly prevalent amongst transfusion associated infection. [107]

Conclusion.

This study on 127 β-thalassemia patients provided valuable insights on HCV, HBV, and HIV prevalence. Half of the patients were HCV-positive, while HBV and HIV cases were negligible. Demographically, more males were enrolled, with blood group B being the most common. Higher infection rates were observed in older age groups. No significant gender or blood category differences were found.

Recommendation

Large population and multiple areas are recommended for sample size of β-halassemia along with family back ground, further tests like, Malaria parasite and syphilis are also added to evaluate the appropriate results for future studies.

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