CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Clinical and Demographic Variables of Beta Thalassemia Patients from Islamabad and Rawalpindi

by

Sufyan Sohail Khan

A thesis submitted in partial fulfillment for the degree of Master of Science

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This thesis is devoted to my Parents who are the best gift for me from Almighty Allah; there is nothing in this world which can become a substitution of them. I also dedicate it to my wife for her support.

CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY
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CERTIFICATE OF APPROVAL

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Abstract

Thalassemia was seen in the territory of the Mediterranean Sea. Inhabitants of Mediterranean, Central Eastern, African, and Southeast Asian ancestry are at an extortionate jeopardy of exchanging the genes in lieu of thalassemia. It was initially cognized clinically by Dr. Thomas Cooley in 1925. Thalassemia is the name of a collection of hereditary blood malady portrayed by anemia. Hemoglobin, the oxygen-conveying segment of the red platelets comprises of four discrete protein chains. The subsequent anemia is normally extreme with a few medical issues like augmented spleen, bone disfigurements, weariness and requires systematic enduring transfusion, remedy and therapeutic management. Thalassemias can't be averted on the grounds that they're inborn. Nevertheless, these haemorrhage affliction can be perceived preliminary to parturition owing to pre-birth screening. Thalassemia is a recurrent innate ailment on the planet. The primary urban settlements that rose as Indus Valley Civilization can be followed back in history to past 2500 B.C. The total population of Pakistan in 2017 inflated upto 207.8 million with an annual growth rate of 2.40%. Pakistan encompasses four predominant ethnic classifications namely, Punjabi, Pathans, Sindhi, and Balochi. In Pakistan, nationwide pervasiveness of consanguineous marriages varies from 31.1 to 62%. Thalassemia was imparted from the Indian subcontinent as premature as 1938. The inaugural delineation of thalassemia in Pakistan was given by Raheemtoola in 1960. The ubiquity of thalassemia in Pakistan lingered under documented attributable to the devilishly scanty diagnostic amenities. The primary pre-birth perusal of thalassemia was consummated in May 1994. The underlying in-depth portrayal of molecular pathology of β-thalassemia in the consequential ethnic classes in Pakistan was promulgated in 1996. In 2003 more than three dozen NGOs partook in the administration of thalassemic procured conjointly under the aegis of Thalassemia Federation of Pakistan (TFP). β-thalassemia is the most rife hereditary illness. Near to 1.5-3% of the inhabitants is guesstimated to be carriers for β-thalassemia with 50 60,000 newly discovered thalassemic neonate being conceived every year. The assessed pervasiveness is 3 8% in Pakistan. Roughly

5000-6000 offsprings are diagnosed every year to have beta thalassemia in Pakistan. The prime intent of this dissertation is to inaugurate an efficacious prophylactic program with a goal to make alertness of thalassemia among the general masses of Pakistan particularly Islamabad and Rawalpindi. All the thalassemia patients included in this research work are from the twin cities of Islamabad and Rawalpindi. A total of 100 thalassemic patients who went to and were enlisted at the Pakistan Thalassemia Centre and Jamila Sultana Foundation in Islamabad and Rawalpindi during May 2018 were included. Thalassemic patients were interviewed with regard to their clinical and demographics. Inferential statistics was used for statistical analysis. The data was recorded and tabulated in MS Excel spreadsheet. Chi square test was computed by Graph Pad Prism version 5 for determining the significance of distributions of the data at $p < .05$. From clinical perspective, bulks of patients in this research were thalassemia major suffers, diagnosed by Hb electrophoresis within 6 months. Clinical characteristics were found statistically non-significant ($p < .05$). Manifestations of paleness, pallor, episodes of fever, amplification of spleen, and hepatosplenomegaly linked with jaundice are seen in β-thalassemia major and intermedia. From demographics aspect, β-thalassemia youngsters conceived to consanguineous guardians was higher than non-consanguineous guardians. It can be inferred that the demographic features are statistically non-significant ($p < .05$) with an exception of origin of thalassemia patients which is considered to be very statistically significant ($p < .05$). . It is vital to take into heed with respect to this disease as it could manifest pernicious, one. Moreover, the severity of this disorder can be diminished by means of analyzing and intriguing bona fide remedy.

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List of Abbreviations

Acronym	**W**hat (it) **S**tands **F**or
$\boldsymbol{\beta}$	Beta
$\boldsymbol{\alpha}$	Alpha
$\boldsymbol{\gamma}$	Gamma
$\boldsymbol{\delta}$	Delta
Hb	Hemoglobin
B.C	Before Christ
A.D	Anno Domini
KPK	Khyber Pakhtunkhwa
Ref	Reference
NGO	Non-Governmental Organization
RBC	Red Blood Cell
QTL	Quantitative Trait Loci
IVS	Intervening Sequences
Kb	Kilobase
HPLC	High Performance Liquid Chromatography
g/dl	Grams per Decilitre
TF	Transcription Factor
PCR	Polymerase Chain Reaction
HIV	Human Immunodeficiency Virus
n.d	No Date
TV	Television
QAU	Quaid-i-Azam University

Chapter 1

Introduction

1.1 Introduction

Thalassemia is a term that alludes to a collection of hereditary blood malady portrayed by anemia because of enriched red platelet obliteration. Hemoglobin, the oxygen-conveying segment of the red platelets comprises of tetrad discrete protein chains, 2 α globin and 2 β globin. Tetrad genes are entailed to produce ample alpha globin protein chains while duplet genes (one from both progenitors) are required to form adequate β globin protein chains. The two prominent categories of thalassemia α and β, are refer to in the wake of flaw in these protein chains.

On the off chance that the bod does not yield ample quantity of these twain proteins, the red platelets end up noticeably deficient and can't convey adequate oxygen. The subsequent anemia is normally extreme with a few medical issues like augmented spleen, bone disfigurements, weariness and requires systematic enduring transfusion, remedy and therapeutic management.

Thalassemia is a hereditary autosomal recessive blood malady. In thalassemia, the hereditary deformity brings about decreased frequency of fusion of one of the globin chains that structure haemoglobin (Gene Tech: Thalassemia Patient Information Material, n.d). Inadequate amalgam of haemoglobin materializes in thalassemia, an assembly of inbred haemolytic anemias. The RBCs are miniature, subtle and ephemeral (Gerard J. Tortora & Bryan H. Derrickson, 2005).

Thalassemia is a quantitative perplexity of unduly trace of globins intermixed, whereas sickle-cell paleness (a hemoglobinopathy) is a qualitative conundrum of uniting an erroneously functional globin. Thalassemias primarily out-turn in exiguous output of typical globin proteins, frequently by way of alteration in regulatory genes.

Hemoglobinopathies entail configurationally aberration in the globin proteins themselves. The dyad forms might partly cover, nonetheless, as a few forms which genesis anomaly in globin proteins (hemoglobinopathy) furthermore modifies their fabrication (thalassemia).Hence, several thalassemias are hemoglobinopathies, yet the majority are patently not. Either or both of these forms may well genesis anemia (K Park, 2005).

β -thalassemia is the most rife hereditary illness. The disease is universal pandemic. Near to 1.5-3% of the inhabitants is guesstimated to be carriers for β-thalassemia with 50 60,000 newly discovered thalassemic neonate being conceived every year (Eleftheriou A, 2003; R. Colah *et al.,* 2010). β-thalassemia is chiefly ubiquitous in masses of the Mediterranean vicinity howbeit forbye observed in Southern Europe, Africa, Indian Subcontinent, Southeast Asia, and the Middle East (V. Viprakasit *et al.,* 2009; Navaneet *et al.,* 2013). Southeast Asia represents around half of the world's carriers (Angastinotis M. Epidemiology In: Galanello R *et al.,* 2005, Vol I: 10-13) with the earth's topmost accumulation of carriers (30% of the community) being in the Maldives.

These days, β-thalassemia is perceived in communities living in Africa documented to be betwixt 3-7% within the lion shares of North Africa (Weatherall DJ *et al.,* 2001), the Americas also, Europe mutually represent 10-13% of the world carriers (Angastinotis M. Epidemiology In: Galanello R *et al.,* 2005, Vol I:10-13), and in Tharu individuals in the Terai vicinity of Nepal and India (Modiano, G *et al.,* 1991). It is accepted to represent exceedingly bottommost malaria wog and decease, (Terrenato L *et al.,* 1988) representing the memorable potential of Tharus to live on in sectors with profuse malaria sickness pervasion, where others manifest impuissant. Thalassemias are uniquely cognate with mankind of Mediterranean outset, Arabs (particularly Palestinians and human race of Palestinian lineage),

and Asians (E. Goljan, Rapid Review Series). The Maldives has the superlative frequency of thalassemia globally with a carrier frequency of 18% of the natives. The assessed pervasiveness is 16% in humankind from Cyprus 1%, (E. Goljan, rapid Review Series) in Kingdom of Thailand, and 3 8% in populaces from People's Republic of Bangladesh, People's Republic of China, Republic of India, Federation of Malaysia and Islamic Republic of Pakistan. Roughly 5000-6000 offspring's are diagnosed every year to have β thalassemia in Pakistan (Ansari SH and Shamsi TS, 2010-2011). Thalassemias furthermore ensue in scion of folks from Mediterranean realm (e.g. Greece, Italy, Spain, and others), in Latin America.

In Europe, the uppermost concentration of the affliction are discovered in Greece, coastal territory in Turkey (especially the Aegean Region, for example, Izmir, Balikesir, Aydin, Mugla, and Mediterranean locality, for example, Antalya, Adana, Mersin), in a section of Italy, notably southern Italy and the lower Po valley. The prime Mediterranean archipelago (barring the Balearics) for example, Sicily, Sardinia, Malta, Corsica, Cyprus, and Crete are distinctly affected specifically; depite that, it is scarcely endemic at the western end of the Mediterranean and seems, by all accounts, to be hardly any in France exclusive of those of Italian or Spanish origin.

Pandemic, sick person with hemoglobin E-beta-thalassaemia (Hb E/β-thalassaemia) constitute about half of those influenced with extreme β-thalassaemia (Higgs DR, 1993; Weatherall DJ *et al.,* 1996; Chui DH *et al.,* 1998; Nanacy *et al.,* 1999; Nancy *et al.,* 2011; Galanello R *et al.,* 2010; P Sandhya rani P *et al.,* 2013). The most elevated incidences are seen in India, Bangladesh as well as all through Southeastern Asia, particularly in Kingdom of Thailand, Lao People's Democratic Republic and Kingdom of Cambodia, where typically for individuals to amass alleles for both hemoglobin E (Hb E) and β-thalassaemia. All through these locales, Hb E/β-thalassaemia has turned into an inexorably serious general medical issue. In Thailand, around 3,000 influenced youngsters are conceived every year and appraisals of around 100,000 living patients have been given (Nancy F Olivieri *et al.,* 2011). The bona fide rifeness has guesstimated 5-7% of the populace globally

conveys clinically notable hemoglobin mutant. Figure 1.1 shows the geographical distribution of thalassemia.

Hence, it is noteworthy to take into thought about this affliction as it might demonstrate lethal one. Furthermore consequently the potency of this infirmity can be brought down by diagnosing and taking appropriate medicines.

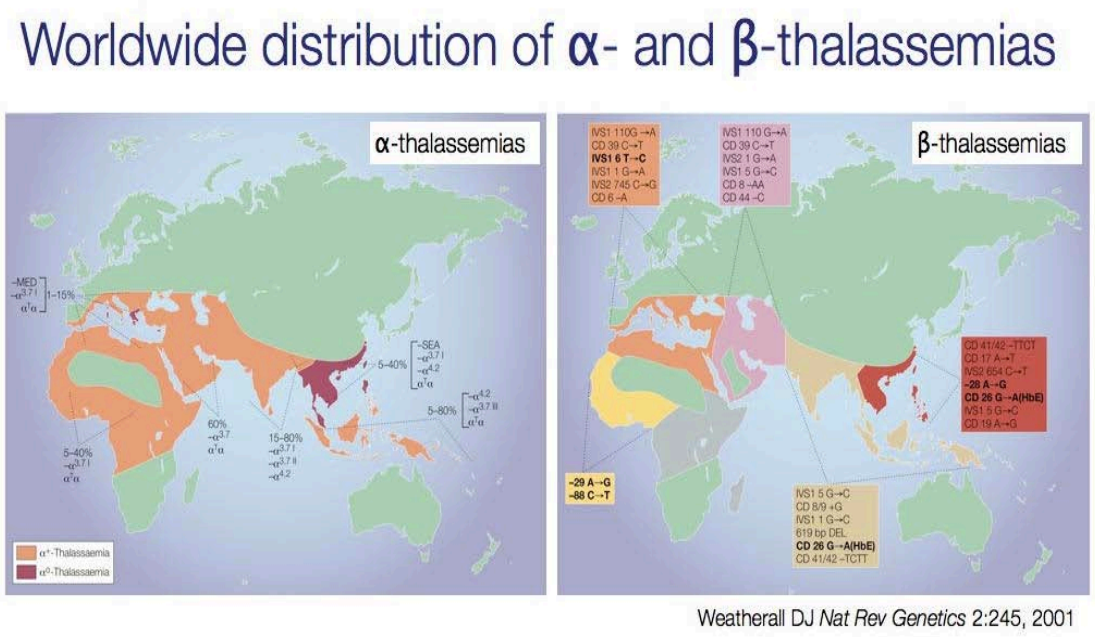

FIGURE 1.1: Epidemiology of Thalassemia Worldwide (Adopted from Weatherall DJ, 2001)

1.2 Aim of Study

Thalassemia is a perilous malady which is propagating globally. Studies on the clinical and demographic profile have been determined in several populations such as India, Iran etc. In Pakistan, most of the research has been done on consanguineous marriages in different regions such as Sargodha, Rahim Yar Khan etc. and its association on demographic variables and outcomes but no studies have been done on the clinical and demographic variables of thalassemia patients from Islamabad and Rawalpindi.

Now there is a need to determine the clinical and demographics variables of thalassemia patients from Islamabad and Rawalpindi in order to inaugurate an efficacious prophylactic program with an objective to make consciousness of thalassemia among the general masses of Pakistan particularly Islamabad and Rawalpindi.

1.3 Objectives

- To determine the clinical and demographics variables of thalassemia patients from the twin cities of Islamabad and Rawalpindi.
- To inaugurate an efficacious prophylactic program.
- To make consciousness of thalassemia among the general masses of Pakistan particularly Islamabad and Rawalpindi.

Chapter 2

Literature Review

2.1 History of Thalassemia

Thalassemia was seen within the territory of the Mediterranean Sea (Cooley T B *et al.,* 1946). Inhabitants of Mediterranean, Central and Eastern Europe, Africa, and Southeastern Asian ancestry are at an extortionate jeopardy of exchanging the genes in lieu of Mediterranean anemia (Weatherall D J, 1997). It had been ab initio cognized clinically by Dr. Thomas Cooley in 1925, which depicted an ailment of iron dearth with microcytic erythrocytes. At that juncture it had been cleped Cooley's anaemia. Thereupon Wipple and Bradford gave a brand new name to this affliction as Thalassemia (Cooley T B *et al.,* 1946).

2.2 Pakistan and Thalassemia

In this section the history of Pakistan, population and ethnic groups, caste and consanguineous marriages will be discussed.

2.2.1 History of Pakistan

The inhabitants of Pakistan are originated from all those individuals who, at several occasions and for several reasons, have travelled to the land that presently well

known as Islamic Republic of Pakistan. The initial urban colony that emanated as Indus Valley Civilization is often trailed back in history to past 2500 B.C (Stanley Wolpert, 1977). Despite that, it had been with the arrival of the Aryans from Eurasia between 1500- 500 B.C. that a new era in the history of Pakistan actually commences. Thereupon, the area has witnessed consecutive seizures by Persians in 530 B.C., Greeks in 330 B.C., Bactrians, Scythians, and Parthians between 185 B.C. and 75 B.C., Kushans in 1st century A.D., Huns in 500 A.D., Arabs in 700 A.D., Turks in 977 A.D., Mongols in 1221 A.D., and Mughals from Central Asia in 1504 A.D. (Rapson E.J, 1955).

Regardless of the continuous attacks and ancestral relocations, principally from the Central Asia, the tremendous lion's share of the populace, nonetheless, comes from a hereditary pool that has its underlying foundations in South Asia. The prior perception of the Pakistanis probable to be picked up by guests to a major city in Pakistan is of its stunning assortment (Halliday, 1990).

2.2.2 Population and Ethnic Groups

The total population of Pakistan in 2017 inflated up to 207.8 million with an annual growth rate of 2.40% (Pakistan Census, 2017). Pakistan encompasses four predominant ethnic classifications namely, Punjabi, Pathans, Sindhi and Balochi, which are widely disseminated into different provinces of Punjab, KPK, Sindh, Balochistan, and Federal Islamabad Capital Territory correspondingly (Suhaib Ahmed, 1998). Based on the Pakistan Census 2017 the extent of the inhabitants in distinct territories was as per the following: Islamabad Capital Territory 2.01 million (4.91%), Punjab 110 million (2.13%), KPK 30.5 million (2.89%), Balochistan 12.3 million (3.37%), and Sindh 47.9 million (2.41%). Furthermore, a fifth classification of individuals called Mohajirs, who emigrated from different parts of India at the epoch of formation of Pakistan in 1947, can likewise be perceived. Figure 2.1 represents the demographics of Pakistan.

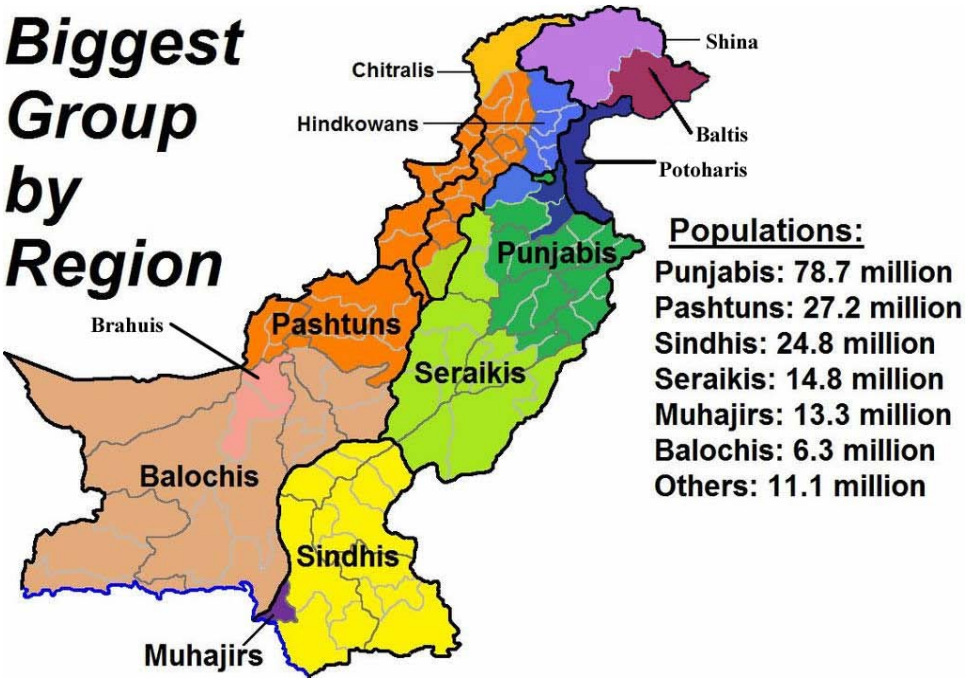

FIGURE 2.1: Demographics of Pakistan (Ref: Demographics of Pakistan, n.d)

2.2.3 Caste, Biradri and Tribe

The remains of the orthodox caste practice, that took its outset in a similar manner as Hinduism in the Aryan time, can as well be observed in Pakistani culture. Howbeit, societal bigotry on the underpinning of caste, yet prevalent in India, isn't blatant. The Biradri is a fraternity whose individuals are interconnected either intimately or remotely by blood or marriage with the goal that individuals consider each other as family (Pauline Kolenda, 1978). It is significantly bigger when contrasted with a family relationship net work of a person's related in the Western Society. Biradris ordinarily don't have any regional restrictions. There are various sizeable Biradris in Pakistan that range in numbers from a couple of hundred to even millions. Portions of the bigger Biradris are subdivided in to mini sub- Biradris. What might as well be called a Biradri among the Pathans and Baluchis is a clan.

2.2.4 Consanguineous Marriages

Consanguineous matrimony is a bond between first and second cousins, the prior being more typical. A cousin nuptial, the more frequently utilized expression, has been carried out over an extensive stretch of time yet it is more illustrious in developing Asian nation state in particular Pakistan (Gavin W. Jones, 2010).

In Pakistan, the circumstance hasnt altered to date and the nationwide pervasiveness varies from 31.1 to 62% (Riaz HF *et al.,* 2016). Pakistan has one of the most astounding rampancy of consanguineous conjugal bond globally and remains dormant (Gavin W. Jones, 2010; Agha N, 2016). It is a multicultural nation with heterogeneous civilizations, miscellaneous stratums and unique religious groups living in its five discrete areas. To date, every one of the investigations from various territories of Pakistan has demonstrated a high number of consanguineous wedlock though some locales of Pakistan have demonstrated a disturbing proportion of consanguineous unions, for example, the Sargodha area of Punjab and Khyber Pakhtunkhwa (Hina S *et al.,* 2015; Sthanadar AA *et al.,* 2016). Table 2.1 depicts the percentages of consanguineous marriage from various territories of Pakistan.

TABLE 2.1: Percentages of Consanguineous Marriages from various areas of Pakistan (Ref: Hina S *et al.*, 2015; Riaz HF *et al.*, 2016)

Area	Consanguineous Marriage %
Quetta	31
Lahore	46
Rawalpindi	48.10
Jhelum	44.3
Gujrat	48.50
Faisalabad	52.1
Peshawar	55
Malakand District	66
Sargodha	56.72
Rahim Yar Khan	58.46

Cousin conjugal bond are connected to numerous atypical recessive hereditary maladies; individuals from a similar family convey certain recessive genes that are typically suppressed in their genotype. At the point when interfamily matrimony occurs, the odds of these genes to be expressed in the posterity are incredibly expanded due to a homozygous condition (Modell B *et al.,* 2002). Many dire illnesses are additionally analyzed later in youth, for example, inner deformities, learning impediments and numerous single gene disorders such as neurological afflictions, thalassemia and cystic fibrosis (Modell B *et al.,* 2002). Autosomal recessive psychological diseases are extremely widespread in societies with recurrent parental consanguinity (Musante L *et al.,* 2014). Every year 700 youngsters are conceived with hereditary inabilities because of cousin wedlock (Rebecca Lefort, 2010).

2.3 Thalassemia in Pakistan

Thalassaemia was imparted from the Indian subcontinent as premature as 1938 (Weatherall DJ *et al.,* 2001). The inaugural delineation of thalassaemia in Pakistan was given by Raheemtoola in 1960 (Raheemtoola RJ, 1960). In 1968 Stern and comrade were the prefatory to disclose 4% carrier rate of β-thalassaemia amid Pathans (Stern MA *et al.,* 1968). The ubiquity of thalassaemia in Pakistan lingered under documented attributable to the devilishly scanty diagnostic amenities (Saleem M, 1974). The epoch of seventies and the eighties descried a moderately sizeable amount of scrutinies on the study of disease transmission of inbred hemoglobin infirmities in Pakistan.

In the mid eighties twain non-administrative managements (NGO) inaugurated blood transfusion facilities for the youngsters with thalassaemia with an incessantly expanding number of patients advent to these centers, it became overt that thalassaemia would pronto be twigged as a remarkable medical issue in Pakistan.

The primary pre-birth perusal of thalassaemia was consummated in May 1994 (Ahmed S *et al.,* 1994). The underlying in-depth portrayal of molecular pathology of β-thalassaemia in the consequential ethnic classes in Pakistan was promulgated

in 1996 (Ahmed S *et al.,* 1996). The molecular pathology of α-thalassaemia was blazoned in 2003 (Khan SN *et al.,* 2003).

The bone marrow graft for thalassaemia was emanated in 2000 first at Karachi, stalked by a centre at Rawalpindi (Shamsi TS *et al.,* 2008). In 2003 more than three dozen NGOs partook in the administration of thalassaemics procured conjointly under the aegis of Thalassaemia Federation of Pakistan (TFP) with a notion to espouse invariable administration entente for thalassaemics all throughout Pakistan.

2.4 Beta Thalassemia

β-thalassaemia is a monogenic disorder that prunes the formation of haemoglobin. Hb is the iron-containing protein within the blood platelet that transports O_2 to cells throughout the body (Genetics Home Reference: Beta thalassemia, 2018).

An individual with β-thalassemia, negligible degree of hemoglobin prompts a dearth of oxygen in umpteen regions of the body. Influenced people likewise undergo a paucity of red platelets (iron deficiency), which can beget pallid dermis, frailty, lethargy, and moreover alarming intricacies (Genetics Home Reference: Beta thalassemia, 2018). Folks with beta thalassemia are at an augmented menace of engendering aberrant blood clots (Rund D *et al.,* 2005; Galanello R *et al.,* 2010; Origa R, 2015; Genetics Home Reference: Beta thalassemia, 2018).

Beta thalassemia is categorized into twain kinds relying upon the solemnity of prodrome: thalassaemia major (otherwise called Cooley's anaemia) and thalassaemia intermedia. From these pair, thalassaemia major is some more agonizing (Rund D *et al.,* 2005; Galanello R *et al.,* 2010; Origa R, 2015; Genetics Home Reference: Beta thalassemia, 2018).

2.4.1 Inheritance Pattern

In this section the inheritance of beta thalassemia and hemoglobin globin genes will be discussed.

2.4.1.1 Inheritance of Hemoglobin

The genes affiliated with thalassemia regulate the causation of a protein in red cells called hemoglobin. Hemoglobin affixes oxygen in the lungs and exonerates it when the erythrocytes attain surrounding tissues like liver. The attaching and extricating of oxygen through the agency of hemoglobin is imperative for continued existence.

Every hemoglobin atom encompasses tetrad subunit proteins. Dyad of the subunit proteins are called alpha and duad are called beta. Hemoglobin appropriately fixes and unbridles oxygen wholly when twain alpha subunits are equated with two beta subunits. A doubleton of genes based on chromosome number 16 governs the creation of the alpha subunits of hemoglobin. A solo gene sited on chromosome number 11 modulates the formation of the hemoglobin beta subunit ("how do people get thalassemia", 1998). This is represented in figure 2.2.

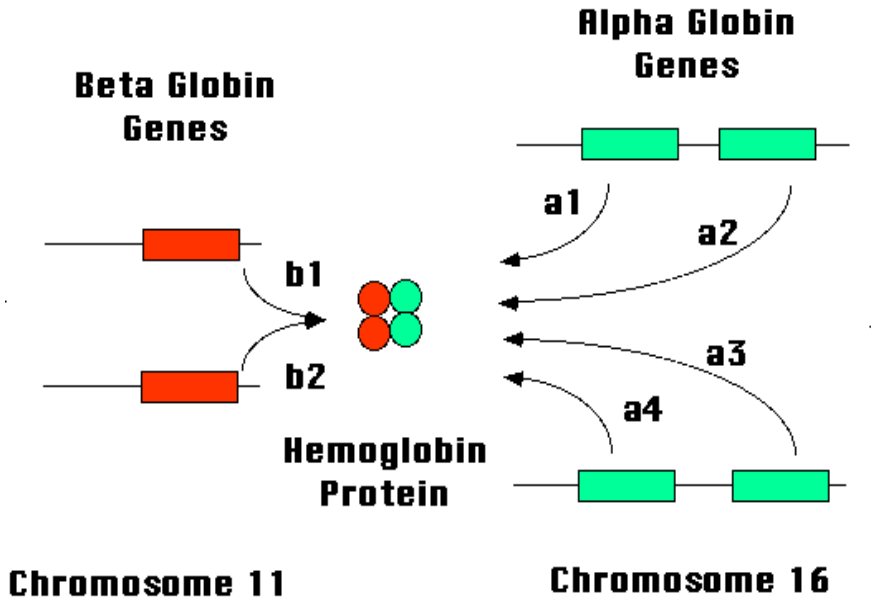

FIGURE 2.2: Beta and Alpha Globin Genes (Ref: "how do people get thalassemia", 1998)

Thalassemia betides when at least one of the genes neglects to make protein, prompting a deficit of one of the subunits In the event that one or both genes are impaired, β-thalassemia will materialize. Grievousness rest upon what numbers

of genes are permuted (Christian Nordqvist, 2018). Beta thalassemia, wherefore, is ascribed to a meagerness of beta subunits. ("how do people get thalassemia", 1998).

2.4.1.2 Inheritance of Beta Thalassemia

Thalassemia minor (or hallmark) is typically a benignant wog that engenders exclusively a humane weariness. Figure 2.3 demonstrates the plausible denouements for progeny of twosome human beings with thalassemia minor. A 25% prospect subsists that a tyke will acquire two wonted genes from the progenitors. A 25% likelihood forbye exists that a scion will receive two thalassemia genes; moreover encounter a drastic type of thalassemia major or thalassemia intermedia. A 50% odds exists that the youngster will procure a typical gene from one genitor and a thalassemia gene from the biological mother. This would generate thalassemia minor (or feature) ("how do people get thalassemia", 1998).

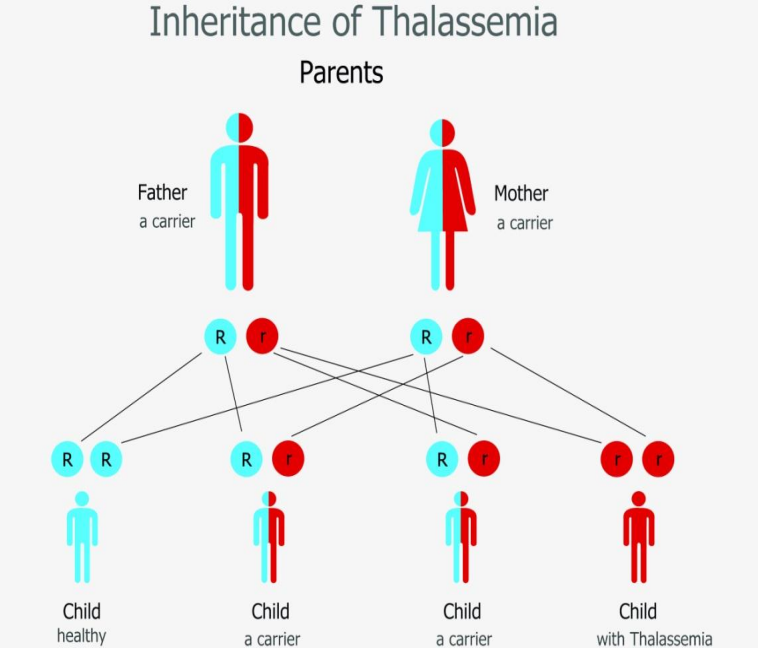

FIGURE 2.3: Inheritance of Hb genes from progenitor with thalassemia trait (Adopted from Genetrace: Beta Thalassemia Test (HBB Genotyping), n.d)

2.4.2 Causes of Beta Thalassemia

Twain globin genes are necessitated to produce beta globin chains, one from both rents. In the event that a single or twain genes are impaired, β-thalassemia will materialize. Grievousness rest upon what numbers of genes are permuted (Christian Nordqvist, 2018).

On the off chance that one of the beta globin genes be defective the quantity of beta globin in the cell is alleviated markedly. This state of affairs is known as thalassemia quirk or thalassemia minor. In the event that the two genes cease to function no beta globin protein is formed. This is called thalassemia major ("how do people get thalassemia", 1998).

Sporadically, genes manufacture a miniature number of conventional beta protein. Once in a while, a man derives two beta thalassemia genes in which the creation of beta globin protein from each is minimized, however isn't zero. The indisposition is less acute than thalassemia major. The clinical abnormality is described as, thalassemia intermedia ("how do people get thalassemia", 1998). Table 2.2 portrays the genotypes and phenotype of β-thalassemia.

TABLE 2.2: Genotypes and Phenotypes of β-Thalassemia (Ref: Charles T Quinn & Satheesh Chonat, 2017)

Common genotypes	Name	Phenotypes
β/β	Normal	None
β/β^0	Beta Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β/β^+		
β^+/β^+	Beta Thalassemia intermedia	Variable severity
β^+/β^0		Mild to moderate anemia
β^E/β^+		Possible extramedullary hematopoiesis
β^E/β^0		Iron overload
β^0/β^0	Beta Thalassemia major	Sever anemia
	(Cooley's Anemia)	Transfusion dependence
		Extramedullary hematopoiesis
		Iron overload

2.4.3 Symptoms

The manifestation of thalassemia fluctuates influenced by the sort of thalassemia (Christian Nordqvist, 2018). Prodrome won't emanate till 6 months of age in almost all weans with beta thalassemia. This is in view of the fact that newborns possess a disparate kind of hemoglobin termed fetal hemoglobin. Subsequent to 6 months quotidian hemoglobin embarks on supplanting the fetal kind; what's more manifestations may become apparent (Christian Nordqvist, 2018).

These comprehend:

- jaundice and pale skin
- drowsiness and fatigue
- chest pain
- cold hands and feet
- shortness of breath
- leg cramps
- rapid heart beat
- poor feeding
- delayed growth
- headaches
- dizziness and faintness
- greater susceptibility to infections (Christian Nordqvist, 2018)

Bone disfigurements may upshot while the body endeavors to give rise to additional bone marrow. Presuming that there is oodles ferritin, the carcass will seek to imbibe extra ferric to counterpoise. Ferritin may likewise accrue from blood transfusions. Exorbitant iron is able to incapacitate the spleen, heart, and

liver (Christian Nordqvist, 2018). Extortionate iron can similarly induce diabetes, hypo and hyper thyroidism/parathyrodism, low levels of estrogen (female hormone) or testosterone (male hormone) (Wikipedia: Thalassemia, 2017). Sufferers having hemoglobin H are prospective to develop gallstones and an augmented spleen (Christian Nordqvist, 2018). Other manifestations of thalassemia incorporate truncated fertility (Wikipedia: Thalassemia, 2017).

2.5 Types of β-Thalassemia

In this section different types of β-Thalassemia will be discussed.

2.5.1 Thalassemia Trait

Carriers of β-thalassemia are clinically symptomless. The hallmark medical specialty call attention to blood disorder (alleviated erythrocytes volume), hypochromic (abated thrombocyte hemoglobin quantity) and expanded HbA2 level (Galanello *et al.,* 2010; Sandhya *et al.,* 2013; Grow *et al.,* 2014).

2.5.2 Thalassemia Major

People with thalassemia major generally display inside the initial two long periods of existence with serious pallor, in need of standard red platelet [RBC] transfusions. Research of untreated people having thalassemia major has disclosed an innumerable manifestations like development hindrance, anemia, jaundice, poor musculature, liver & spleen swelling, leg ulcers, enlargement of masses from extramedullary haematopoiesis, what's more, skeletal changes that outcome from extension of the bone marrow. Fringe blood streak seems, nonetheless blood disorder and hypochromic, anisocytosis, poikilocytosis [speculated tear drop and stretched cells] and nucleated thrombocytes [i.e. immature RBC's]. Hb arrangement [by cellulose acetate electrophoresis or high performance liquid chromatography [HPLC]] fluctuates as indicated by the sort of β-thalassemia. In β-thalassemia, described

by the deprivation of β-globin chain amalgamation, HbA is truant, HbF is 95 98%, and HbA2 is 2 5% (Galanello *et al.,* 2010; Sandhya *et al.,* 2013; Grow *et al.,* 2014).

2.6 Thalassemia (delta-β) Intermedia

Sufferers have a bearable frailty and demonstrate an awfully heterogeneous haematological image, fluctuating in distress from β-thalassemia carrier state thereto of Cooley's anemia (Galanello *et al.,* 2010; Sandhya *et al.,* 2013; Grow *et al.,* 2014).

2.6.1 Dominant β-Thalassemia

Interestingly with the standard recessive forms of β-thalassemia, that prompts a diminished creation of normal β globin chains, some uncommon changes end in the union of greatly precarious β globin variations that encourage in blood cell precursors inflicting ineffectual erythropoiesis. These transformations are connected with a clinically recognizable thalassaemia phenotype within the heterozygote and are thusly implied as prevailing β thalassaemia (Thein, 1992). The proximity of hyper-unsteady haemoglobin should be presumed in any person with thalassaemia intermedia once the 2 guardians are haematologically normal, or in families with a case of autosomal dominant transmission of the thalassaemia intermedia phenotype. β globin gene sequencing builds up the finding (Galanello *et al.,* 2010).

2.6.2 Silent β-Thalassemia

Primarily by negligible deficit of β-globin sequence, silent kind β-thalassaemia is made. Generally in homozygous condition, a standard β-thalassaemia like condition is found or if there ought to be an event of compound heterozygous thalassaemia intermedia like sign is recognized. It is to a great degree unprecedented to find the alleles for silent β-thalassaemia, with the exception of CT and CG transformation in β-globin sequence at one hundred and one position, IVS II 844 (CG) in heterozygous carrier state nineteen (Bianco *et al.,* 1917; Panja *et al.,* 2012).

2.7 Clinical Aspect of Beta Thalassemia

The β-thalassemias comprises quartet clinical ailments of inflating acuteness: two ailments are predominantly asymptomatic, the silent carrier state and β-thalassemia trait, typically out-turn from the bequest of one variant β-globin gene, plus twain in need of medicinal administration, thalassemia intermedia and thalassemia major. The further divergent configurations the lion share's brought about by homozygosity or compound heterozygosity for a variant beta globin allele also from heterozygosity for dominant alterations (Thein *et al.,* 1990). Homozygous or compound heterozygous β-thalassemia as a rule introduces no symptomatic impediments. In other two conditions untimely outset of iron deficiency, idiosyncrasy blood permutates and raised fetal hemoglobin concentrations aren't seen in other syndrome. The prognosis can be affirmed with the aid of demonstration of the β-thalassemia attribute in the two rents. This indisposition is typified by clement anemia, pared mean cell volumes and mean cell hemoglobin concentrations and inflated concentrations of the usual lesser grown-up segment of hemoglobin (normally surpassing 3.5 percent), hemoglobin A2 (a2d2). Thalassemia major and thalassemia intermedia possess hardly any distinct molecular associate yet embody a spacious gamut of clinical furthermore, research facility anomalies (Camaschella *et al.,* 1995; Nancy F Olivieri *et al.,* 1999).

The phenotypes of homozygous otherwise hereditary heterozygous compound Mediterranean anemia comprehend Cooley's anemia and thalassaemia intermedia. People possessing Cooley's anemia in general desire medicinal cognizance in the initial two long periods of life and be in need of routine RBC transfusions to endure. Thalassemia intermedia encompass convalescent who exhibit subsequently and don't have need of habitual transfusion. Barring in the unfamiliar dorminant structures, heterozygous β-thalassemia brings about the clinically silent carrier state. Hemoglobin E/Beta-thalassemia and Hemoglobin C/β-thalassemia unveil an extraordinary extent with reference to assortment variety of phenotypes and range of seriousness (Galanello *et al.,* 2010).

2.7.1 Genetic Modifier Factors

Over 200 variations have been up 'til now put forward; the epic prevalence is point mutants in essentially imperative districts of the β-globin gene (Titus H.J. Huisman *et al.,* 1997; Giardine *et al.,* 2007). Erasures of β-globin sequence are rare. The β-globin gene alterations give rise to a mitigated or non-existent creation of β-globin chains. Modifier genes are expounded as hereditary variations that prompt dissimilitude's in ailment phenotype. In homozygous β-thalassaemia, fundamental inherited modifiers, impacting the clinical acuteness of the infirmity, consolidate hereditary transformation prepared to alleviate the globin chain disproportion, in this way achieving a lighter sort of thalassaemia. These elements are the incidence of unexpressed or gentle β-thalassaemia alleles connected with a high outstanding yield of β-globin, the coinheritance of alpha hypochromic anaemia or presumably of innate determinants capable of to succour with a constant formation of γ globin chains (HbF) in grown-up life (Galanello *et al.,* 1998). Many β-thalassaemia changes (i.e. erasure and non- erasure δ beta-thalassaemia, elimination of 5' section of the β globin gene) increment "basically" the gamma globin gene production. Further transformations extending HbF creation are those related with deletional and non-deletional intrinsic perseverance of fetal hemoglobin linked with the beta globin gene group.

Lately, the genome-wide association approach, notably analyzing quantitative trait loci (QTL) which induce raised HbF, have revealed innate components (i.e. polymorphism in BCL11A gene and in the HBS1LCMYB intragenic locale) not connected to beta globin gene pack, prepared to alter the severeness of the homozygous beta zero thalassemia. The clinical phenotype of homozygous β-thalassemia may moreover be permutated by the co-inheritance of other genetic variations mapping exterior the globin assemblage. These peripheral inherited modifiers affect mostly the intricacies of the thalassemia phenotype. Numerous inborn modifiers have been distinguished in the ongoing years. Little predictable information have been accounted for genes associated with iron metabolism (i.e. C282Y and H63D HFE gene mutant), most likely in light of the fact that their impact on iron over-load is concealed because of treatment, and for genes linked with bone metabolism

(Uda M *et al.,* 2008). Latterly, a polymorphism in glutathione-S transferase M1 gene has been connected with an intensified hazard of heart iron over-load in thalassemia major (Origa R *et al.,* 2008). In a few cases, heterozygous β-thalassemia may give rise to thalassemia intermedia phenotype in preference to asymptomatic carrier state. The lion share's of these infirms have plethora operative alpha globin genes (alpha gene triplication or quadruplication) which augment the disproportion in the proportion of alpha/non-alpha globin chain fusion (Galanello *et al.,* 1998; Sollaino MC *et al.,* 2009).

2.8 Pathophysiology of Beta Thalassemia

The thalassemias are classified by which chain of the Hb particle is influenced. In alpha thalassemia, creation of alpha globin chain is wedged, whereas in β hypochromic anemia creation of beta globin chain is influenced. Hypochromic anemia yields insufficiency of alpha or beta globin, as opposed to sickle-cell frailty that creates a specific variant variety of beta globin. Beta globin chains are encoded by a sole gene on chromosome number 11, alpha globin chains are encoded by duplet succinctly secure genes on chromosome number 16. The thalassemias are inborn affliction of Hb unification that repercussion from transformation within the share of globin chain manufactured. An abate within the quantitative relation of manufacturing unequivocal globin chain alternatively chains (α, β, γ, δ) hampers haemoglobin amalgamation as well as originate disparity with naturally induced globin chains. Since two classes of chains (alpha and non-alpha) meld together at an extent near 1:1 to supply typical Hbs, an overabundance of usually formed type is accessible and amasses within the cell as a precarious outcome, prompting the decimation of the cell. This variation is the attribute of all sorts of thalassaemia (Harsh Mohan, 2006; Yaish HM, 2017; Wikipedia: Thalassemia, 2017).

Phenotypically these duplet forms of β- thalassaemia: beta zero-no beta-globin chain merger, and beta positive with a few beta globin chain union. Clinically, Mediterranean anemia exhibits as β-thalassemia trait (beta positive or beta zero),

intermedia (beta positive/beta positive; beta positive/beta zero) or Cooley's anemia (beta zero/beta zero). β-thalassemia trait is generally symptomless moreover, is cognate with the legacy of a solitary gene blemish. Beta-thalassemia major outcomes in drastic transfusion over-reliant iron deficiency and is caused by the patrimony of two β-globin gene mutants either in an exceedingly compound heterozygous or homozygous state. β-thalassaemia intermedia is of endurable acuteness with the lion's share of influenced individuals not be in need of wonted blood transfusions (Elizabeth *et al.,* 2010).

The disparity between α and β globin chains of hemoglobin brings about thalassemia. The diminished proportion or nonappearance of beta globin chains in β - thalassemia upshot in a relative overabundance of unbind α globin chain that provoke within the RBC precursors in the bone marrow, this inhibits with development of the red cells and its annihilation in the bone marrow (ineffective erythropoesis), and it additionally brings about marrow enlargement. The resultant hypertrophy of erythroid marrow is described by misshapening of the bone of the face; it could likewise bring about osteoporosis with pathologic break of long bones (Taher A T *et al.,* 2010). The diminution deviates from an imperceptible wane to an utter truancy of formation. The ramification of undermined fabrication of globin chains in due course outcome in the dumping of fewer Hb into every RBC, eliciting hypochromasia. The Hb paucity leads to RBCs of becoming miniature, triggering the paradigm hypochromic and microcytic picture of thalassemia. Despite that, this doesn't come about in the silent carrier state, as both hemoglobin level and erythrocyte indices linger ordinary. Prodigious erasures that may incorporate the whole β gene, or to a greater extent, elongate to expunge the adjoining δ gene, have been formerly published (Harsh Mohan, 2006; Yaish HM, 2017; Wikipedia: Thalassemia, 2017).

Fringe haemolysis adding to sickliness is not as much apparent in thalassemia major in contrast to thalassaemia intermedia, plus happens once alpha globin chains initiate membrane detriment to the red cell. The subsequent pallor empowers the generation of erythropoietin with resulting serious yet ineffectual development of the bone marrow which causes the said bone distortions (frontal bossing, with

developed maxilla). Haemolysis from time to time begets gallstones however this additionally arises often in thalassaemia intermedia than major (Galanello R *et al.,* 2001). In spite of the fact that people with thalassaemia intermedia are in menace of ferritin over-burden, subordinate to inflated intestinal assimilation, hypogonadism, hypothyroidism and diabetes are not popular in them. Figure 2.4 summarizes the pathophysiology of β- thalassemia.

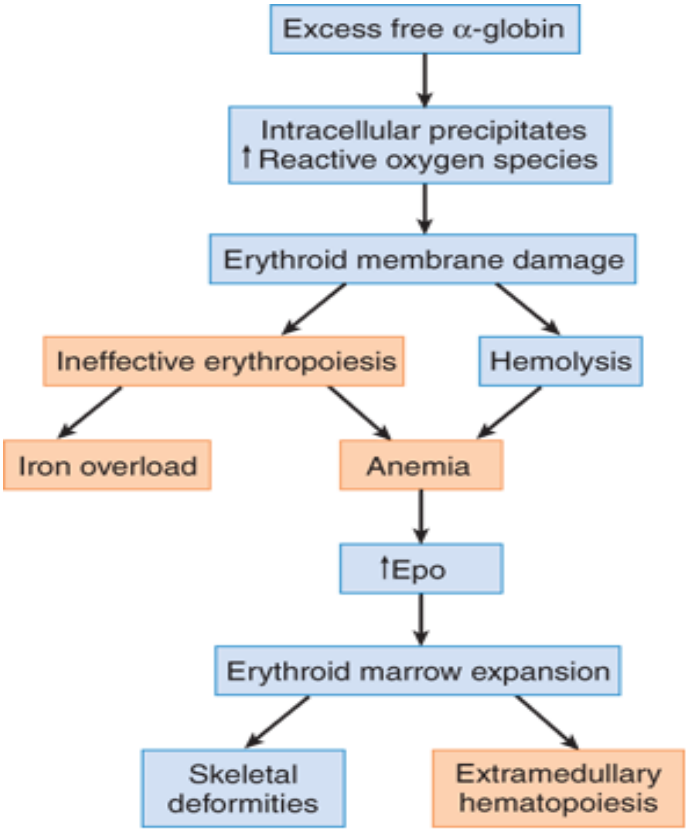

FIGURE 2.4: Pathophysiology of β-thalassemia (Adopted from H. Franklin Bunn and Jon C. Aster, n.d)

2.9 Molecular Biology

Every globin gene is made up from a sequence of nucleotide bases separated into three coding sequences, named exons, and a pair of noncoding areas, referred to as introns or interceding sequences (IVS) (Ghodekar *et al.,* 2010; Yaish HM, 2017).

2.9.1 Molecular Pathology

Hitherto, surplus 1000 inborn modifications that impact the structure or else amalgamation of alpha and beta-globin chains are recognized. Transformations that out result in beta or alpha thalassaemia is analogous in theory yet heterogeneous within their paradigms. Currently, over 200 molecular deformities typically well-known that down regulate the expression of beta globin has been represented. Such flaw outcome in several forms of beta thalassaemia (Ghodekar *et al.,* 2010; Yaish HM, 2017).

2.9.2 Genetic Changes

Every one of the genes that regulate the generation of globin chains exist in one of two groups located on twain heterogeneous chromosomes. Chromosome number 11 is that position of five utilitarian b-like globin genes organized in an interconnection conglomeration larger than 60 kilobases (kb) (Ghodekar *et al.,* 2010; Yaish HM, 2017).

An acute curb space of the d-globin gene (promoter) is acknowledged to glitch; it suppresses mRNA operations, giving rise to minute amount of Hb A2 ($\alpha 2/\delta 2$) output, consequently representing below 3% of whole Hb in grown-up RBCs (Ghodekar *et al.,* 2010; Yaish HM, 2017).

2.10 Beta - Thalassemia Affiliation with Other Hemoglobin Aberration

Interconnection between Hemoglobin E and beta-thalassaemia brings about thalassaemia phenotypes extending from Cooley's anemia thereto a mild kind of thalassaemia intermedia. Contingent upon the acuteness of manifestations trio categories can be recognized.

2.10.1 Forbearing Hemoglobin E/Beta-Thalassemia

Roughly 15% of total cases are discerned in Southeastern Asia. This gathering of sufferers sustains haemoglobin levels within the locality of 9 and 12 g/dl and more typically do not progress clinically critical problems. No medical care is needed (Galanello *et al.,* 2010).

2.10.2 Passably Agonizing Hemoglobin E/Beta-Thalassemia

The predominance part of Hemoglobin E/Beta-Thalassemia cases lies within this classification. The hemoglobin levels stay at 6-7 g/dl and the clinical indications is same as thalassaemia intermedia. Patients do not require transfusions except if the diseases hasten to promote weakness. Ferritin overload may happen (Galanello *et al.,* 2010).

2.10.3 Acute Hemoglobin E/Beta-Thalassemia

The hemoglobin level falls well below 4-5 g/dl. Patients within this category evince prodrome like thalassaemia major and are treated as thalassaemia major patients (Galanello *et al.,* 2010; Panja *et al.,* 2012).

2.10.4 Hemoglobin C/Beta-Thalassemia

Patients suffering HemoglobinC/Beta-thalassemia for the most part experiences iron deficit and splenomegaly. Hbc crystal bodies can be distinguished in blood film, microcytosis and hypochromic is discovered (Panja *et al.,* 2012). Sufferers of HbC/β-thalassaemia can live freely

without experiencing any manifestations and be analyzed amid schedule assessments. Nonetheless, whenever it begins to develop, it results in symptoms such as anemia & swelling of the spleen. Only in this case blood transfusions become necessary.

Microcytosis & hypochromic are found for each situation. The blood film indicates unmistakable hemoglobin C gems with straight parallel edges, target cells and unpredictably contractedcells with highlights of thalassaemia, for example, microcytosis (Galanello *et al.,* 2010; Panja *et al.,* 2012).

2.11 Beta-Thalassemia Affiliated with Other Trait

In uncommon occasions the β- hypochromic anaemia imperfectness doesn't occur within the β globin sequence bunch. In cases during which the β- hypochromic anaemia gene is expounded with completely different highlights, the sub-atomic injury has been discovered either within the cistron coding the transcription factor TFIIH (beta-thalassaemia characteristic connected with tricothiodystrophy) or within the X-linked transcription factor GATA-1 (X-linked blood disease with thalassemia) (Viprakasit *et al.,* 2001, Freson *et al.,* 2002; Galanello *et al.,* 2010).

2.12 Prognosis of β-Thalassemia

Physicians analyze thalassemias employing blood tests, inclusive of a Complete Blood Count (CBC) along with unique Hb assay. Thalassaemia mitigates our erythrocytes tally to facilitate RBCs to become miniature than normal. This will provide medical practitioner with an assessment of magnitude as well as the appearance of RBCs at hand, moreover named as red cell indices. This typifies Mean Corpuscular Volume (MCV), an estimation of whole of red blood cells.

2.12.1 Hemoglobinopathy Assessment

This test quantifies the quality and comparative quantity of hemoglobin extant in the RBCs.

2.12.2 DNA Examination

These examinations are deployed for scrutinizing erasures as well as modifications in the alpha and beta globin making genes. Intermittently emerging changes of the β- globin gene can be seen by virtue of Polymerase Chain Reaction (PCR) - based frameworks. The widely utilized procedures are reverse dot blot analysis or primer-specific amplification, with a course of action of probes or complementary to bulk of recognized transformations discerned in the masses as of which the influenced person commenced (Old J *et al.,* 2005). Pedigree research can be carried out to analyse carrier status and the kinds of variations existing. Pre-birth screening implies of taking a specimen of amniotic liquid or else tissue from the placenta.

2.13 Issues of β-Thalassemia

Transfused thalassemic patients may create difficulties identified with ferritin overload. Entanglements of ferritin surplus within youngsters incorporate development impediment and disappointment or deferral of sexual development. Subsequently iron overabundance associated intricacies embody involvement of the heart, hepatocyte (fibrosis and cirrhosis), and endocrine organs (diabetes mellitus, hypogonadism and deficiency of the parathyroid, thyroid, pituitary, and, less usually, adrenal glands (Higgs DR *et al.,* 2012; Mohammad Ali Mashhadi *et al.,* 2014). Confusions are splenomegaly, ceaseless hepatitis (coming about because of disease with infections that reason hepatitis B and additionally C), Human Immunodeficiency Virus (HIV) contamination, venous thrombosis, and osteoporosis (Pippard M, 1996). The hazard for hepatocellular carcinoma is expanded in patients with liver, viral disease and iron over-burden. People who have not been frequently transfused typically pass on before the second-third decade; survival of people who have been frequently transfused and treated with proper chelation reaches out past age of 40 years. Heart ailment engendered by myocardial siderosis is extremely imperative life-constraining complexity of ferritin plethora in Mediterranean anemia. Honestly, heart perplexities are the rationale for mortalities in 71% of patients suffering from Cooley's anemia (Taher A *et al.,* 2013).

2.14 Therapy of β-Thalassemia

Treatment of thalassaemia depends upon the nature and graveness of the complaint. Remedy for inpatients with Cooley's anemia encompasses recurrent blood transfusion prophylactic, iron chelation, splenectomy, and allogeneic hematopoietic transplantation.

2.14.1 Prevention Schemes

Avoidance of β - thalassemia is hinged on general public cognizance of the affliction, diagnosis of carriers, hereditary counseling, and pre-birth testing (Peters M *et al.,* 2012).

2.14.2 Abode Remedy

Iron Multivitamins. Ascorbic acid, inflates the extent of iron assimilate from sustenance (Iron-rich Foods and Anemia: Management and Treatment, 2017).

2.14.3 Blood Transfusion

Convalescents with thalassaemia major are transfusion addicted nonetheless this isn't so in thalassaemia intermedia. The preponderance troublesome curative decision that should be made while treating a patient with thalassaemia intermedia is regardless of whether to instigate a long-term transfusion scheme (Borgna-Pignatti C, 2007). RBC transfusions on a continual basis in beta-thalassemics intent at repressing the erythropoietic reaction which is influenced by tissue hypoxia (Vasileiadis I. *et al.,* 2013). It ought to likewise be noticed that transfusion regimen inaugurated in youth to support development, can be ceased after adolescence. Patients with thalassemia major entail spasmodic and enduring blood transfusions each 2-3 weeks to keep up a hemoglobin level higher than 9.5 gm/dl and succour wonted development.

2.14.4 Iron Chelation Cure

Owing to the fact that the Hb in red blood cells is an iron- prolific macromolecule, continual blood transfusions will call forth an accumulation of iron within the blood. This malady is thought as hemochromatosis. It detriments the hepatocyte, heart and alternative components of the body. To avert this prejudice, iron chelation therapeutic is necessitate eliminating surfeit iron from the physique. Twain medicaments are plied for iron chelation remedy. Deferoxamine is a fluid drug that's given gradually beneath the dermis, primarily with a miniature movable pump utilized nightlong. This treatment requires some investment and can be somewhat agonizing. Symptoms comprise of vision loss plus hearing impairment. Deferasirox tablet is taken one a day. Symptoms encompass migraine, sickness, retching, loose motions, joint agony, and enervation (NIH: Thalassemias, n.d).

2.14.5 Splenectomy

When the spleen turns out to be additionally dynamic and begins to obliterate the RBCs, transfusions turn out to be less effectual. At that point it comes to be imperative to take the spleen out called "Splenectomy" (Iron-rich Foods and Anemia: Management and Treatment 2017). In the event that the annual erythrocyte required transcends 180-200 ml/kg of red blood cell (inferring that the haematocrit of the whole of erythrocytes is something like 75%), removal of spleen should be pondered, equipped that alternative motives for augmented depletion, for instance, lysis responses, are dominated out. Furthermore manifestations for removal of spleen are prodrome of lymphatic tissue growth, reduction in white cells as well as deficit of thrombocytes and increasing hemochromatosis irrespective of great chelation (Weatherall, D J *et al.,* 2001).

2.14.6 Folic Acid Adjuncts

Folic acid is a synthetic type of vitamin B9, moreover referred to as B vitamin. It is imperative that succour in erythrocytes formation as well as salubrious cell

growth and performance. One could be in need of folate supplement regardless of blood transfusions or potentially iron chelation treatment (NIH: Thalassemias, n.d; Deborah Rund *et al.,* 2000; Wikipedia: Thalassemia, 2017; Yaish HM, 2017).

2.14.7 Stem Cell Transplant

Stem cell transplant alludes to a method that displaces our aberrant alternatively defunct stem cells with salubrious ones as of somebody else (a giver). Hematopoietic stem cells are a kind of adult stem cell that can be found inside a bone marrow which produces red blood cells and different thrombocytes. A stem cell transplant is the exclusively medical aid which will rectify Mediterranean anemia. Despite that, just marginal inhabitants can locate a decent match among benefactors and have the unsafe technique (NIH: Thalassemias, n.d).

2.14.8 Gene Therapy

This proposes a prospective antidote for β-thalassaemia moreover would portray an exemplary substitute to both customary remedy and bone marrow transplantation. Gene therapy yet give rise to several difficulties amid which is the fluctuation and poor-quality articulation of retroviral vectors conveying the human β - globin tape. Significant advancement has now been made to avail oneself of lentiviral vectors which steadily transfer the β-globin articulation tape. HbF reactivation by 5 azacytidine, Butyrate and Hydroxycarbamide (Hydroxyurea) has been discerned to be correspondingly fruitful in thalassaemia inpatients. Recombinant Human Erythropoetin (rHuEPO) can increment hemoglobin level in a few patients with thalassaemia Intermedia (Borgna-Pignatti C, 2007).

Chapter 3

Methodology

3.1 Research Approval & Patients Identification

In the wake of getting consent from supervisor and ethical committee of CUST, Islamabad Pakistan the research was conducted. All the thalassemia patients included in this research work are from Islamabad and Rawalpindi.

3.2 Study Domain and Place

The domain of this current research is β-thalassemia which was carried out at the Human Genetics Lab, Department of Animal Sciences, Quaid-i-Azam University, Islamabad.

3.3 Data Collection

The data was collected by interviewing 100 thalassemic patients or family informer with regard to their clinical and demographics from Pakistan Thalassemia Centre (a project of Pakistan Bait ul Mal, Government of Pakistan) Islamabad, and Jamila Sultana Foundation Rawalpindi.

3.4 Statistical Analysis

Statistical Analysis of the information was implemented by utilizing Graph Pad Prism version 5 and MS Excel. The data was recorded and tabulated in MS Excel spreadsheet and then imported in Graph Pad Prism version 5. The entered statistics was dissociated into Clinical and Demographic classifications where graphs for each variable were drawn in MS Excel. Chi square test was computed by Graph Pad Prism version 5 for determining the significance of distributions of the data at $p < .05$. Inferential statistics was used to deduce the chi square and p values of all the variables related to clinical and demographic.

Chapter 4

Results and Discussion

4.1 Data Collection

In this research work, a total of 100 thalassemic patients suffering from β-thalassemia who were enlisted at the Pakistan Thalassemia Centre and Jamila Sultana Foundation in Islamabad and Rawalpindi during May 2018 were included in this research.

4.2 Demographics

In this section the results of the demographic variables will be discussed

4.2.1 Age, Gender and Marital Status

(Figure 4.1, 4.2, 4.3) presents the age, gender and marital status of the thalassemia patients. Among 100 patients, 57 were males and 43 were females (Figure 4.1). Out of these, 25 males and females (50%) lie within the age group between 10-19 years (Figure 4.2). A chi-square test was computed. The result were found statistically non-significant, X^2 (2, n=100) = 2.47, p < .05. Most of these patients (98%) are single with an exception of two who is married (Figure 4.3). The result were found statistically non-significant, X^2 (1, n=100) = 0.04, p < .05.

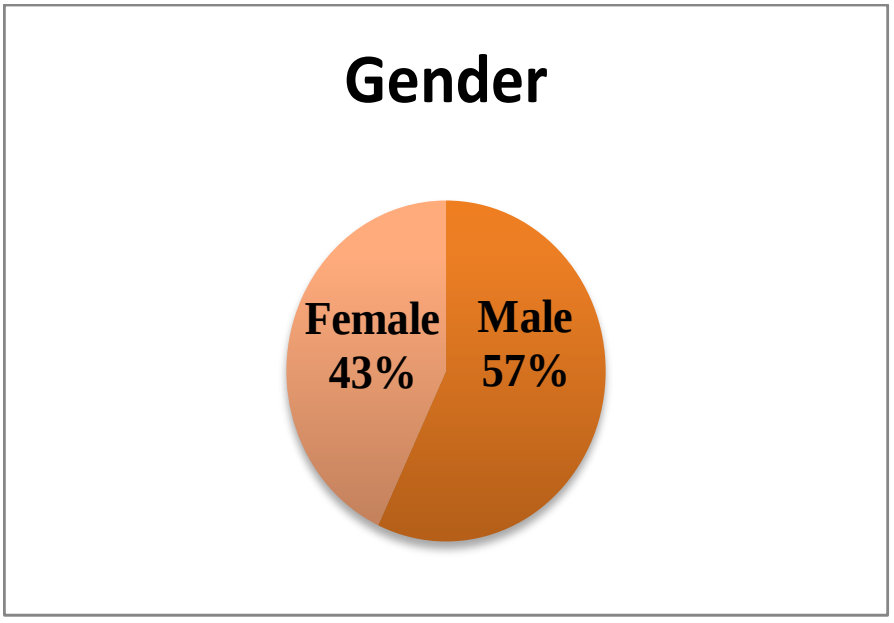

FIGURE 4.1: Gender of Thalassemia Patients

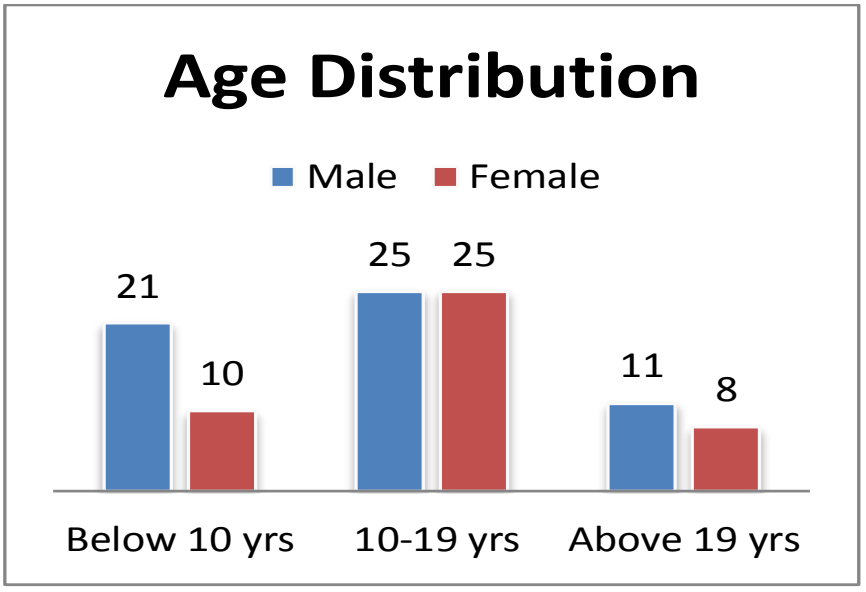

FIGURE 4.2: Age Distribution

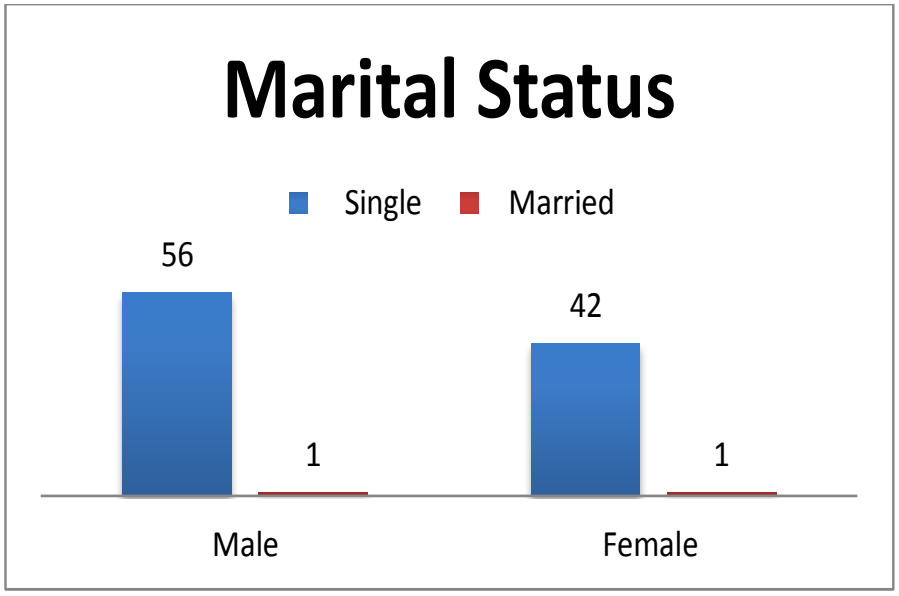

FIGURE 4.3: Marital Status of Thalassemia Patients

4.2.2 Domicile

(Figure 4.4) represent the domicile of thalassemia patients. The predominance of our patients was from the districts of Rawalpindi (52%) and Islamabad (15%). This was trailed by KPK (13%), Attock and Chakwal (4%) and 12% came from other districts. The result were found statistically non-significant, X^2 (5, n=100) = 2.95, p < .05.

In addition, 84% reside in urban areas while the remaining 16% reside in rural areas (figure 4.5). The result were found to be very statistically significant, X^2 (1, n=100) = 7.23, p < .05.

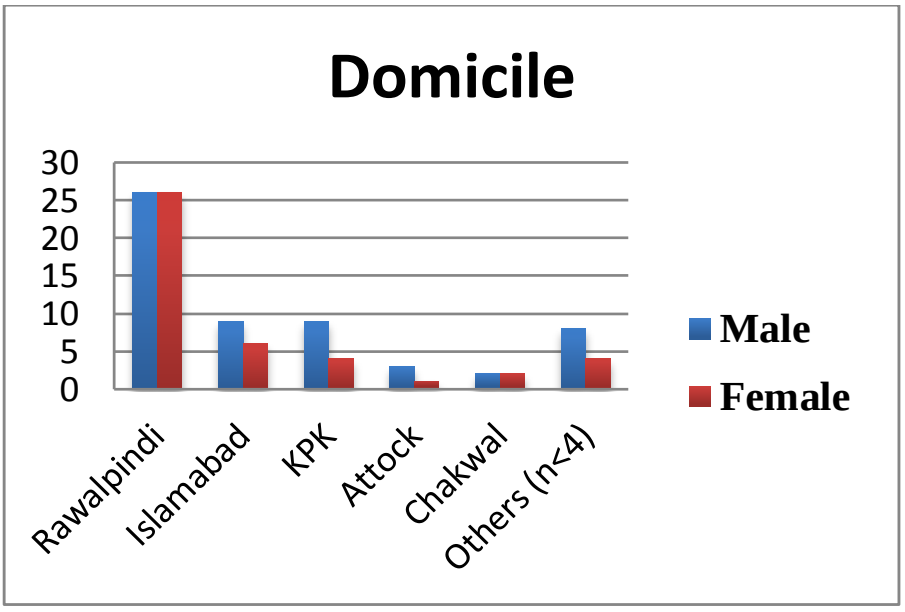

FIGURE 4.4: Domicile of Thalassemia Patients

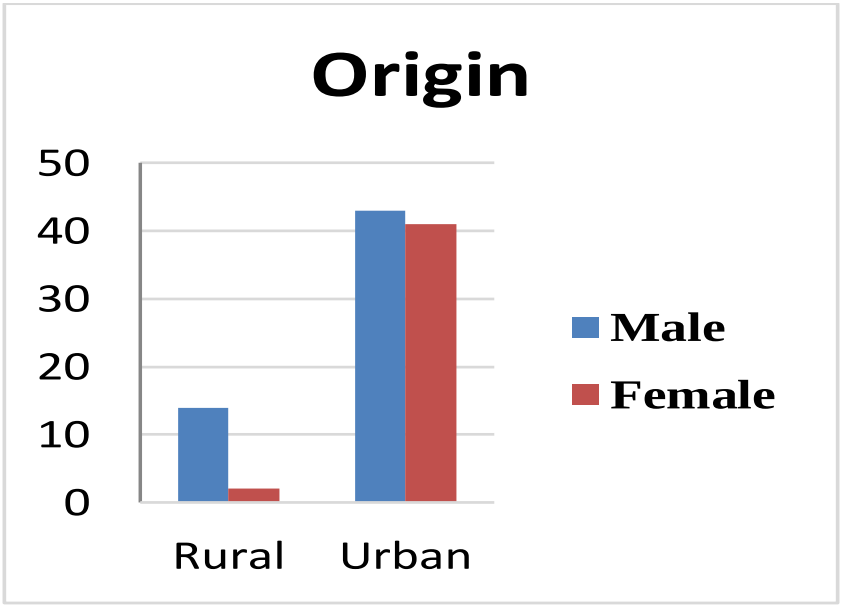

FIGURE 4.5: Origin of Thalassemia Patients

4.2.3 Ethnicity

(Figure 4.6) portrays the ethnicity ancestry amid the thalassemia patients. Concerning ethnic ancestry, 13 (14.6%) were Awan, 10 (11.2%) were Rajpoot, 9

(10.1%) were Qureshi, 6 (6.74%) were Malik, 4 (4.49%) were Pathan and Sheikh, 3 (3.37%) were Abbasi, Balouch, Bhatti and Mughal and 31 (34.8%) were from other ethnic group. The result were found statistically non-significant, X^2 (10, n=89) = 5.52, p < .05.

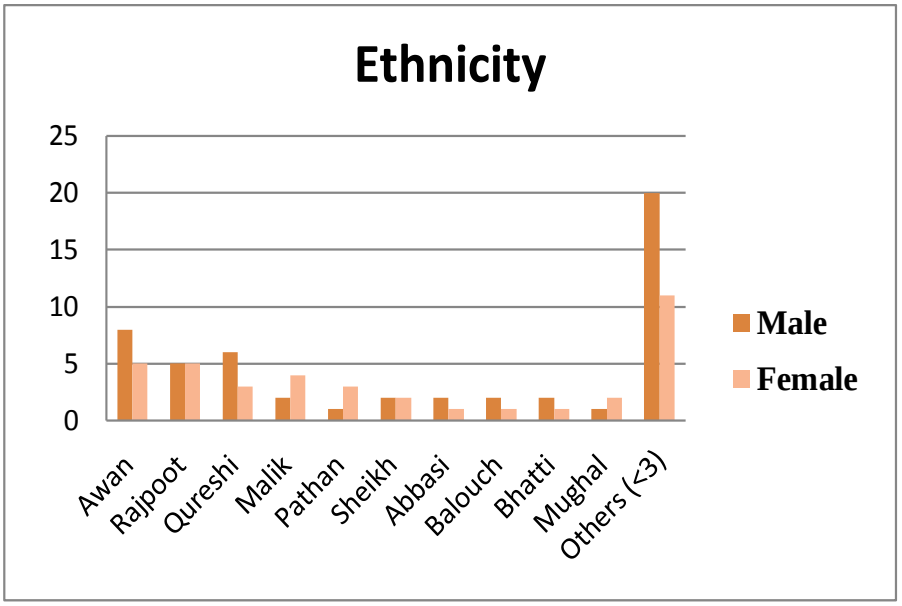

FIGURE 4.6: Ethnicity Ancestry amid the Thalassemia Patients

4.2.4 Economic Status

(Figure 4.7) depicts the economic status amid the thalassemia patients. The predominance of our patients belongs to middle income family 41 (46.1%). This is trailed by low income family 24 (27%), poor family 21 (23.6%) and high income family 3 (3.37%). The result were found statistically non-significant, X^2 (3, n=89) = 3.05, p < .05.

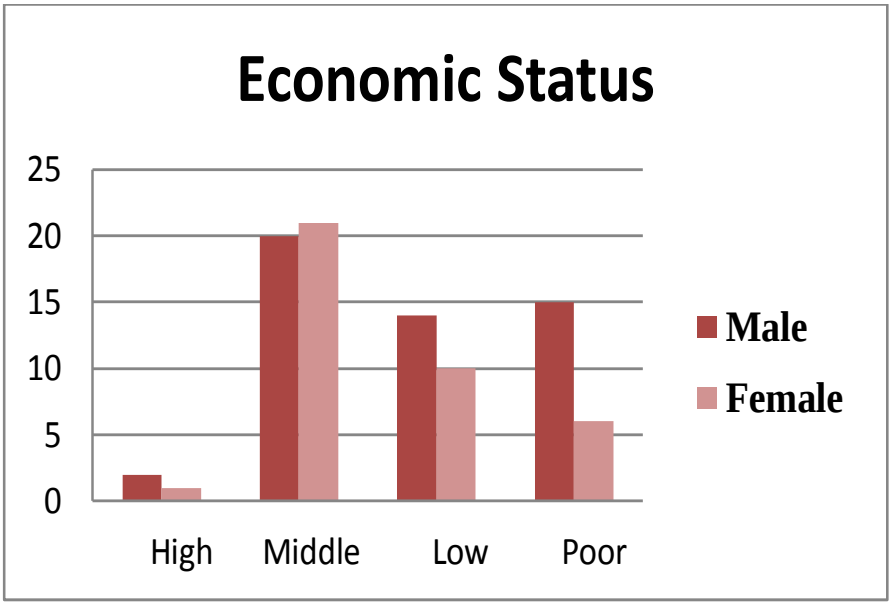

FIGURE 4.7: Economic Status amid the Thalassemia Patients

4.2.5 Family Type

(Figure 4.8) demonstrates the family type of the thalassemia patients. The bulk of our patients (57) live in an extended (joint) family accounting for 62% while the remaining 35 (38.04%) resides in nuclear (traditional) family. The result were found statistically non-significant, X^2 (1, n=92) = 0.26, p < .05.

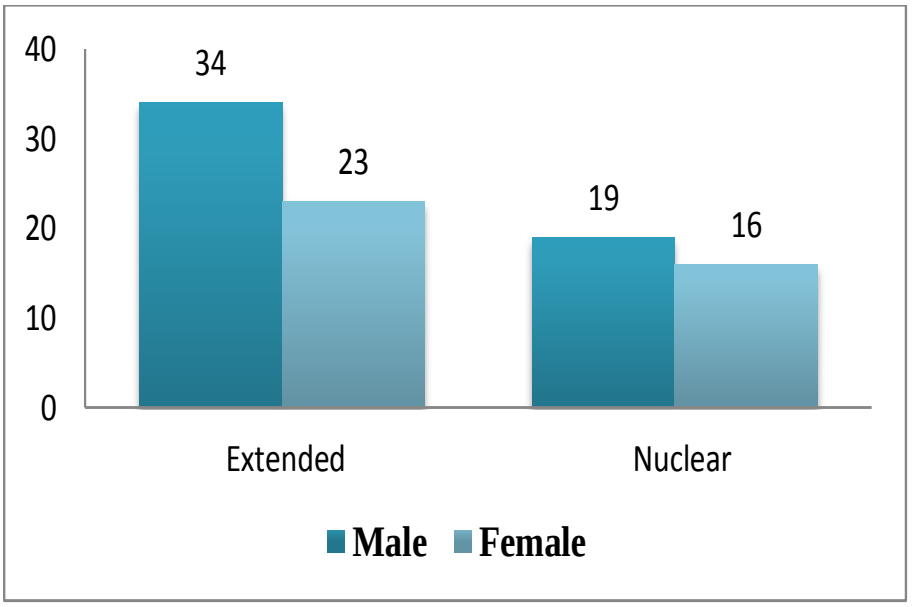

FIGURE 4.8: Family Type of the Thalassemia Patients

4.2.6 Parental Consanguinity

(Figure 4.9) delineates the parental consanguinity. Among the 100 thalassemic patients, 67 (70.5%) progeny were conceived to consanguineous rents plus, remaining 28 (29.5%) to non-consanguineous progenitors. The result were found statistically non-significant, X^2 (1, n=95) = 0.03, p < .05.

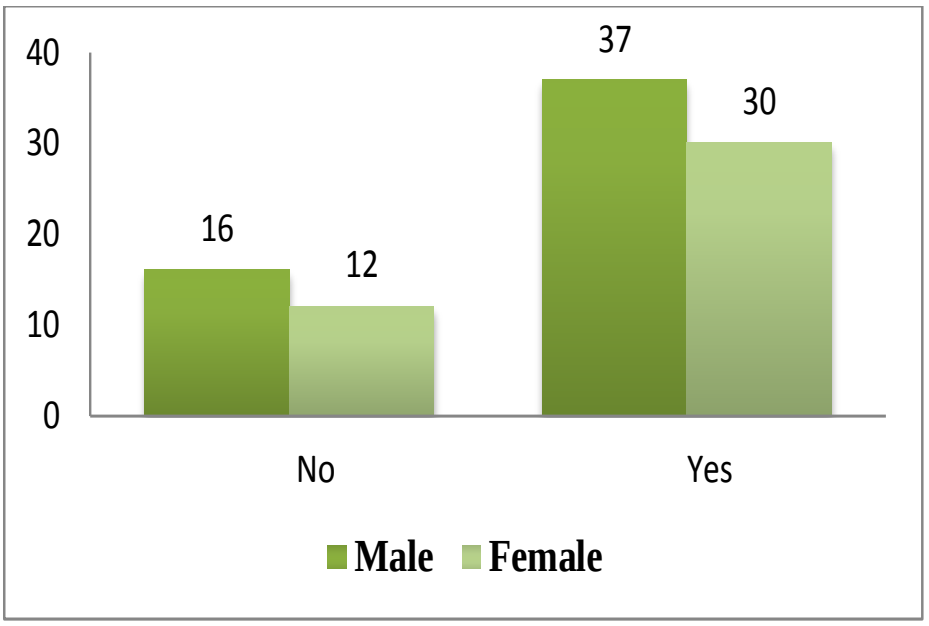

FIGURE 4.9: Parental Consanguinity among Thalassemia Patients

4.2.7 Family History and Mortality in Family with Thalassemia

(Figure 4.10) shows the family history of thalassemia sufferers. The lion share's of our patients (49) does have a family history of thalassemia representing 52.1% in comparison to 43(45.7%) that doesn't have a family history of thalassemia. The remaining 2 (2.13%) doesn't know. The result were found statistically non-significant, X^2 (2, n=95) = 4.43, p < .05.

Moreover, preponderance 64 (68.1%) of our thalassemic patients hasnt witnessed any death in the family due to thalassemia as compared to 30 (31.9%) who has witnessed death in the family (figure 4.11). The result were found statistically non-significant, X^2 (1, n=94) = 3.32, p < .05.

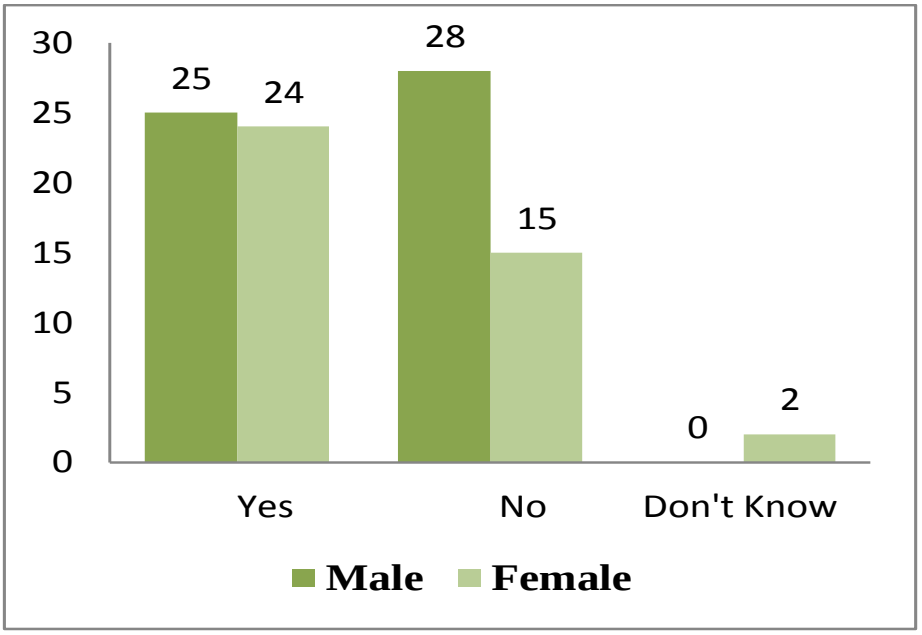

FIGURE 4.10: Family History of Thalassemia Sufferers

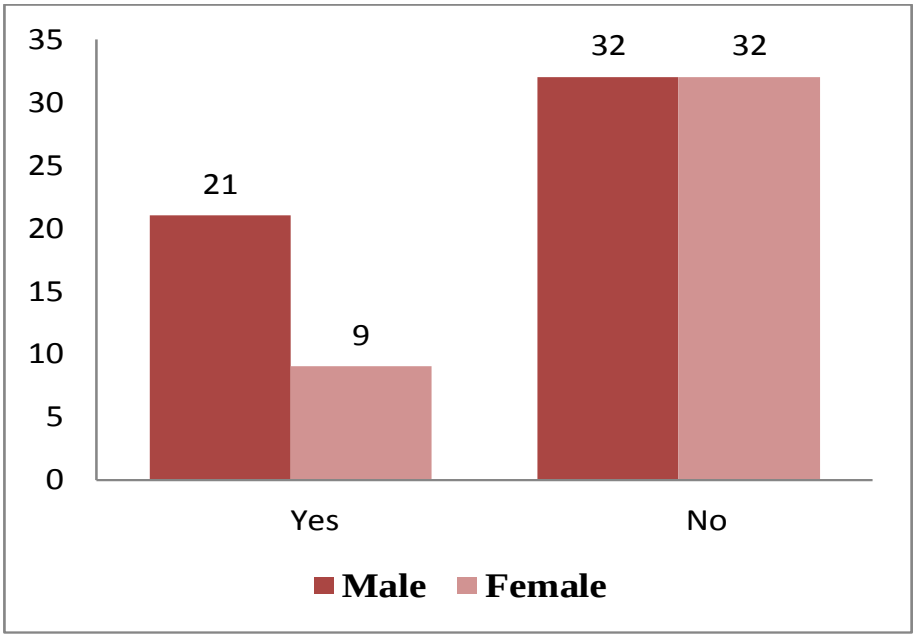

FIGURE 4.11: Mortality in Family with Thalassemia

4.3 Clinical Characteristics

In this section the results of the clinical characteristics will be discussed.

4.3.1 Thalassemia Types

(Table 4.1) represents the thalassemia types. Most of the thalassemic patients in this study were thalassemia major (94%), trailed by (4%) thalassemia intermediate and (2%) thalassemia minor. The result were found statistically non-significant, X^2 (2, n=100) = 4.58, p < .05.

TABLE 4.1: Thalassemia Types

Thalassemia Type	Male	Female	% ages
Major	56	38	94
Intermediate	1	3	4
Minor	0	2	2
Total	57	43	100

4.3.2 Onset of Thalassemia

(Table 4.2) presents the onset of thalassemia. Of 100 patients, (76%) were determined to have thalassemia were in the vicinity of 0 and 6 months and (24%) were diagnosed with thalassemia late between 1-3 years. The result were found statistically non-significant, X^2 (1, n=100) = 0.10, p < .05.

TABLE 4.2: Onset of Thalassemia

Onset of Symptoms	Male	Female	% ages
Congenital	44	32	76
Late	13	11	24
Total	57	43	100

4.3.3 Blood Groups

(Table 4.3) represents the blood groups of thalassemia sufferers. The results obtained demonstrated that the lion share's of the patients (38%) were from group B+, the second prime group of the patients (25%) were from group O + and (15%) were from group A + though different groups AB+ (9%), B- (4%) , O- (5%), A-(4%), B- (4%) indicated low rates. The result were found statistically non-significant, X^2 (6, n=100) = 5.45, p < .05.

TABLE 4.3: Blood Groups of Thalassemia Sufferers

Blood Group	Male	Female	% ages
A +	6	9	15
A -	3	1	4
AB+	7	2	9
B+	23	15	38
B -	3	1	4
O+	12	13	25
O -	3	2	5
Total	57	43	100

4.3.4 Age at 1^{st} Transfusion

(Table 4.4) portrays the age at 1^{st} transfusion of thalassemia patients. The bulk (72%) of our thalassemic patients received their 1^{st} transfusion below 1 yr of age. This is followed by 17% between 1-2 yrs, 5% between 2-3 yrs and 6% above 3 yrs. The result were found statistically non-significant, X^2 (3, n=100) = 1.12, p < .05.

TABLE 4.4: Age at 1^{st} Transfusion of Thalassemia Patients

1^{st} Transfusion (age)	Male	Female	% ages
Below 1 yr	41	31	72
1-2 yrs	11	6	17
2-3 yrs	2	3	5
Above 3 yrs	3	3	6
Total	57	43	100

4.3.5 Disease Complications of Thalassemia

(Table 4.5) shows the disease complications of thalassemia. All the enlisted patients' sufferer from various kinds of entanglements although the majority 29 (56.9%) of the sufferer are stable trailed by 15 (29.4%) increasing by age, 5 (9.8%) decreasing by age and 2 (3.9%) just started. The result were found statistically non-significant, X^2 (3, n=51) = 1.57, p < .05.

TABLE 4.5: Disease Complications of Thalassemia

Disease Complications	Male	Female	% ages
Decreasing by age	3	2	9.8
Increasing by age	9	6	29.4
Just Started	2	0	3.9
Stable	16	13	56.9
Total	30	21	100

4.3.6 Mode of Diagnosis of Thalassemia

(Table 4.6) represents the mode of diagnosis of thalassemia. The preponderance 59 (98.3%) of patients was diagnosed for thalassemia by Hb electrophoresis and a minority 1 (1.7%) was diagnosed by PCR. The result were found statistically non-significant, X^2 (1, n=60) = 0.89, p < .05.

TABLE 4.6: Mode of Diagnosis of Thalassemia

Mode of Diagnosis	Male	Female	% ages
Hb Electrophoresis	31	28	98.3
PCR	1	0	1.7
Total	32	28	100

Hence, it can be deduced that demographic and clinical characteristics are not statistically significant (p < .05) with an exception of origin of thalassemia patients which is analyzed to be very statistically significant (p < .05).

4.4 Discussion

Thalassemia is a noteworthy medical issue in the twin urban communities of Islamabad and Rawalpindi. In this research we analyzed the patients identified to have thalassemia as proportion to their clinical and demographic profile. In this investigation, 100 patients [57 males and 43 females] were taken into consideration. This may demonstrate that thalassemias are more typical in males than in females on the grounds that the rents give more regard for their male tyke and prepared to spend more cash on a male tyke contrasted with a female one. We found that the vast majority of the patients (76%) were analyzed for β-thalassemia in the

vicinity of 0-6 months (Table 4.2). Comparative outcome was accounted for by Modell and Berdoukas (1984) and MB Agarwal (2001). It is anticipated that the malfunction of converting, from γ-chain to β-chain at the 3^{rd} month brings about the manifestation of clinical prodrome ascribed to β-thalassemia.

Manifestations of paleness, pallor, episodes of fever, amplification of spleen, and hepatosplenomegaly linked with jaundice are seen in β-thalassemia major and intermedia. Comparative clinical highlights for β-thalassemia major were seen by Weatherall and Clegg (1981) and for β-thalassemia intermedia by Weatherall also, Clegg (2001), and Dedye *et al.,* (2003). The extensive variety of clinical prodrome observed in the current study is in accordance with Weatherall and Clegg (1981) who detailed that to a great degree extensive variety of clinical manifestations came about because of interconnection of a wide range of sub-atomic types of the β-thalassemia and auxiliary hemoglobin variations.

The geological and social spread of thalassemia in this locale of Islamabad and Rawalpindi still can't seem to be considered. The geological spread of the thalassemic patients mirrors the nearness of β-thalassaemia within various locales of Rawalpindi and Islamabad. The outcomes show that β-thalassaemia can happen in all areas, races and ethnic classes. The most elevated frequency was in the regions of Rawalpindi (52%) and Islamabad (15%) that might be because of the high populace in this district. Also, 84% live in urban zones while the remaining 16% dwell in country zones.

The outcomes likewise demonstrate that -thalassemia youngsters destined to consanguineous guardians (70.5%) was higher than non-consanguineous guardians, unmistakably shows that consanguinity, which is common from time in the twin urban communities, has a more prominent part to play in the expression of different types of β-thalassemia and can be considered as a fundamental factor of rifeness of thalassemia in Rawalpindi and Islamabad. The autosomal recessive attributes will be more prevalent in the offspring of consanguineous guardians since they have a more noteworthy shot of acquiring indistinguishable duplicates of a mutant gene or genes from typical progenitors (Radha Rama Devi *et al.,* 1982).

Studies have demonstrated that in Pakistan nationwide pervasiveness of consanguious marriage varies from 31.1 to 62% (Riaz HF *et al;* 2016) and has one of the most astounding rampancy of consanguineous conjugal bond globally and remains dormant (Jones GW, 2010) (Agha N, 2016). Of the β-thalassemia youngsters destined to consanguineous guardians, 80% were destined to the first cousins (Hussain R *et al.,* 1998, Qidwai W *et al.,* 2003, Akram DS *et al.,* 2008), which is higher when contrasted with other degrees of consanguinity. An investigation in Pakistan found that the most racial conjugal bond happened among Panjabies (60.7%), and trailed by Sarakies (25.5%) (Ain Q *et al.,* 2011).

A large portion of the patients (38%) were from group B+, that might be expected to the bulk of B+ blood group in the twin cities of Islamabad and Rawalpindi. A notable increment in ferritin was discerned in all β-thalassemia patients (data not shown), which might be because of erythrocyte hyperhemolysis or/and to incessant blood transfusion (Kosman D.J, 2010).

As appeared in Table 4.5, numerous patients suffer from various kinds of entanglements. This might be because of iron overload. The noteworthy increment of ferritin in thalassemic patients demonstrated a current iron over-burden. Iron over-burden may prompt an iron intestinal hyperabsorption and to an anomaly atomic iron shaping non-transferrin bound (NTBI) gathering which adds to the development of free radicals and increments hemolytic process (Widad N.M *et al;* 2003). Furthermore, the critical inflation of ferritin was a vital jeopardy factor for myocardial infarction (Piga A *et al;* 2009). Iron over-load likewise comes about in an unavoidable difficulty that hastens the multiorgan irregularities, particularly organs that collect abundance iron, including liver, pituitary organ, pancreas and heart (Lobo V *et al;* 2010, Riaz H *et al;* 2011).

The high predominance of β-thalassemia also, that of consanguineous unions in the network and the impact of that on the wellbeing administrations in Islamabad and Rawalpindi, ought to elevate the wellbeing specialists to build up a viable preventive program. This thorough program includes carrier discoveries, sub-atomic diagnostics, hereditary guiding, and pre-birth analysis. Such program can just be conceivable by starting an exploration concentrate to decide the atomic premise of

the sort thalassemia in Islamabad and Rawalpindi, which will be the accompanying advance of the present research.

Chapter 5

Conclusion and Future Approach

5.1 Conclusion

From the above-stated analysis of data it can be noted that thalassemia is a perilous malady that is propagating globally since this is an easygoing thing to ponder as the assessed pervasiveness is 3– 8% in Pakistan and the cases may increment as it is an innate ailment. Near to 1.5-3% of the inhabitants is guesstimated to be carriers for β-thalassemia with 50– 60,000 newly discovered thalassemic neonate being conceived every year. Roughly 5000-6000 offspring's are diagnosed every year to have beta thalassemia in Pakistan (Ansari SH *et al.,* 2011). Hence, it is noteworthy to take into thought about this affliction as it might demonstrate lethal one. Furthermore consequently the potency of this infirmity can be brought down by diagnosing and taking appropriate medicines, for example,

- Blood Transfusions
- Iron Chelation Therapy
- Folic Acid Supplements
- Blood and Marrow Stem Cell Transplant
- Certain Medications as Deferoxamine

- Deferasirox

From this research it can be deduced that

1. An increasing number of thalassemia sufferers predominance of males over females between 10-19 years of age visit their thalassemia centres in Rawalpindi and Islamabad on daily basis for routine blood transfusion and therapy of other medical maladies.

2. From demographics aspect, marital status of most of the patients are single, belongs to middle income family and live in an extended (joint) family. In addition, are conceived to consanguineous rents and does have history of thalassemia within family.

3. From clinical perspective, bulks of patients in this research were thalassemia major suffers, diagnosed by Hb electrophoresis within 6 months.

5.2 Future Approach

In the near future, this study can ameliorate in stimulating the wellbeing experts and legislature of Rawalpindi and Islamabad to verbalize general public cognizance, public coaching agendas at secondary school/school levels and give lectures to wellbeing experts including specialists and medical caretakers working in the neighborhood and relatives through intermittent gatherings, holding symposium, discussion groups and compiling articles in the everyday papers and telecasting in TV and radio.

It is important to take it as a general medical issue in light of the fact that thalassemia causes an enormous mental and budgetary deplete on patients and their families. Thalassemia patients furthermore, their folks require deep rooted mental help for anticipation of psychological well-being issues.

This lethal illness can be intercepted by inaugurating an efficacious prophylactic program which includes silent carrier identification, molecular diagnostics, hereditary advising, pre-birth prognosis and pre-marriage screening.

To the best of my insight, this investigation is the underlying of its genre from the lookalike metropolis of Pakistan, which inspects both clinical and demographic features of thalassemia with an objective to make consciousness of thalassemia among the general masses of Pakistan particularly Islamabad and Rawalpindi.

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Appendix

CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY (CUST)

Proforma for Thalassemia Patients Date:_____

I. Demographics

1) Family ID:
2) Patient Name:
3) Gender:
4) Fathers Name:
5) Thalassemia Type: (Major/Minor)
6) Description of Anomaly:
7) Other Associated Diseases:
8) Onset: (Cong/Late)
9) Nature of disease: (Progressive/Not Progressive)
10) Age of Diagnosis:
11) Family History of Thalassemia: (Yes/No)Affected Males:
Affected Females:

If yes then (Please draw three generation pedigree with correct parental relationship)

1) Medical Records: (Attach file when available)

2) Age of the Patient (Please mention Date of Birth, if possible):

3) Origin: (R/U)

4) Present Town: (R/U)

5) Contact No:

6) Caste and Language:

7) Education; Occupation of Patient:

8) Marital Status: (Single/ Married)

9) Economic State: (Poor/ Low/ Mid/ High)

10) Family Type: (Nuclear/ Extended)

11) Parental Consanguinity: (Yes/ No)

12) Spouse Relationship:(1st /2nd cousin/Distant relatives/Out of family)

13) Parents present Age: Father: Mother:

14) Parental Age at the Birth of Patient: Father: Mother:

15) Family History with any other Disease:

16) Any Mortality in the Family with Thalassemia:

17) Mortality in: Males: Females:

II. Pathological / Disease Profile

1) Blood Group:

2) Child Weight (Currently):

3) Child Height (Currently):

4) Age at Registration:

5) Age at 1st Transfusion:

6) Transfusion (How frequent):

7) Ferritin Level (mg/g):

8) Iron Level (mg/g):

9) Chelating level:

10) Chelating Therapy:

11) Hemoglobin Level:

12) Complications of the Disease:

13) Disease Complications are: (Increasing by age /Stable /Decreasing by age)

14) Vaccination of Hepatitis:

15) Any other Infection:

16) Mode of Diagnosis of Disease: (PCR/Simple liver tests/Name, if any other)

17) Any Data or Blood samples taken by any other: (Yes/No)

18) MOST IMP: Reasons of Mortality in Thalassaemic ones (Open ended Q):

Reasons:

(Splenomegaly, Hepatomegaly, Any Infection, Unavailability of Blood, Economic Status, Any other reason)

Mortality Age: