2 Pakistan and thalassaemia

Historical background:

The people of Pakistan are made up from all those who, at various times and for various reasons, have come to the land that today is known as Pakistan. The first urban settlements that emerged as Indus Valley Civilization can be traced back in history to beyond 2500 B.C. (Wolpert 1977). However, it was with the arrival of the Aryans from Eurasia between 1500-500 B.C. that the new chapter in the story of Pakistan really begins. Subsequently, the region saw successive invasions 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 1962).

In spite of the frequent invasions and tribal migrations, mainly from the Central Asia, the vast majority of the population, however, stems from a genetic pool that has its roots in South Asia. The first impression of the Pakistanis likely to be gained by visitors to a big city in Pakistan is of its amazing variety (Halliday 1990).

Population and the ethnic groups:

According to the census conducted in 1981 Pakistan's population was 84.25 million. Presently it is estimated at 137 million and its growth rate is 2.8% (Economic survey 1995-1996).

There are four main ethnic groups (Fig 2.1) i.e. Punjabi, Pathan, Sindhi, and Baluchi who are mainly distributed in the provinces of Punjab, North West Frontier Province (NWFP), Sindh and Baluchistan respectively. In addition, a fifth group of people called Mohajirs, who migrated from various parts of India at the time of creation of Pakistan in 1947, can also be recognized. According to the census (1981) the proportion of the population in various

provinces was as follows: Punjab 56.4%, Sindh 22.5%, NWFP 15.8%, and Baluchistan 5.1%.

Fig: 2.1. Four Provinces of Pakistan. The predominant population in each province consists of Pathans in the North West Frontier Province (NWFP), Punjabis in Punjab, Sindhi and Mohajirs in Sindh, and Baluchis in Baluchistan.

Punjabi:

The name Punjab is derived from "punj" meaning five, and "aab" meaning waters. Five major rivers cross the province of Punjab. The inhabitants of Punjab are known as Punjabis. Agriculture, because of an extensive network of rivers and canals, is the most important industry in Punjab. Punjabis are the largest ethnic group numbering approximately 80 million. The racial origin of Punjabis is considered to be from Aryans. However, a mixture from South Indian civilizations is also present (Joshi and Singh 1976).

Pathan:

In the mountainous terrain of the North West Frontier Province (NWFP) the people are mostly Pathans, divided into clans or into families connected with certain villages or districts. The Pathans number approximately 15-20 million. They are perhaps the world's largest tribal society (Quddus 1987). Some of the tribes residing in Pakistan are also distributed over the neighbouring Afghanistan. Many origins have been attributed to Pathans i.e. Aryan, Greek, Persian, Arab and Jewish (Bokhari 1975). What ever their origin may be, they can be distinguished by their sharp features and fair skin.

Sindhi:

The province of Sindh derives its name from the mighty river “Sindh” or “Indus” that is also the reason for the name "India" for the region as a whole. Geographically speaking “Sindh” denotes the lower half of the Indus Valley. Ethnically Sindhi society has been cosmopolitan and its origin ranges from the descendants of the ancient Aryans, Scythians, Arabs, Turks, Persians, Rajputs, and Baluhis (Khan 1980). There are approximately 15 million Sindhis and almost all of them are confined to the province of Sindh.

Baluchi:

Baluchis, the smallest ethnic population numbering about 5 million, are scattered over the largest province of the country accounting for about 42% of its area. Most of Baluchistan has more in common with Western Asia than with the Indian subcontinent. Although there is evidence that the area was inhabited in the stone age (7000-3000 B.C.) the name Baluchistan only came into existence with the arrival of tribes from Iran called "Baluch". There is evidence that this tribal migration took place around 700 B.C. (Bokhari 1975).

Mohajir:

This group is not well defined. It includes mostly individuals and their descendants who migrated from various parts of India during partition of the subcontinent in 1947. These include predominantly Urdu speaking individuals from various parts of Northern India. In addition, a large number of people from Gujrat and Bihar are also included in this group. Unofficial estimates for the number of Mohajirs are around 15 million. They mostly reside in the province of Sindh.

Caste Biradri and Tribe:

The remnants of the traditional caste system, that took its origin in common with Hinduism in the Aryan era, can also be seen in Pakistani culture. However, social discrimination on the basis of caste, still widespread in India, is not prominent. The Biradri is a community whose members are related either closely or distantly by blood or marriage so that members think of each other as kinsmen (Kolenda 1978). It is much larger as compared to a kinship net work of an individual's kindred in the Western Society. Biradris usually do not have any territorial confinements. There are a number of sizeable Biradris in Pakistan that range in numbers from a few hundred to even millions. Some of the larger Biradris are subdivided in to smaller sub-Biradris. The equivalent of a Biradri amongst the Pathans and Baluchis is a tribe.

The rules for behaviour within the Biradri or a tribe are well defined and agreed upon. The elders usually perform the duties of enforcing the rules, doing justice on disputes and carrying out social planning (Punjabi 1976).

Consanguineous marriages:

A consanguineous marriage is defined as marriage between individuals who have at least one, not too remote, common ancestor. In practice the relationships beyond 2nd cousins are taken as non-consanguineous (Bittles 1994). The offspring of a consanguineous marriage is said to be inbred. The main genetic consequence of consanguinity is an increase in the proportion of homozygotes. Recessive genes, that are unable to express themselves in the heterozygous state, are thus brought to the fore (Bodmer and Cavalli-Sforza 1976).

Marriages in Pakistani culture are usually stable and arranged by family members of the two spouses. Consanguineous marriages are a frequent occurrence. Pakistan is a Muslim country so a marital relationship closer than 1st cousin is prohibited (Al-Quran, Sura Al-Nisa). Studies in the major urban cities of Punjab show that almost 50% of the marriages are between close relatives (Shami and Zahida 1982; Shami and Iqbal 1983; Shami and Hussain 1984; Shami and Minhas 1984; Shami and Siddiqui 1984; Darr and Modell 1988; Bittles et

al, 1993). A study of 9,520 couples in the urban Punjab by Bittles (1994) showed that 50% of the marriages are consanguineous, 34% are between unrelated but Biradri members, and only 16% are between completely unrelated individuals (Table 2.1). Average coefficient of inbreeding, the probability that an individual inherits two genes at a locus that are identical by descent i.e. from a common ancestor, in Punjabis is estimated to be 0.0280 (Bittles 1994).

A study of consanguineous marriages amongst Pathans of Swat (Table 2.1) showed that it was 31% (2^{nd} cousin or closer) in the urban areas and 37% in the rural areas (Wahab and Ahmed 1996). Comparable data from other regions and ethnic groups are lacking. Unpublished reports on consanguineous marriages amongst other ethnic groups are summarized in Table: 2.1. A rural Sindhi sample of 202 couples showed that 73% marriages were consanguineous (Dr. Rafique Memmon, personal communication). In 189 couples from rural Baluchis 87% marriages were consanguineous (Dr. Jaleel Anwar, personal communication). Consanguineous marriages in 120 Mohajir couples, however, were 47.5% (Mr. Mohammad Iqbal personal communication).

Darr and Modell (1988) have shown that consanguineous marriages amongst the British Pakistanis were more common in the present generation of couples as compared to their previous generation. Wahab and Ahmad (1996) also observed similar trend in a study of Pathan couples in Pakistan.

The main effect of parental consanguinity is to increase the risk of recessively inherited disorders in the offspring. It particularly favours the manifestation of rare recessive disorders, because the chance that one carrier will marry another is low unless they are related. When the recessive disorder is common the carrier has a relatively high risk of marrying another however they choose their partner, and the risk is approximately doubled in a first cousin marriage (Modell and Kuliev 1992).

Table: 2.1. Pattern of consanguineous marriages in the five main ethnic groups of Pakistan.

Ethnic Group:	**Number Studied:**	**Urban/ Rural:**	**DIC:**	**1st Cousins:**	**1 ½ Cousins:**	**2nd Cousins:**	**Biradri Member:**	**Unrelated:**	**Coefficient of inbreeding:**	**Reference:**
Punjabi	9,520	Mostly urban	86 (0.9%)	3,529 (37.1%)	1,115 (11.7%)	54 (0.6%)	3,230 (33.9%)	1,506 (15.8%)	0.0280	Bittles 1994
Pathan	2,037	Mixed	-	448 (22.0%)	108 (5.3%)	139 (6.8%)	169 (8.3%)	1173 (57.6%)	0.0164	Wahab and Ahmad 1996
Sindhi	202	Rural	10 (5.0%)	112 (55.4%)	12 (5.9%)	14 (6.9%)	43 (21.3%)	11 (5.4%)	0.0437	Dr. Rafique Memmon Personal communication.
Baluchi	189	Rural	-	159 (84.1%)	-	5 (2.6%)	?	25 (13.2%)	0.0532	Dr. Jaleel Anwar personal communication.
Mohajir	120	Urban	1 (0.8%)	29 (24.2%)	10 (8.3%)	17 (14.2%)	?	63 (52.5%)	0.0209	Mr. Mohammad Iqbal personal communication

DIC: Double 1st cousin

Haemoglobin disorders in Pakistan:

β-thalassaemia trait:

Stern et al, (1968) reported a 4% carrier rate for β-thalassaemia amongst 129 Pathans. The incidence of thalassaemia in Pakistan remained under reported due to very limited diagnostic facilities (Saleem 1974). The studies on carrier rate of β-thalassaemia in Pakistani population have widely variable results (Table 2.2). Farzana et al, (1975) reported 2.6% incidence in 610 healthy blood donors and medical staff at a hospital in Karachi. In another study of 1224 adults from Karachi, Hashmi and Farzana (1976) found 1.4% carrier rate for β-thalassaemia. A study on 67 Pakistanis in Paris reported an incidence of 3.0% (Coquelete et al, 1983). Latif (1983) found 9.6% carrier rate for β-thalassaemia in a selected sample of 437 individuals from Lahore who had hypochromic and microcytic anaemia. In another study Hameed and Chaudhry (1984) found a carrier rate of 1.6% in 300 healthy adults from Lahore. Ihsanullah et al, (1985) found 6.3% β-thalassaemia carriers amongst 256 individuals in a hospital based study at Karachi. The carrier rate amongst the British Pakistanis is around 6% (Modell and Berdoukas 1984). Dash (1985) has found 3.6% carrier rate for β-thalassaemia in 2000 healthy blood donors from East Punjab in India. In a more recent population based study, conducted at the Armed Forces Institute of Pathology Rawalpindi by Khattak and Saleem (1992a), 5.4% of the 500 individuals were found to be carriers of β-thalassaemia. The later study also identified that the carrier rate in Punjabis was 3.3% and amongst Pathans it was 8.0%. A recent survey of 1000 individuals from Rawalpindi showed 3.9% carrier rate for β-thalassaemia (Hassan et al, 1997).

Variability in results of the studies on thalassaemia carrier rate is mostly due to lack of adequate laboratory facilities, technical difficulties in carrier detection, and inappropriate selection of the target population (Khattak 1987). WHO (1985) has estimated that the overall carrier rate of β-thalassaemia in Pakistan is 5%.

α-thalassaemia:

Little is known about the prevalence of α-thalassaemia in Pakistan. Modell and Berdoukas (1984) have anticipated that the carrier rate for α^+-thalassaemia (-α/αα) may be as high as 50% and about 6% of the population may be homozygous for α^+-genotype (-α/-α).

Population based data on α-thalassaemia in Pakistan are very scanty (Table: 2.2). Khan and Hayee (1986) screened 320 cord blood samples for red cell indices and Hb-Barts at a hospital in Lahore. They found low MCV and MCH with raised level of Hb-Barts, indicating α-thalassaemia trait, in only 3 (1%) of the new born babies. In another study of Hb-Barts in the cord blood of 500 new born babies in a hospital at Rawalpindi 2.4% had raised level indicating a carrier status for α-thalassaemia (Zuhur-ur-Rehman 1991). Hb-H disease is an uncommon disorder in Pakistan. At the Armed Forces Institute of Pathology, Rawalpindi, each year approximately 150 new cases of β-thalassaemia major are diagnosed and during the same period approximately 3-4 cases of Hb-H disease are also seen (Saleem 1996).

Abnormal haemoglobins:

Lehman and Ager (1961, cited by Stern et al, 1968), were the first to describe sickle haemoglobin in a native of Pakistan. The study of 129 Pathans by Stern et al, (1968) also revealed an individual having Hb-D Punjab. Saleem (1974) reported compound heterozygotes of Hb-S/β-thalassaemia and Hb-E/β-thalassaemia in Punjabi subjects. Hashmi and Farzana (1976) found that 0.9% of 1224 individual from Karachi were carriers for Hb-D (0.65%), Hb-E (0.16%), and Hb-S (0.08%). The largest study of 5000 individuals from Pakistani Armed Forces by Sharma et al, (1976) showed that 39 (0.78%) had an abnormal haemoglobin including Hb-D (0.42%), Hb-E (0.18%), and Hb-S (0.18%). A recent study of 500 healthy individuals from the northern parts of Pakistan showed that 1.2% carried Hb-D trait, and 0.2% were carriers of Hb-E (Khattak and Saleem 1992b).

β-thalassaemia major:

Raheemtoola (1960) was the first to document a case of Cooley's anaemia in Pakistan. Since then relatively few studies have described thalassaemia major (Saleem 1974, Raheemtoola 1981, PMRC 1982, Saleem et al, 1985).

Table: 2.2. Studies on the carrier rate of thalassaemia in Pakistani population.

Disorder:	Ethnic group:	Type of study:	Place:	Subjects:	Methods used:	Carriers:	95% CI	Reference:
β-thalassaemia	Pathan	Population based	Peshawar	129	CEM Electrophoresis	5 (3.9%)	0.56-7.24%	Stern et al, 1968
β-thalassaemia	Mixed, all groups	Population based	Karachi	1224	CEM Electrophoresis	17 (1.4%)	0.73-2.04%	Hashmi and Farzana 1976
β-thalassaemia	?	Population based	Paris	67	CEM Electrophoresis	2 (3.0%)	1.08-7.08%	Coquelet et al, 1983
β-thalassaemia	Mostly Punjabi	Hospital based	Lahore	437	CEM Electrophoresis	42 (9.6%)	6.83-12.36%	Latif 1983
β-thalassaemia	Mostly Punjabi	Population based	Lahore	300	CEM Electrophoresis	5 (1.6%)	0.24-3.16%	Hameed and Chaudhry 1984
β-thalassaemia	?	Hospital based	Karachi	256	CEM Electrophoresis	16 (6.3%)	3.24-9.16%	Ihsanullah et al, 1985
β-thalassaemia	?	?	London	?	?	? (6%)	?	Modell and Berdoukas 1984
β-thalassaemia	Mixed, all groups	Population based	Rawalpindi	500	CEM Electrophoresis	27 (5.4%)	3.42-7.38%	Khattak and Saleem 1992a[@]
β-thalassaemia	Punjabi	Population based	Rawalpindi	245	CEM Electrophoresis	8 (3.3%)	1.14-5.46%	Khattak and Saleem 1992a[@]
β-thalassaemia	Pathan	Population based	Rawalpindi	201	CEM Electrophoresis	16 (8.0%)	4.26-11.74%	Khattak and Saleem 1992a[@]
β-thalassaemia	Mixed	Hospital based	Rawalpindi	1000	CEM Electrophoresis	39 (3.9%)	2.7-5.1%	Hassan et al, 1997
α-thalassaemia	Mostly Punjabi	Population based	Lahore	320	CEM Electrophoresis	3 (0.94%)	0.12-2.0%	Khan and Hayee 1986
α-thalassaemia	Mixed	Population based	Rawalpindi	500	CEM Electrophoresis	12 (2.4%)	1.06-3.74%	Zuhur-ur-Rehman et al, 1991

CEM: Cellulose acetate membrane electrophoresis.
CI: Confidence interval.
[@] Same study (total subjects are 500 that included 245 Punjabi, 201 Pathans, and 54 other groups.

Estimated number of thalassaemia major cases in Pakistan:

The number of cases of a recessive disorder in a population can be calculated from its carrier rate by Hardy Weinberg law which states that $p^2 + q^2 + 2pq = 1$, where p and q are the frequencies of the two alleles under question. Hardy Weinberg equation needs a correction for consanguineous marriages. In a population where mating is not random, the proportion of homozygotes is increased by Fpq. Where F is the inbreeding coefficient (Bodmer and Cavalli-Sforza 1976).

Alwan and Modell (1997) have estimated that in Pakistan with a population of 115 million and 3% annual birth rate, 5% carrier rate for β-thalassaemia, and 50% consanguineous marriages, a little over 3000 β-thalassaemia homozygotes will be born each year (Fig: 2.2).

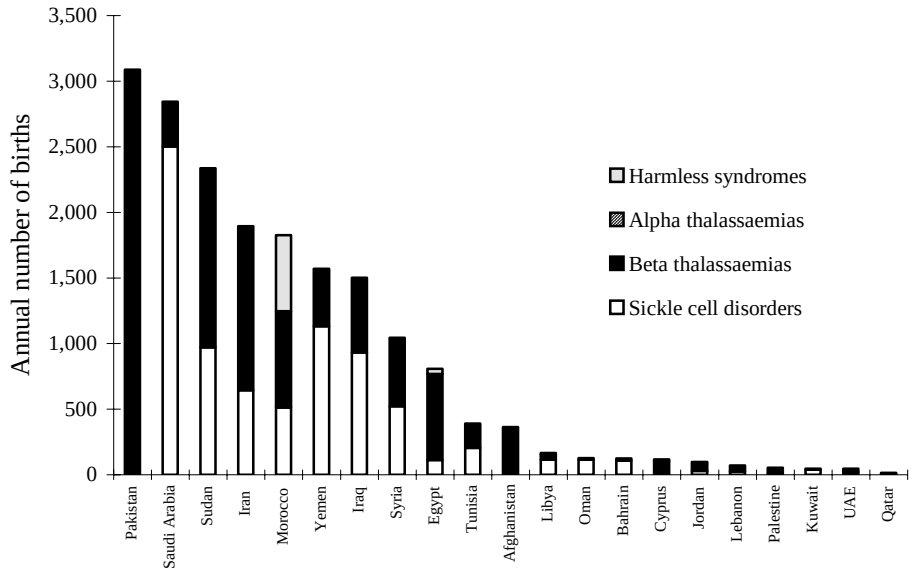

Fig: 2.2. Annual number of infants born with a haemoglobin disorder in the Eastern Mediterranean region (Total=18,600) (Alwan and Modell 1997).

Treatment facilities for thalassaemia:

The Government has paid very little attention to thalassaemia (Saleem 1996). Consequently, there is a lack of awareness amongst the general public and the health professionals. Fatimid Foundation, a charity organization working since 1982, is providing blood transfusion services to over 6000 of its registered patients. This organization runs its centres in Karachi,

Lahore, Multan, and Peshawar. A few other charity organizations in different parts of the country look after an additional 1500 registered cases. Fig: 2.3 summarizes the total number of registered cases in various cities of the country. The registered cases are less than 25% of the estimated 30,000 total in the whole of the country.

Saleem (1996) has comprehensively reviewed thalassaemia at the National level. Retrospective analysis of over 900 cases seen at the Armed Forces Institute of Pathology (AFIP) Rawalpindi showed that the average number of new cases diagnosed per year has steadily increased from 58 in 1984 to 173 in 1995. This is primarily due to increasing awareness amongst the local medical practitioners who refer the cases. The mean age at presentation was 32 months. Most of the children were severely anaemic and had developed moderate to marked hepato-spleno-megaly. Most patients come from the rural areas or smaller cities where specialist doctors and adequate facilities for diagnosis are not available. Therefore, a substantial delay in reaching a specialized medical centre for treatment is not unusual.

The quality of treatment that is provided to these patients is far from ideal or even optimum. There is no audit on the treatment protocols being followed at the transfusion centres. Pre-transfusion haemoglobin of patients receiving regular blood transfusions at one of the treatment centres (Fig: 2.4) showed a mean level of 6.5 g/dl with values ranging between 3.0-9.5 g/dl (Saleem 1996). A low pre-transfusion haemoglobin is due to poorly developed transfusion services, inadequate voluntary blood donations, and the lack of awareness amongst the treating physicians and the affected parents about the benefits of hyper-transfusion regimen.

A survey of thalassaemic children receiving treatment at a centre in Pakistan showed that 33% had never received iron chelation, 42% were occasionally getting one injection of desferal at the time of transfusion, 18% were getting desferal at irregular intervals and only 1.5% were on regular desferal therapy (Saleem 1996). The importance of screening of blood for hepatitis B and C has just started to be realized. In a recent study by Bhatti (1995) it was shown that 60% of the multiply transfused thalassaemics are positive for Hepatitis-C.

Transmission of HIV, fortunately, is not a significant problem at least in the northern areas of Pakistan (Tariq and Hussain 1995).

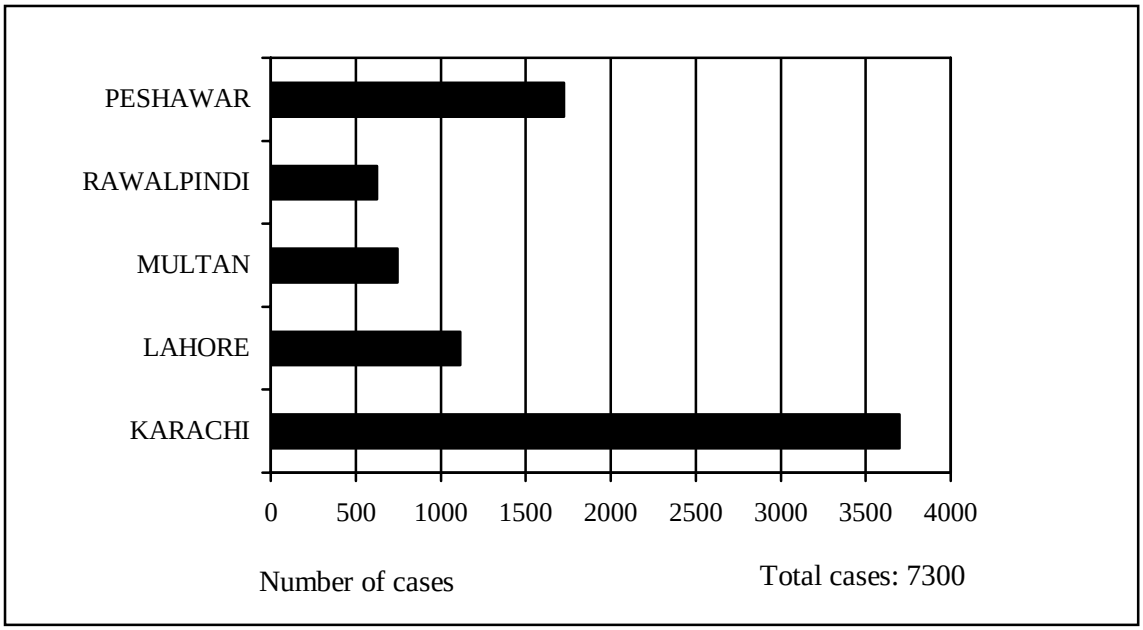

Fig: 2.3. The number of registered cases of β-thalassaemia major in various cities of Pakistan (Saleem 1996).

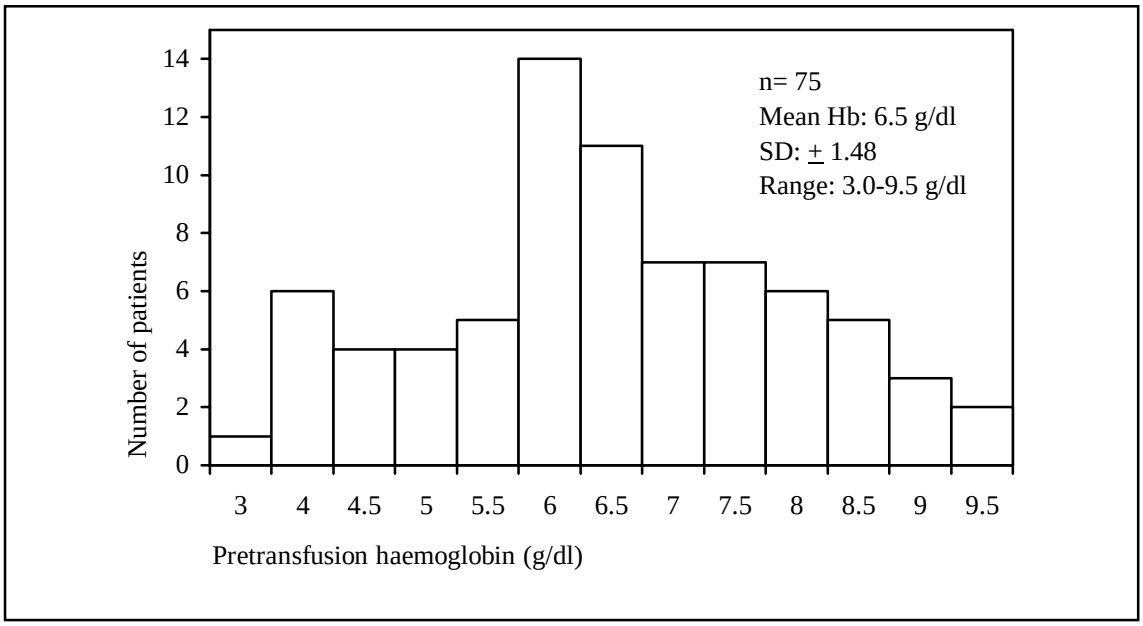

Fig: 2.4. Frequency distribution of pretransfusion haemoglobin in 75 patients of β-thalassaemia major at one of the treatment centres in Pakistan (Saleem 1996).

Poor management of thalassaemics is reflected by an average survival of 10.5 years (Fig 2.5) in 76 deceased children getting treatment at one of the centres in Pakistan (Saleem 1996). The average survival of 10.5 years is for patients who receive treatment, whereas at least 2/3rd of thalassaemics in Pakistan do not have an access to a treatment centre and they probably die during very early childhood. As a result of poor survival and poor quality of life the affected families experience tremendous psycho-social burden.

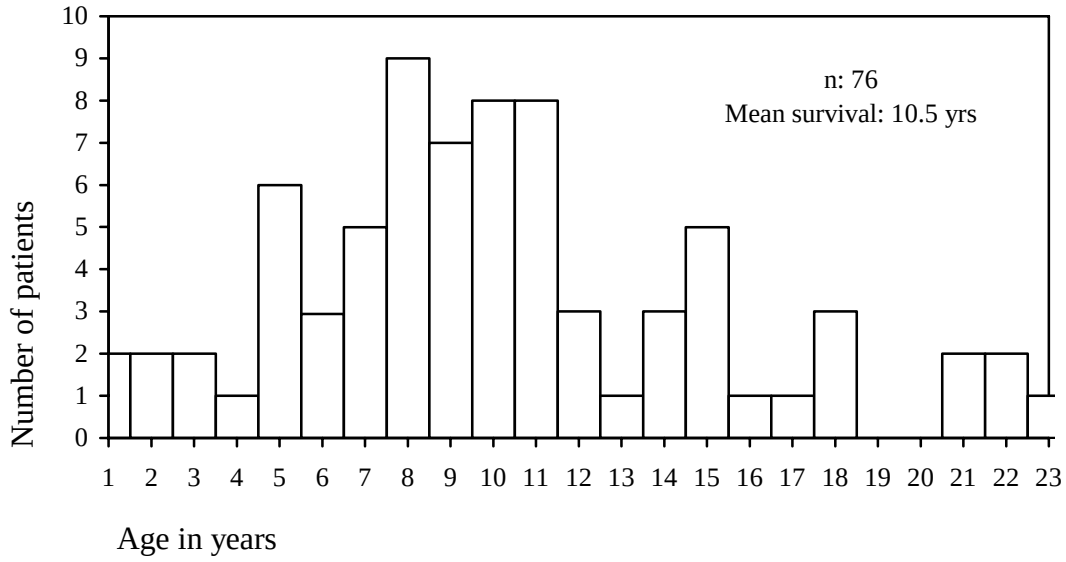

Fig: 2.5. Age distribution at the time of death in 76 patients with thalassaemia major who received blood transfusions at one centre in Pakistan (Saleem 1996).

Thalassaemia and consanguineous marriage:

When a recessive disorder is common, 5% β-thalassaemia carriers in Pakistan for example, the carriers have a relatively high risk of marrying another carrier however they choose their partner, and the risk is approximately doubled in a first cousin marriage (Modell and Kuliev 1992). Saleem (1996) in a retrospective analysis of 602 patients of β-thalassaemia major observed that the parents of 357 (59%) patients were 1st cousins, 145 (24%) had more distant related parents, and only 100 (17%) had completely unrelated parents.

Prevention of thalassaemia in Pakistan:

There is very little awareness about the concept of prevention of thalassaemia amongst the health professionals and the general public in Pakistan. Genetic counselling is available to only a fraction of the couples attending various treatment centres. Carrier screening although available at many private laboratories is not widely used. The main reason for the under utilization of the screening facilities is the lack of awareness amongst the health professionals dealing with thalassaemic families and the high cost. The quality of work at the private laboratories is also dubious. There is a general lack of counselling of the carriers. Inadequate counselling of the affected families is also due to the non-availability of prenatal diagnostic facilities within the country.

Alwan and Modell (1997) have calculated (Fig: 2.6) that the number of pregnancies at risk of a clinically significant haemoglobin disorder in Pakistan is in excess of 12000 every year. These figures are the highest for β-thalassaemia in the Eastern Mediterranean Region. Screening of such large number of pregnancies would require tremendous financial and administrative resources. The first step would be to identify a pregnancy at risk. In a Pakistani setting, where the majority of the pregnancies escape medical attention, it will be a challenging task. The next problem would be to establish centre(s) where facilities for prenatal diagnosis are available. This would also involve in depth studies of β-thalassaemia mutations in all ethnic groups of Pakistan and a cost-effective strategy for carrying out prenatal diagnosis. Varawalla et al, (1991a; 1991b), have studied the mutations causing β-thalassaemia in 167 subjects from the northern region of Pakistan. They have identified seven different mutations in the study population (Fig: 2.7).

Another important issue that would determine the success of a thalassaemia prevention programme in Pakistan would be the attitude of the affected couples towards prenatal diagnosis and termination of pregnancy. In the Mediterranean, extensive data now exists about the relative acceptability of the options open to the at risk couples. The absence of equivalent objective information for the predominantly Muslim countries of the Eastern Mediterranean Region, including Pakistan, introduces considerable uncertainty about the success of a prevention programme (Alwan and Modell 1997). Some information about the attitude of the British Pakistanis towards prenatal diagnosis and termination of pregnancy is

available. The British Pakistanis have a greater difficulty requesting prenatal diagnosis than most other ethnic groups in the UK. However, when couples are adequately counselled, they often make use of prenatal diagnosis, although they have particular problems with late termination of pregnancy (Petrou 1994).

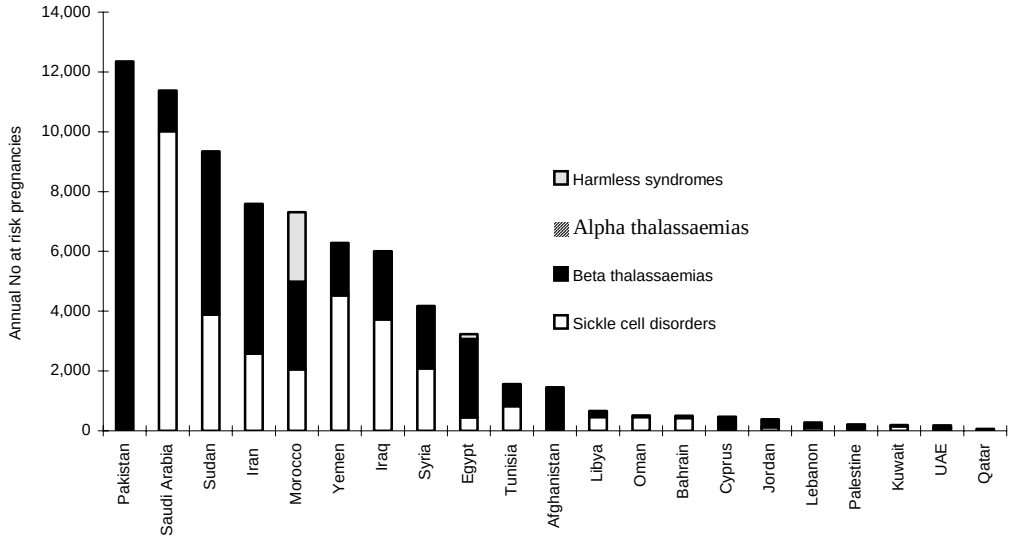

Fig: 2.6. Annual pregnancies at risk of a haemoglobin disorder in the Eastern Mediterranean region (Total=74,250) (Alwan and Modell 1997).

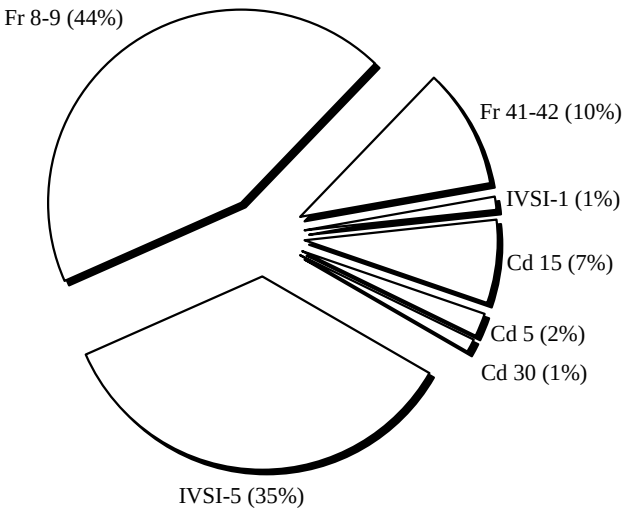

Fig: 2.7. β-thalassaemia mutations in 167 individuals from the Northern region of Pakistan (Varawalla et al, 1991a and b).