4 Epidemiology of haemoglobin disorders

Introduction:

Haemoglobin disorders are the commonest inherited disorders in Pakistan. Analysis of the situation is essential before a large scale community based prevention programme for these disorders can be initiated (Alwan and Modell 1997). The results of several studies on haemoglobin disorders in Pakistan are available (Stern et al, 1968; Hashmi and Farzana 1976; Sharma et al, 1976; Latif 1983; Saleem et al, 1985). But many of these surveys have limitations regarding the methods used, and the selection of the subjects (Khattak 1987). This chapter aims at studying the epidemiology of haemoglobin disorders in the major ethnic groups of Pakistan. The information is used to provide a basis for calculating the number of affected children born each year.

Subjects:

In this study 1345 adult males from the five major ethnic groups of Pakistan were screened for β-thalassaemia. Due to resource constraints haemoglobin electrophoresis was done only in those cases where Hb-A_2 estimation was required i.e. the cases that had MCV ≤75 fl or MCH ≤ 25pg. The subjects included, 290 (21.6%) Punjabi, 307 (22.8%) Pathan, 223 (16.6%) Sindhi, 300 (22.3%) Baluchi, and 225 (16.7%) Mohajirs. The subjects were sampled at several different places and the staff was provided by the local military hospitals. The samples from Punjabis were collected from an infantry battalion stationed at Rawalpindi. The samples from Sindhis and Baluchis were collected at the regimental centres in Hyderabad and Quetta respectively. The samples from Pathans were collected from the students of Khyber Medical College, Peshawar by a team of two volunteer doctors. The samples from Mohajirs were collected at Karachi by a mobile team of two volunteers who went from door to door for collection of samples. These samples were transported to AFIP, Rawalpindi within 12 hours of collection.

α-thalassaemia screening was carried out in one hundred randomly selected patients with β-thalassaemia major. These included 67 Punjabis and 43 Pathans. In addition, red cell indices from 264 cases of heterozygous β-thalassaemia, diagnosed by PCR, were investigated for any co-incidental α-thalassaemia. The later group of subjects comprised of the couples who requested prenatal diagnosis for thalassaemia and they all had at least one affected thalassaemic child.

Results:

β-thalassaemia:

The haematological values of 1345 adult males from the five ethnic groups are presented in Table: 4.1. The mean Hb for each ethnic group varied from 13.7 to 15.0 g/dl. Baluchis had the highest mean Hb (15.0 SD ±1.5). The number of individuals who had Hb <13.5 g/dl, varied from, 94/290 (32.4%) in Punjabis, 102/307 (33.2%) in Pathans, 98/223 (43.9%) in Sindhis, 41/300 (13.7%) in Baluchis, to 77/225 (34.2%) in Mohajirs. There were 164/1345 (12.2%) subjects who had MCV ≤75 fl or MCH ≤25 pg and required Hb-A_2 estimation (Table 4.2). Raised Hb-A_2 was found in 71/164 (43.3%) cases selected by low red cell indices. Molecular genetic analysis by multiplex PCR was done in 15 out of the remaining 96 cases because these subjects in addition to low red cell indices (MCH ≤ 25 pg or MCV ≤ 75 fl) also had Hb <9.0 g/dl. This analysis did not find any carriers.

The results of carrier screening in the ethnic groups are summarized in table 4.2. There were 71/1345 (5.3%) carriers of β-thalassaemia in all ethnic groups (95% confidence limit 4.1-6.5%). The proportion of β-thalassaemia carriers out of the individuals who had hypochromic microcytic red cell indices ranged from 39% in Punjabis, 53% in Pathans, 14% in Sindhis, 42% in Baluchis, and 52% in Mohajirs. The carrier rate in the ethnic groups was as follows: Punjabis 4.5%, Pathans, 5.2%, Sindhis 1.3%, Baluchis 9%, and Mohajirs 5.2%.

Abnormal haemoglobins:

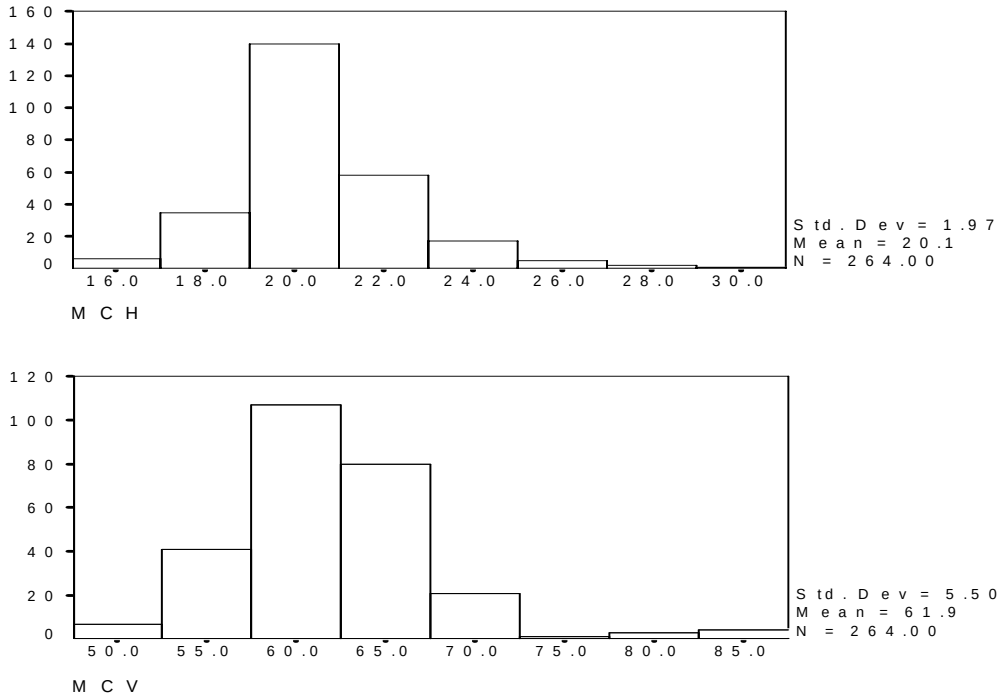
Since haemoglobin electrophoresis was done only in cases that had MCV ≤75 fl or MCH ≤25 pg, many cases of abnormal haemoglobins could not be identified. Out of the 57

Baluchi subjects, in whom electrophoresis was done, 5 (8.7%) were found to have heterozygous Hb-S. Similarly, 2/30 (6.6%) Pathans, in whom electrophoresis was done, had heterozygous Hb-S. No other abnormal haemoglobin was detected in any of the ethnic groups.

α-thalassaemia:

Heterozygous -$\alpha^{3.7}$ (-α/αα) deletion was found in 5/100 (5%) cases. One case with homozygous -$\alpha^{3.7}$ (-α/-α) deletion was also seen. No case of -$\alpha^{4.2}$ deletion was observed.

The red cell indices of 264 β-thalassaemia carriers confirmed by ARMS PCR showed that 259/264 (98%) had MCH ≤ 25 pg while 256/264 (97%) had MCV ≤ 75 fl (Fig: 4.1). In the cases with MCV >75 fl or MCH > 25 pg, 5/264 (1.9%) had Cap+1 (A-C) mutation and 4/264 (1.5%) had a severe β-thalassaemia mutation. The subjects with the Cap+1 mutation had normal Hb-A_2 whereas the level in the other 4 subjects was ≥3.5%. The later four cases were suspected to have coincidental α-thalassaemia trait.



ig: 4.1 Histogram of MCV and MCH in 264 cases of β-thalassaemia trait proven by PCR.

Table: 4.1. Haematological parameters in 1345 adult males from the five major ethnic groups of Pakistan.

Ethnic group:	**Hb (g/dl):**		**TRBC ($X10^9$/L):**		**MCV (fl):**		**MCH (pg):**	
	Range:	**Mean ± SD:**	**Range:**	**Mean ± SD:**	**Range:**	**Mean ± SD:**	**Range:**	**Mean ± SD:**
Punjabi: (n=290)	7.0-16.8	13.9 ± 1.9	3.0-6.2	4.95 ± 0.54	54-101	85.2 ± 7.7	16-34	28.1 ± 3.3
Pathan: (n=307)	7.6-19.8	14.2 ± 1.7	4.0-6.9	5.10 ± 0.51	61-103	87.5 ± 7.1	17-35	29.1 ± 3.1
Sindhi: (n=223)	9.4-18.4	13.7 ± 1.6	3.4-7.2	4.83 ± 0.53	64-110	87.4 ± 7.6	18-39	28.6 ± 3.2
Baluchi: (n=300)	8.9-19.4	15.0 ± 1.5	3.9-7.2	5.41 ± 0.55	59-108	83.1 ± 8.0	19-36	28.1 ± 3.1
Mohajir: (n=225)	9.6-17.3	14.6 ± 1.6	3.8-7.0	4.91 ± 0.53	60-102	85.1 ± 7.2	18-33	29.2 ± 3.0
All groups: (n=1345)	7.0-19.8	14.3 ± 1.7	3.0-7.2	5.04 ± 0.53	54-110	85.7 ± 7.5	16-39	28.6 ± 3.1

Table: 4.2. Frequency of hypochromic and microcytic red cell indices and the carrier rate for β-thalassaemia in the five major ethnic groups of Pakistan.

Ethnic group:	**MCV≤75fl or MCH ≤25pg:**	**β-thalassaemia carriers:**			**95% Confidence interval:**
		Number:	**% of low MCV or MCH**	**% of total**	
Punjabi: (n=290)	33 (11.3%)	13	39.4%	4.5%	2.1-6.9%
Pathan: (n=307)	30 (9.8%)	16	53.3%	5.2%	2.7-7.7%
Sindhi: (n=223)	21 (9.4%)	3	14.3%	1.3%	0-2.8%
Baluchi: (n=300)	57 (19.0%)	27	47.4%	9.0%	5.7-12.3%
Mohajir: (n=225)	23 (10.2%)	12	52.2%	5.2%	2.3-8.1%
All ethnic groups: (n=1345)	164 (12.2%)	71	43.3%	5.3%	4.1-6.5%

Discussion:

This study is a population based survey of thalassaemia in the ethnic groups in Pakistan. Its main strengths are appropriate sample selection, representation by the major ethnic groups, and reliable methodology. However, its limitations include a relatively small sample size and the inability to screen for the abnormal haemoglobins.

Screening for thalassaemia:

β-thalassaemia trait can be suspected when MCV and/or MCH are low. Its main differential diagnosis includes iron deficiency and α-thalassaemia trait. There is a considerable overlap in the haematological picture of the three conditions. β-thalassaemia trait in most situations can be confirmed by raised Hb-A_2 and iron deficiency can be confirmed by serum ferritin (Steinberg and Adams III 1991). Identification of α-thalassaemia trait, however, requires either globin chain synthesis ratios or DNA analysis (Higgs et al, 1989). The distinction between the three conditions is important not only for treatment but also for identification and subsequent counselling of carriers. Iron deficiency is by far the commonest cause of low MCV and MCH in Pakistanis. This impression, unfortunately, has cast a shadow on the importance of low MCV and MCH caused by thalassaemia.

In this study 12% of the individuals were found to have MCV $\leq$75 fl and/or MCH $\leq$25 pg and 43% of the later population had β-thalassaemia trait. It shows that β-thalassaemia, at least in the adult healthy males, is a significant cause for low MCV and MCH. The frequency of low MCV and MCH was similar amongst all ethnic groups except in Sindhis where although 9% had this abnormality only 1.3% had β-thalassaemia trait. This may be due to concomitant α-thalassaemia in Sindhis that can mask the red cell indices in β-thalassaemia carriers (Kanavakis et al, 1982). Study of red cell indices in the British Pakistanis shows that about 11% of the adult males have MCH <23 pg, of whom about half have β-thalassaemia trait (Modell and Berdoukas 1984). MCH data from the adult male population of this study also confirm this observation (Fig: 4.2). Modell and Berdoukas (1984) have suspected that most Pakistanis who have low MCH but do not have β-thalassaemia trait may have α-thalassaemia trait. The results of low red cell indices in this study are in agreement with those of Modell and Berdoukas (1984). However, it is questionable whether all individuals with low MCV or MCH who are not β-thalassaemia carriers have α-thalassaemia because many of them might

also be iron deficient. Screening for the common α-thalassaemia determinants in 100 individuals investigated in this study showed that 6% carried -$\alpha^{3.7}$ deletion (-α/αα:5 and -α/-α:1). The study of α-thalassaemia although very small indicates that the majority of the individuals who have low MCV or MCH and are not β-thalassaemia carriers may have iron deficiency. Population-based data on α-thalassaemia in Pakistan are very scanty. The only two studies by Khan and Hayee (1986) and Zahur-ur-Rehman et al, (1991) show carrier rates of 0.94% and 2.4% respectively. These studies also support that α-thalassaemia is not a significant factor in causing low MCV or MCH in the Pakistanis.

The carrier rate for deletional α-thalassaemia-2 was found to be 6%. At this rate one would expect 0.2% of the population to be homozygous for α-thalassaemia-2. However, red cell indices in 264 β-thalassaemia carriers (confirmed by PCR) showed that 97% had MCV≤75fl and 98% had MCH ≤25pg. In the 3% with red cell indices in the normal range half had Cap+1 mutation. This suggests that interference due to co-inheritance of α-thalassaemia (-α/-α) may be present in only 1.5% of the cases. If the deletional forms of α-thalassaemia is seen in 0.2% then the remaining 1% must be non-deletional in type. The combined incidence of deletional as well as non-deletional forms of α-thalassaemia in Pakistan may be around 20%, a rate at which about 1.5% of the population may be homozygous for α-thalassaemia trait (deletional or non-deletional).

In populations where α-thalassaemia is common it can cause significant problems in identification of β-thalassaemia carriers (Kanavakis et al, 1982). Another diagnostic problem may be caused by silent mutations (Cao et al, 1994). The results of this study indicate that both α-thalassaemia and silent mutations may not be very significant problem in Pakistani population. However, in working out a strategy for detection of at risk couples it may be useful to investigate by DNA methods all those individuals whose partners are known carriers of β-thalassaemia.

Modell and Berdoukas (1984) suggested that for all practical purposes the finding of MCH ≥25 pg should exclude β-thalassaemia trait in Pakistanis. The same cut off limit for MCH was used in this study for identifying individuals requiring Hb-A_2 estimation. This approach,

although cost effective, might have missed about 3% of β-thalassaemia carriers who either had concomitant α-thalassaemia or silent mutations. However, in an epidemiological survey this would not make a significant difference.

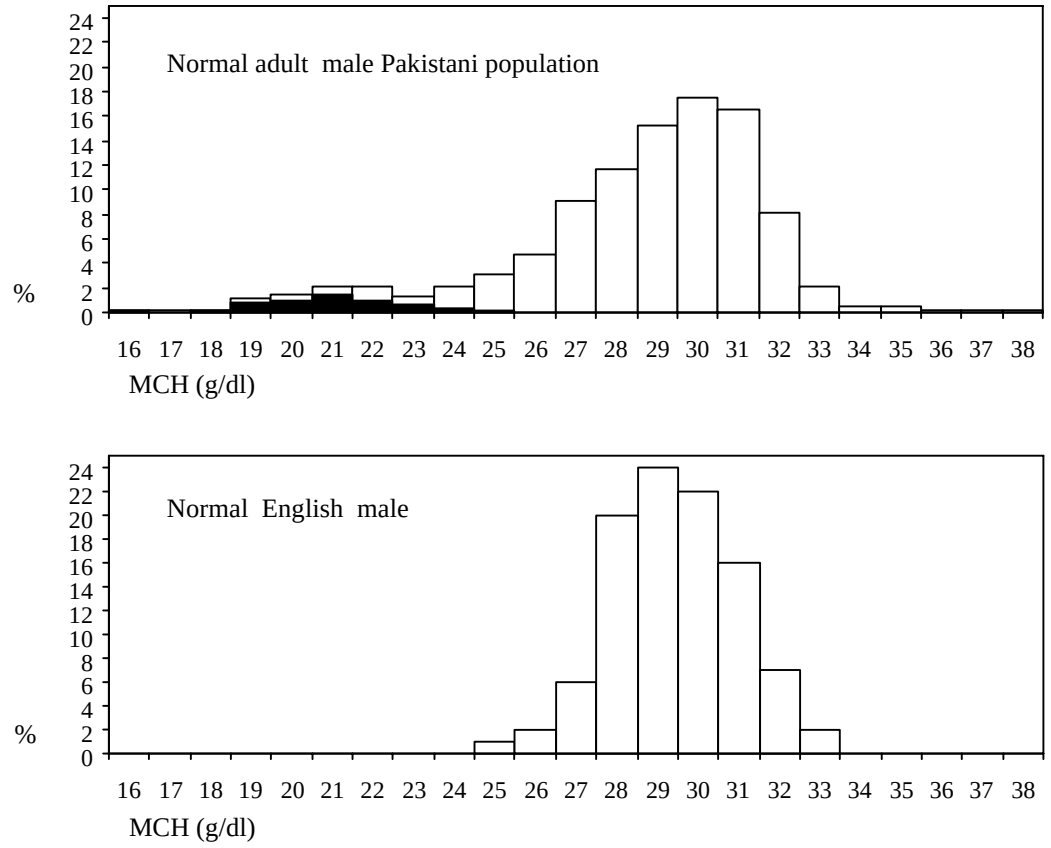


Fig: 4.2. A comparison of MCH values from the normal adult male Pakistanis (this study) and a similar English population (Modell and berdoukas 1984). The Pakistanis have a long tail in the low MCH region. The solid area in the histogram represents the proportion of β-thalassaemia carriers.

β-thalassaemia carrier rates in the ethnic groups:

The study by Khattak and Saleem (1992a) gave an indication that the β-thalassaemia carrier rate is different in Punjabis (3.3%) and Pathans (8.0%) (Table: 4.1). Apart from the later study no information is available on the carrier rate in any of the ethnic groups in Pakistan. It is important to investigate the carrier rate in each group because β-thalassaemia genes may be unevenly distributed amongst the ethnic groups. Moreover, the frequency of consanguineous marriage is also different between the ethnic groups (Bittles 1994; Wahab and Ahmad 1996). This could affect the calculation of annual birth rate of thalassaemia major in each group. The overall β-thalassaemia carrier rate of 5.3% (95% confidence limits: 4.1-6.5%) found in this

study is in agreement with the generally accepted figures (WHO 1985). The carrier rate in the ethnic groups varied considerably. The rate was lowest in Sindhis (1.3%) and highest in Baluchis (9%). The carrier rate in Sindhis may be spuriously low due to coincident α-thalassaemia. This issue, however, remains open for further investigations. Baluchis are a well-defined group who are mostly descendants of a few tribes that originally migrated from Iran to their present location in 700 B.C. (Bokhari 1975). It is likely that the high gene frequency for thalassaemia in Baluchis is due to a founder effect (Bodmer and Cavalli-Sforza 1976).

Abnormal haemoglobins:

A major limitation of this study is the inability to screen for abnormal haemoglobins. However, other published reports indicate that abnormal haemoglobins are not a significant problem as compared to β-thalassaemia. Hashmi and Farzana (1976) found the following carrier rates in 1224 individuals from Karachi: Hb-D 0.65%, Hb-E 0.16%, and Hb-S 0.08%. The largest study of 5000 Pakistani Armed Forces personnel 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 study of 500 individuals from the northern parts of Pakistan showed that 1.2% carry heterozygous Hb-D, and 0.2% were carriers of Hb-E (Khattak and Saleem 1992b).

Calculation of the number of annual births of thalassaemia major:

Carrier frequency of thalassaemia can be used to calculate the annual number of births of affected children. This information is essential prerequisite in planning a national programme for control and prevention of the disorder (Alwan and Modell 1997).

Theoretical considerations:

The annual birth rate for thalassaemia major can be calculated by the Hardy Weinberg analysis of the carrier rate (Bodmer and Cavalli-Sforza 1976). According to the Hardy Weinberg law, the number of heterozygotes and the homozygotes for a trait in a randomly mating population is: $p^2 + q^2 + 2pq = 1$. Where p and q are the frequencies of the two alleles under study. If p and q are the frequencies for the normal and β-thalassaemia genes in a population then p^2, q^2 and $2pq$ will represent the proportion of normals, homozygotes and heterozygotes respectively. In a population where mating is not random, the proportion of

heterozygotes is reduced in comparison to Hardy-Weinberg proportions by an amount *2Fpq*, while that of homozygotes is increased by *Fpq* where *F* is the inbreeding coefficient. The actual number of homozygotes will be equal to $q^2 + Fpq$ (Bodmer and Cavalli-Sforza 1976).

Taking the carrier rate for β-thalassaemia as 5% (0.05) its gene frequency (*p*) will be 0.025 (1/2 of the carrier rate because each carrier has one normal and one β-thalassaemia gene) and the frequency of normal gene (*q*) will be 1-*p* (0.975). The number of β-thalassaemia homozygotes (p^2) per thousand will be 0.625. This figure would require a correction for the frequency of consanguineous marriages. The average inbreeding coefficient (*F*) of the Pakistani population is 0.028 (Bittles 1994). The actual number of β-thalassaemia homozygotes will be equal to $p^2 + Fpq$ i.e. 0.625 + 0.683 = 1.308 per thousand.

Calculations based on the already published work:

Several studies have focused attention on carrier rate of β-thalassaemia in Pakistan. Table 2.2 gives a summary of the studies so far carried out on Pakistanis. Stern et al, (1968) were the first to report a 4% carrier rate for β-thalassaemia amongst 129 Pathans. The studies conducted thereafter have reported carrier rates ranging from 1.4-9.6%. Variability in the results 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) have reported an overall β-thalassaemia carrier rate of 5%.

The estimated number of annual thalassaemia major births in Pakistan, based on 5% carrier rate in 137 million population with annual birth rate of 2.8% (Economic survey 1995-1996) and a coefficient of inbreeding of 0.028 (Bittles 1994) will be 5014. This estimate is based on the assumption that the carrier rate and the frequency of consanguineous marriage in all ethnic groups is similar.

Revised calculations based on the results of this study:

This study shows that the carrier rates amongst the ethnic groups are different. The coefficient of inbreeding also varies considerably between the groups (Table: 2.1). It is therefore expected that estimates of the annual birth rate for thalassaemia major would

also be different in each ethnic group. Table: 4.3 gives a detailed picture of the calculation of number of annual births of thalassaemia major in each ethnic group. The population of Pakistan is estimated at 137 million and the annual birth rate is 2.8% (Economic Survey 1995-1996). The last census was carried out in 1981. The latest information on the population of ethnic groups is not available. For the purpose of calculation the population of each group has been taken in the same proportion as these were found in the 1981 census.

The revised calculations show 4550 annual new births of thalassaemia major (1.2/1000) in Pakistan (Table 4.3). The maximum number of births is expected in Punjabis (1.124/1000) which also is the largest ethnic group (80 million). The highest birth rate is expected in Baluchis (4.3/1000) due to a high carrier rate and high frequency of consanguineous marriages. The birth rate amongst Sindhis (0.324/1000) is low because of a very low carrier frequency (1.3%) found in this group. As discussed earlier the carrier rate in Sindhis might be spuriously low because of possible coincidental α-thalassaemia. Unpublished data from the Fatimid thalassaemia centre in Karachi, that looks after the majority of Sindhi patients, suggests that the number of Sindhi patients with thalassaemia are not less than the patients from other ethnic groups.

Consanguineous marriage and thalassaemia:

Consanguineous marriage increase the frequency of recessive disorders. The increase, however, is most marked in disorders that are rare (Modell and Kuliev1992). Table: 4.3 gives an estimate of the number of thalassaemia births with and without correction for the consanguineous marriage. It is clear that the number of births in the absence of consanguineous marriage is lower (2442) than in their presence (4550). It is apparent that even in the complete absence of consanguineous marriage there would still be approximately 2500 annual births of thalassaemia major.

Table: 4.3. Calculation of the expected number of births of children with β-thalassaemia major per year in the five ethnic groups.

Ethnic group:	**Population: (millions)**	**β-thalassaemia Carrier rate:**	**β-thalassaemia gene frequency (*p*)**	**Normal gene frequency (*q*)**	**Coefficient of inbreeding (*F*)[$]**	**Number of children with thalassaemia major per 1000 new births**		**Annual Birth rate (%)**	**Total new births per year (millions)**	**Number of children with thalassaemia major born per year with reference to the practice of customary consanguineous marriages**	
						p^2	$(p^2 + Fpq)$			Absent	Present
Punjabi	78	4.5%	0.0225	0.977	0.028	0.506	1.124	2.8%	2.184	1365	2454
Pathan	20	5.2%	0.026	0.974	0.0164	0.676	1.091	2.8%	0.56	378	610
Sindhi	15	1.3%	0.0065	0.994	0.0437	0.042	0.324	2.8%	0.42	18	136[£]
Baluchi	7	9.0%	0.045	0.955	0.0532	2.025	4.311	2.8%	0.196	397	844
Mohajir	15	5.2%	0.026	0.974	0.0209	0.676	1.205	2.8%	0.42	284	506
Total	135	-	-	-	-	-	-	-	-	2442	4550

[$] *F* values are based on Table: 2.1.
[£] See page 78 para 2 for comments.

Calculation of the number of patients with abnormal haemoglobins:

Since abnormal haemoglobins were not studied, their calculations are based on the already published data. The largest study of abnormal haemoglobins in Pakistanis is that of Sharma et al, (1976) which reported an incidence of 0.18% each for Hb-S and Hb-E. According to these figures it is estimated that only three new homozygotes each of Hb-S and Hb-E will be born every year if there was no consanguineous marriage. However, the expected number of homozygotes in the presence of consanguineous marriage is estimated at 99 each for Hb-S and Hb-E. The number of new births of compound heterozygotes of abnormal haemoglobins and/or thalassaemia per year is estimated at 368 (Hb-S/β-thalassaemia: 181, Hb-E/β-thalassaemia: 181, and Hb-E/Hb-S: 6). The correction for consanguineous marriages on the birth rate of compound heterozygotes is more difficult to apply. In fact, it is expected that consanguineous marriage would reduce their incidence. The total number of new cases of a clinically significant abnormal haemoglobins, including compound heterozygotes with thalassaemia, is expected to be 560.

The effect of consanguineous marriage on the birth rate of Hb-S and Hb-E is marked because both are rare in Pakistan. Only three homozygous SS or EE births per year are expected without any correction for consanguineous marriage as compared to 99 per year when the correction is applied. This highlights the importance of consanguineous marriages in affecting the birth rate of rare recessive disorders.

Estimated total number of patients and pregnancies at risk:

The number of births of a clinically significant haemoglobin disorder per year in Pakistan is estimated at 5110 (thalassaemia: 4550 and abnormal haemoglobins 560). This corresponds to an annual birth rate of 1.35/1000 new births. Thalassaemia major represents the bulk of the problem (Fig: 4.3). The estimated number of pregnancies at risk would be around 20440.

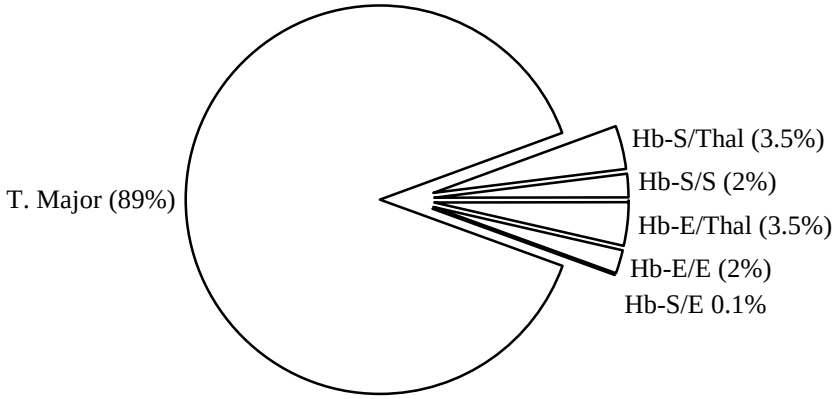


Fig: 4.3. The estimated annual births of a clinically significant haemoglobin disorder in Pakistan.