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Prevalence of β-thalassemic Patients Associated With Consanguinity and Anti-HCV - Antibody Positivity – A Cross Sectional Study

Qurat-ul-Ain, Laiq Ahmad*, Muhammad Hassan, Shahid Mahboob Rana and Farhat Jabeen
Faculty of Science and Technology, Department of Zoology, Government College University, Faisalabad, Pakistan

Abstract.- A cross sectional study was carried out at various hospitals of Faisalabad city. The aim of current study was to investigate the prevalence of β-thalassemia disease in hospital population related to age, consanguinity and anti-HCV antibody positivity. For this purpose, 300 patients were interviewed for the different parameters including specific type of β-thalassemia disease, their family history together with information about their sibs and offspring. Results demonstrated that males were significantly ($P<0.001$) more affected than females. In total hospital population, β-thalassemia major (97%) was more frequent than thalassemia intermedia (7%). The highest representation of β-thalassemic patients (34.33%) was observed between 3-5 years of age groups, whereas affected patients were the highest from 1st birth order (32.90%) and the lowest from 8th birth order (0.64%). The prevalence of anti-HCV antibodies positivity was 65.0% among all β-thalassemic patients. Distribution of β-thalassemia patients among different surnames showed the highest percentage in Rajpoots (25.62%) and lowest in Baloach (3.30%). In consanguine studies, outcome of first cousin marriages result was higher β-thalassemia affliction than in unrelated outcomes. The coefficient of inbreeding among parents of β-thalassemic patient ($F=0.0455$) was also observed high. In conclusion, higher total consanguinity rate and coefficient of inbreeding among thalassemic patients indicated genetic basis of the disease. However, higher anti-HCV positivity among patients reflects the poor facilities of transfusion.

Key words: ß-thalassemia major, ß-thalassemia intermedia, inbreeding, caste.

INTRODUCTION

Thalassemias are inherited disorders characterized by abnormal production of hemoglobin and associated with low hemoglobin production and excessive destruction of red blood cells.

The World Health Organization (WHO) recognizes thalassemia as the most prevalent genetic blood disorder in the world, found in more than 60 countries with a carrier population of upto 150 millions (Cao and Galanello, 2002). Approximately 250 million people worldwide are heterozygotes for β-thalassemia and at least 2,000,000 affected homozygotes are born annually, while some other reports have shown 3% to 10% of the world's population carries a thalassemic gene (Gupta *et al*., 2002; Premawardhena *et al*., 2004). The highest frequency of β-thalassemia trait is reported in Gujarat, followed by Sindh, Punjab, Tamil Nadu, South India and Maharashtra (Ambekar *et al*., 2001).

Pakistan has the highest number of children with transfusion dependent thalassemia in the world due to high frequency of the gene, consanguineous marriages, high birth rate, and large population size (Alwan and Modell, 1997). It has been estimated that over 4000 cases of transfusion dependent β-thalassemia major are born in Pakistan per year (Saleem *et al*., 1996). The carrier rate for β-thalassemia major in Pakistan is reported to be 5.3% (Ahmed, 1998) and in different regions it varies from 1.4 to 8.0% with an average of 5% (Ahmed *et al*., 2000). β-thalassemia is most prevalent in the provinces of Sindh and Baluchistan along the Arabian Sea coast and the North West Frontier Province (NWFP) (now Khyber Pakhtunkhwa) where populations from different parts of the world have settled during various periods in history (Khan *et al*., 1995).

The average life expectancy of β-thalassemic patients in Pakistan is 10 years and in 2003. The disease load was of 90,000 to 100,000 patients throughout the country (Lodhi, 2003) and in northern areas 83 % of the children suffering from

* Corresponding author: laeeq01@yahoo.com
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Refractory anemia has β- thalassemia (Saleem *et al.,* 1985). Number of older patients is less, while the number of younger patients is increasing due to increasing disease load and shortened life expectancy (Rahman and Lodhi, 2004).

Recent studies have suggested that low-income status and lack of awareness are also contributing in increasing the frequency of this disease (Sengupta, 2008). Other social factors, such as a preference to marry within the ethnic groups and consanguineous marriages; have also contributed to the increased incidence of this disease in the Pakistani population (Rafique and Moinuddin, 1991). β-thalassemia was prevalent in Punjabis (60.7%) followed by Saraikees (25.5%). Caste wise, it was most frequent in Rajputs followed by Jatts, Arian, Sheikhs and Pathans (Hafeez *et al*., 2007). Very high (>81%) consanguinity and low literacy rate are the risk factors for high incidence of β-thalassemia in South Punjab (Baig *et al*., 2005).

Current study was conducted to collect baseline information on different types of β-thalassemia in hospital population of Faisalabad. The study envisages such aspects as patient's age at diagnosis of thalassemia, inbreeding effects in association to different surnames. The prevalence of HCV positivity in multitransfused thalassemic patients was also included in this investigation.

MATERIALS AND METHODS

Data collection

The present study was carried out in specific units of thalassemia at various hospitals of Faisalabad city, *i.e.,* District Head Quarter Hospital, Allied Hospital, and Ali Zaib foundation center (Center for thalassemia patients) Faisalabad. Information was collected by interviewing the β-thalassemia patients or their close relatives. The collected information includes the diagnosis *i.e.* specific type of β-thalassemia disease, age at present, age at diagnosis, exact relationship between father and mother, their family history including information about their sibs and offspring's, the same or any other type of disease in the family and the associated disease process.

The data sample was also analyzed for the study of consanguinity. Genetic relationship in marriages was classified into first cousins (1 C), second cousins (2 C), distant relatives (DR), Bradri (B) and unrelated (U) as described by Shami and Iqbal (1983).

Statistical analysis

The statistical analyses carried out for the study included percentages, mean, standard errors of means, Z- test. Mean coefficient of inbreeding was calculated by Wright's methods (Wright, 1992).

RESULTS

The present study was carried out on β-thalassemia hospitalized population (300 patients) of Faisalabad including male (n=197) and female (n=103) patients. Among all patients, male were found significantly more affected with β-thalassemia major (TM) ($P<0.001$) and β-thalassemia intermedia (TI) ($P=0.0067$) compared to female (Table I). The incidence of β-thalassemia was the highest in 1st birth order (32.90%) and the lowest in 8th birth order (0.64%) (Table II). Thalassemia major patients were the highest (n= 146, 96.05%) in 1st birth order and the lowest in 8th birth order (n=26, 6.66%). However, thalassemia intermedia patients were the highest (n= 6, 3.94 %) in 1st order while the lowest (0%) in 5th and 7th birth orders. The incidence of β-thalassemia was significantly ($P<0.001$) higher in urban population (80.66%) than in rural population (19.33%). Almost 70% families have more than one β- thalassemic child (two or three) and there may be carriers and even clinically normal children. There were four β-thalassemic children living together from 16 families (5.39%) (Fig. 1A). The percentage distribution of TM and TI in rural areas were 93.10% and 6.89% respectively (Fig. 1B). Similarly, patients from urban area had more prevalence of TM (92.97%) compared to TI (7.02%).

Age related gender specific prevalence of β-thalassemia and anti HCV antibody positivity

Percentage distribution of β-thalassemia patients among eight different age groups, <2, 3-5, 6-8, 9-11, 12-14,15-17,18-20 and 21> years, separately in male and female, are presented in Table III.

Table I.- Percentile distribution of β-thalassemia major (TM) and intermedia (TI) in male and female with sex ratio/100 females.

β-thalassemia Types	Male patients % (n)	Female patients % (n)	Z-Test	P Value	Sex ratio/100 females
TM	92.38 (182)	94.17 (97)	7.11	<0.001	187.62
TI	7.60 (15)	5.82 (6)	2.47	0.0067	250.00
Total	65.66 (197)	34.33 (103)	7.59	<0.001	191.26

Table II.- Percentage distribution of β -thalassemia (TM and TI) patients in different birth order.

β-thalassemia types	Birth order							
	1	2	3	4	5	6	7	8
TM	96.06 (146)	98.24 (112)	96.15 (75)	96 (48)	100 (36)	88.88 (16)	100 (13)	66.66 (2)
TI	3.94 (6)	1.75 (2)	3.84 (3)	4.00 (2)	-	11.11 (2)	-	33.33 (1)
Total	32.9 (152)	24.56 (114)	16.81 (78)	10.77 (50)	7.75 (36)	3.87 (18)	2.8 (13)	0.64 (3)

Values presented the percentages and numbers are in parentheses

Table III.- Percentage distribution of β-thalassemia male and female patients and with anti HCV antibody positivity among different age groups.

Age groups (years)	Male patients			Female patients			Z-Test	P value
	Number	HCV +	Percentage	Number	HCV +	Percentage		
<2	39	15	38.46	24	10	41.66	1.13	0.129
3-5	77	40	51.94	26	15	57.69	4.58	<0.001
6-8	30	23	76.66	29	22	75.86	-0.00	0.500
9-11	25	24	96.00	12	1	91.66	2.87	0.002
12-14	8	8	100.00	7	7	100.00	-0.00	0.500
15-17	11	9	81.81	4	4	100.00	1.57	0.058
18-20	6	5	83.33	0	0	0.00	2.53	0.006
21>	1	1	100.00	1	1	100.00	-1.00	0.159
Total	197	125	63.45	103	70	67.96	7.59	<0.001

The highest percentage (n=77, 39.08%) of β-thalassemia male patients was observed in 3-5 years of age groups. Whereas, among female patients the highest (n=29, 28.15%) incidence percentage was found in a 6-8 years of age group. Among all years age groups, male patients were significantly (P<0.001) higher compared to females. The number of the male β-thalassemia patients from age groups <2, 3-5, 9-11, 15-17 and 18-20 years were significantly (P<0.001) higher than the females.

The frequency of anti HCV antibody positive cases amongst male and female over various age groups has also been detected very high (n=195, 65.0%) (Table III). Percentage of thalassemic males with HCV positivity was higher (n=125, 64.10%) than females (n=70, 35.89%). In 3-5, 9-11, 15-17 and 18-20 years of age group, the percentage of males with HCV positivity were significantly higher (P<0.001) compared to the female, while there was no appreciable difference in 0-2, 6-8, 12-14 and >

21 years of age groups. It was also observed that number of patients with HCV was increased with increase in age regarding the total number of thalassemic patients in each age group (Table III).

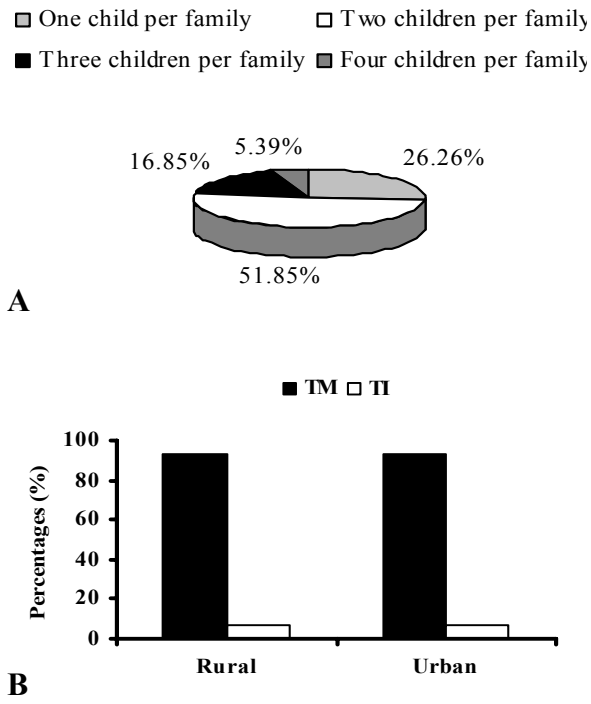

Fig. 1. Percentage distribution of β - thalassemic A) cases in families and B) percentage incident of thalassemia major (TM) and thalassemia intermedia (TI) in study population from rural and urban area of Faisalabad.

Consanguinity and β-thalassemia disease

The percentage distribution of β-thalassemia patients according to parental genetic relationships is shown in Table IV. The incidence of β-thalassemia was significantly higher ($P<0.001$) in 1st cousin marriages compared to unrelated. Incidence of β-thalassemia was the highest in outcome of first cousin marriages (69.66%) and the least in outcome of distant relation marriages (1.0%). Males were highly affected with disorder in 1 C ($P<0.001$) and 2C ($P=0.0003$) marriages outcome compared to females. Total consanguinity rate including 2nd cousins relationship was 77.39%.

Regarding familial aggregation in thalassemia major disease, the risk of thalassemic out comes in first cousin marriages was 70.35% whereas lowest in unrelated ones, 21.07%. Thalassemia intermedia showed 60.0% risk of thalassemic outcome in first cousin relation compared to 35.0% unrelated once.

In first cousin relations' outcomes, male TM patients were more affected 72.67% than female patients 65.97%. Whereas, TI in males indicated a contribution of 64.28% risk of β-thalassemia outcome of first cousins compared to 50.0% female patients.

Caste wise distribution of the disease

The highest percentage of β-thalassemia patients was seen in Rajpoot > Arian > Sheikh > Mughal = Pathan > Jat = Gujar > Khokhar (Malik, Awan) > Baloach.

The distribution of β-thalassemia patients and F value in relation to parental consanguinity among different caste groups is shown in Table V. The highest percentage of β-thalassemia disease in patients was associated to first cousin relations compared to unrelated one. The value of F was high in Gujar (0.057), Baloach (0.054) and Rajpoot (0.052), whereas Sheikh had least F-value (0.032).

DISCUSSION

Thalassemia is recognized as the most prevalent genetic blood disorder in the world. However, β-thalassemia, the most common autosomal single-gene disorder worldwide, found in more than 60 countries with a carrier population of up to 150 millions (Wetherall and Clegg, 2001). The overall prevalence of β-thalassemia estimated in Gaza strip was 4.3% (Sirdah *et al.*, 1998) and 25% was found in district of Isparta (Tunc *et al.,* 2002).

Among 300 subjects, incidence of β-thalassemia major and intermedia in present study was 93% and 7% respectively from Faisalabad comparable to that of 86% and 14% in North Americans patients (Pearson *et al*., 1996), 64% and 36% Lebanon (Inati *et al*., 2006) and 54% and 15% in Eastern North America (Vichinsky *et al*., 2005), respectively. In current study the number of affected males (65.66%) was significantly higher ($P<0.001$) than affected females (34.33%). In contrast, Gurbak *et al*. (2006) reported no appreciable difference in

Table IV.- Distribution of β-thalassemia patients in different genetic relationship.

		Genetic relationship					Total
		First cousins	Second cousins	Distant relatives	Bradri	Unrelated	
Thalassemia major	Male	72.67 (133)	7.10 (13)	1.09 (2)	2.73 (5)	16.39 (30)	183
	Female	65.97 (64)	3.09 (3)	1.03 (1)	–	29.89 (29)	97
Thalassemia intermedia	Male	64.28 (9)	7.14 (1)	–	–	28.57 (4)	14
	Female	50.00 (3)	–	–	–	50.00 (3)	6
Grand total		69.66 (209)	5.66 (17)	1.00 (3)	1.66 (5)	22.00 (66)	300

Note: Values presented the percentages and numbers are in parenthesis.

Table V.- Distribution of β –thalassemia patients in relation to parental relationships, F-values and surnames.

Surname	Genetic relationship					Total	F-value
	1C	2C	DR	B	U		
Rajpoot	80.65 (50)	1.61 (1)	3.22 (2)	–	14.51 (9)	62	0.052
Arain	75.00 (42)	3.57 (2)	–	1.78 (1)	19.64 (11)	56	0.046
Sheikh	50.00 (27)	3.70 (2)	3.70 (2)	1.85 (1)	40.74 (22)	54	0.032
Mughal	78.57 (11)	–	–	–	21.42 (3)	14	0.049
Pathan	57.14 (8)	7.14 (1)	–	–	35.71 (5)	14	0.036
Jat	66.66 (8)	8.33 (1)	–	8.33 (1)	16.66 (2)	12	0.042
Gujar	91.66 (11)	–	–	–	8.33 (1)	12	0.057
Khokhar (Malik, Awan)	50.00 (5)	20.00 (2)	–	10.00 (1)	20.00 (2)	10	0.034
Baloach	87.50 (7)	12.50 (1)	–	–	–	8	0.054

Note: Values presented the percentages and numbers are in parenthesis

males (55.5%) and female (44.5%) thalassemic patients from Gaziantep. However, a statistically non-significant (p=0.07) difference between number of male and female thalassemia patients reported by Asadi-Pooya and Doroudchi (2004). This difference in thalassemic patients (males more affected than females) is noteworthy and deserves further investigation considering thalassemia as a single-gene disease transmitted by a recessive mode of inheritance.

The highest percentage of β-thalassemic patients (34.33%) was between 3-5 years of age groups. Less number of older patients was observed, while the number of younger patients was higher due to increasing disease load and shortened life expectancy. Cario *et al*. (2000) reported 20% of the patients were older than 21 years in Germany. However, in Pakistan the age of β–thalassemia patients was generally believed around 10 years (Lodhi, 2003).

Current results also reveal that the incidence of β-thalassemia was the highest in 1st birth order and the lowest in 8th birth order. However, it is suggested that increased number of thalassemic patients in 1st birth order is due to parent's unawareness about disease and consanguinity. Analysis of the data collected in the present study indicated that almost 70% families have more than one β- thalassemic child (i.e. two or three). Shami and Tariq, (1999) reported previously that β - thalassemia patients come mainly from parents with low levels of education and income in Pakistan.

The incidence of β-thalassemia was significantly (P<0.001) higher in urban population (80.66%) than rural (19.33%). In contrast, a study in district of Isparta revealed the low frequency of β-thalassemia in the city center (1.7%) than in neighboring towns (2.2%) (Tunc *et al*., 2002) This decrease might be due to high awareness of disease in city center rather than in neighboring towns.

In the present study, the prevalence of anti-HCV antibodies was 65.0% among the β-

thalassemic patients consistent with the study of Lai *et al*. (1993). The prevalence of anti HCV among thalassemic patients was previously reported as 34.3% in Hong Kong (Lau *et al.,* 1993), 46.8% in Brazil (Covas *et al.,* 1993), 60% in Pakistan (Bhati *et al*., 1995), 17% in Taiwan (Chung *et al*., 1997), 43.6% in India (Choudhury *et al.,* 1998), 22.4% in Malaysia (Jaml *et al*., 1998) and 55.6% in Myanmar (Okeda *et al*., 2000).

Caste wise incidence was most frequent in Rajputs (25.62%) followed by Arain (23.14%), Sheikhs (22.31%), Mughal and Pathans (5.78%) and then least in other castes. These findings are in agreement with those of Hafeez *et al*. (2007) who reported β-thalassemia was most frequent in Rajputs followed by Jatts, Arian, Sheikhs and Pathans.

In current study, incidence of β-thalassemia was the highest in outcomes of first cousin marriages (69.66%) than in second cousins (5.66%) and unrelated (22.0%). Present findings are in line with Baig *et al*. (2005) reported rate of first cousins marriages among the parents of affected children was 63% in Faisalabad. Results contradicting to present study were reported by Asadi-Pooya and Doroudchi (2004) that 40.6% and 8.9% of β-thalassemia patients were outcomes of 1st cousins and 2nd cousins marriages whereas, 50.5% was outcomes of unrelated parents in Shiraz, Iran.

In present study, total consanguinity rate among parents of β-thalassemic children was 77.39%. These results are in agreement with those observed by Baig *et al*., 2005 (81% in Faisalabad). These findings are not in line with Al-Riyami and Ebrahim (2003) reported 58 % total consanguinity in the Sultanate of Oman. It was very interesting that in current study, males were highly affected in 1st ($P<0.001$) and 2nd ($P=0.0003$) cousin marriages compared to females.

The degree of inbreeding for the offspring of a particular couple can be quantified by calculating the Coefficient of Inbreeding (F). The coefficient measures the probability (genetics is a game of statistics) that a child receives two alleles at a given locus that were both from the same ancestor. In the current study, the coefficient of inbreeding (F) for parents of thalassemia patients was F=0.0455, the same results were also reported by Shami and Tariq, (1999) documented F value 0.0466 in southern Punjab. A large multi-national study showed that prereproductive mortality was 4.4% higher in first cousin progeny (F = 0.0625) than in the offspring of non-consanguineous unions (Bittles, 2003).

In conclusion, present cross sectional study clearly showed the common β-thalassemia disease observed was β-thalassemia major and males were more affected than females. First cousin marriages showed high β-thalassemia affliction than in unrelated couples and total consanguinity rate among parents of β-thalassemic children was 77.39% while the coefficient of inbreeding calculated was 0.0455. The prevalence of anti-HCV antibodies was 65.0% among the β-thalassemic patients.

REFERENCES

AHMAD, S., 1998. *An approach for the prevention of thalassemia in Pakistan*. Ph.D. thesis, University of London, pp. 86-98.

AHMED, S., SALEEM, M., SULTANA, N., RAASHID, Y., WAQAR, A., ANWAR, M., MODELL, B., KARAMAT, A. AND PETROU, M., 2000. Prenatal diagnosis of β-thalassemia in Pakistan: experience in a Muslim country. *Prenat. Diagn*., **20**: 378-383

AL-RIYAMI, A. AND EBRAHIM, G. J., 2003. Genetic Blood Disorders Survey in the Sultanate of Oman. *J. Trop. Pediatr*., **49**: 1-20.

ALWAN, A. AND MODELL, B., 1997. Community control of genetic and congenital disorders. WHO Regional Office for the Eastern Mediterranean, Alexandria, Egypt.

AMBEKAR, S. S., PHADKE, M. A., BALPANDE, D. N., MOKASHI, G. D., KHEDKAR, V. A., BANKAR, M.P., GAMBHIR, P.S., BULAKH, P.M. AND BASUTKAR, D.G., 2001. The prevalence and heterogeneity of beta thalassemia mutations in the Western Maharashtra Population: A hospital based study. *Ind. J. Hum. Genet*., **1**: 219-223.

ASADI-POOYA, A.A. AND DOROUDCHI, M., 2004. Thalassemia major and consanguinity in Shiraz city, Iran. *Turk. J. Haematol*., **21**: 127-130.

BAIG, S. M., RABBI, F., HAMEED, U., QURESHI, J. A., MAHMOOD, BOKHARI, S. H., KIANI, A., HASSAN, H., BAIG, J.M., AZHAR, A. AND ZAMAN, T., 2005. Molecular characterization of mutations causing β-thalassemia in Faisalabad Pakistan using the amplification refractory mutation system (ARMS-PCR). *Ind. J. Hum. Genet.,* **11**: 80-83.

BHATI, F. A., AMIN, M. AND SALIM, M., 1995. Prevalence of antibody to hepatitis C virus in Pakistani thalassemics by particle agglutination test utilizing c200 and c22-3 viral antigen-coated particles. *J. Pakistan*

med. Assoc., **45**: 269 -71.

BITTLES, A.H., 2003. Consanguineous marriage and childhood health. *Devel. Med. Child. Neur.,* **45**:571-576.

CAO, A. AND GALANELLO, R., 2002. Effect of consanguinity on screening for thalassemia. *N. Engl. J. Med.*, **347**: 1200-1202.

CARIO, H., STAHNKE, K., SANDER, S. AND KOHNE, E., 2000. Epidemiological situation and treatment of patients with thalassemia major in Germany: results of the German multicenter beta-thalassemia study. *Ann. Hematol*., **79**: 7-12.

CHOUDHURY, N., SARASWAT, S. AND NAVEED, M., 1998. Serological monitoring of thalassemia major patients for transfusion associated viral infections. *Indian J. med. Res*., **107**: 263 -8.

CHUNG, J. L., KAO, J. K., KONG, M. S., YANG, C. P., HUNG, I.J. AND LIN, T.Y., 1997. Hepatitis C & G virus infection in polytransfused children. *Eur. J. Pediatr.,* **156**: 546 -49.

COVAS, D.T., BOTURAO, N.E. AND ZAGO, M.A., 1993. The frequency of blood borne infections in a population of multitransfused Brazilian patients. *Revistado. Sao. Palo*, **35**: 271 –273.

GUPTA, A., HATTORI, Y. AND AGARWAL, S., 2002. Initiation codon mutation in an Asian Indian family. *Am. J. Hemotol*., **71**: 134-136.

GURBAK, M., SIVASLI, E., COSKUN, Y., BOZKURT, A.I. AND ERGIN, A., 2006. Prevalence and hematological characteristics of beta-thalassemia trait in Gaziantep urban area, Turkey. *Pediatr. Hematol. Oncol*., **23**: 419-425.

HAFEEZ, M., ASLAM, M., ALI, A., RASHID, Y. AND JAFRI, H., 2007. Regional and ethnic distribution of beta thalassemia mutations and effect of consanguinity in patients referred for prenatal diagnosis. *J. Coll. Phys. Surg. Pak.,* **17**: 144-147.

INATI, A., ZEINEH, N., ISMAEEL, H., KOUSSA, S., GHARZUDDINE, W. AND TAHER, A., 2006. beta-Thalassemia: the Lebanese experience. *Clin. Lab. Haem*., **28**: 217–227.

JAML, R., FADZILLAH, G., ZULKFILI, S. AND YASMIN, M., 1998. Seroprevalence of hepatitis B, hepatitis C, and HIV in multiple transfused thalassemia patients: results from a thalassemia day care centre in Malaysia. *South Asian J. med. Publ. Hlth*., **29**:792-4.

KHAN, S.N., ZAFAR, A.U. AND RIAZUDDIN, S., 1995. Molecular genetic diagnosis of β-thalassemia in Pakistan. *J. Pak. med. Assoc*., **45**: 66-69.

LAI, M., VIRGILIS, S., ARGIOLU, F., FARACI, P., MAZZOLENI, A. P. AND LISCIV, V., 1993. Evaluation of antibodies to hepatitis C virus in long term Prospective study of polytransfusion hepatitis among thalassemia children: comparison between first and second generation assay. *Pediatr. Gastroenterol. Nutr*., **16**: 458-464.

LAU, Y. I., CHOW, C. B., LEE, A. C., NG, K. W., LIN, W. L. AND CJAN, C. F., 1993. Hepatitis C virus antibody in multiple transfused Chinese with thalassemia major. *Bon. Marr. Trans.,* **12**: 26-28.

LODHI, Y., 2003. Economics of thalassemia management in Pakistan. In: *Thalassemia awareness week* (ed. S. Ahmed) Friends of Thalassemia.

OKEDA, S., TAKETA, K., ISHIKAWA, T., KOJI, T., SWE, T. AND WIN, N., 2000. High prevalence of hepatitis C in patients with thalassemia and patients with liver disease in Myanmar. *Acta Med. Okayama*, **54**: 137-138.

PEARSON, H. A., COHEN, A. R., GIARDINA, P. J. AND KAZAZIAN, H.H., 1996 .The changing profile of homozygous beta-thalassemia: demography, ethnicity, and age distribution of current North American patients and changes in two decades Department of Pediatrics, Yale University School of Medicine, New Haven, USA. *Pedi.,* **97**: 352-356.

PREMAWARDHENA, A., SHANTHIMALA, D.S., MAHINDA, A., NANCY, O., JULIA, M., ANGELA, A., CHRISTOPHER, F., TIMOTHY, P., ELLIOT, V. AND DAVID, W., 2004. Thalassemia in Sri Lanka: a progress report. *Human mol. Genet.,* **13***:* 203-R206.

RAFIQUE, B. AND MOINUDDIN, A., 1991. Haemoglobinopathies and interfamily marriage in Pakistan. *Pak. Paedi. J.,* **15**: 123-131.

RAHMAN, M. AND LODHI, Y., 2004. Prospects and future of conservative management of beta thalassemia major in a developing country. *Pak. J. med. Sci*., **20**: 105-112.

SALEEM, M., AHMAD, P.A., MUBARIK, A. AND AHMAD, S.A., 1985. Distribution pattern of hemoglobinopathies in northern areas of Pakistan. *J. Pak. med. Assoc*., **35**: 106-109.

SALEEM, M., AHMAD, S. AND KHATTAK, M.F., 1996. *An overview of thalassemia in Pakistan*. 4th International conference, Peshawar. *Pakistan Association of Pathologists* 76.

SENGUPTA, M., 2008. Thalassemia among the tribal communities of India: *Int. J. Biol. Anthro.,* **1**: http://ispub.com/ostia/index.php?xmlFilePath=Journals/ijba/valinz/thalassemia.xml

SHAMI, S.A. AND TARIQ, A.K., 1999. Consanguinity and thalassemia. *Pakistan J. Zool.,* **31**: 105-106.

SHAMI, S.F. AND IQBAL, I., 1983. Consanguinous marriages in the population of Shiekupura (Punjab), Pakistan*. Biologia,* **92**: 231-244.

SIRDAH, M., BILTO, Y.Y., EL JABOUR, S. AND NAJJAR, K., 1998. Screening secondary school students in the Gaza strip for beta-thalassaemia trait. *Clin. Lab. Haematol*., **20**: 279-83.

TUNC, B., CETIN, H., GUMRUK, F., ISTANBULLU, B., YAVRUCUOGLU, H., KURT, U. AND GENC, H., 2002. The prevalence and molecular basis of beta-thalassemia in Isparta province and region. *Turk. J.*

Pediatr., **44**: 18-20.

VICHINSKY, E.P., MACKLIN, E.A., WAYE, J.S., LOREY, F., OLIVIERI, N.F., 2005. Changes in the epidemiology of thalassemia in North America: A new monitory disease. *Pedi*., **116**: 818-825.

WETHERALL, D.J. AND CLEGG, J.B., 2001. Inherited haemoglobin disorders: an increasing global health problem. World Health Organization: *Bull. Wld. Hlth. Org*., **79**: 704-712

WRIGHT, S., 1992. Coefficient of inbreeding and relationship. *Am. Nat.,* **56**: 330-338.

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