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A RESEARCH FOR THE DETERMINATION OF (α & ß) GENES AND (α & ß) THALASSEMIA IN THE DIAGNOSIS OF UNEXPLAINED MICROCYTOSIS, ALLIED HOSPITAL, FAISALABAD

[1]Dr. Rashid Mehmood, [2]Dr. Muhammad Arslan Mughal, [1]Dr. Tabassum Ali
[1]DHQ Hospital Faisalabad
[2]Jinnah Hospital Lahore

Abstract:
Objective: *To determine the frequencies of (α & ß) genes and variants number of haemoglobin in patients affected with Microcytic hypochromic anaemia (MCH).*
Methodology: *A total of 340 patients (MCH below 27pg; MCV below 80 fl) out of total 850 were investigated at Allied Hospital, Faisalabad (September, 2016 to November, 2017). 325 individuals were included in which 88 with α-thalassemia, 42 cases of iron-deficiency anaemia, 11 cases of haemoglobin variants, 171 with β-thalassemia and 13 cases of major thalassemia. Fifteen cases were not confirmed about exact the cause of diseases.*
Results: *Gap-PCR was utilised for genotyping for $-\alpha^{3.7}$, $-\alpha^{PA}$, $-\alpha^{4.2}$, $--^{5NT}$ and $--^{MED.}$ In 325 cases, average frequency of $-\alpha^{3.7}$ deletion was recorded twenty %. For direct mutation analysis, Amplification Refractory Mutation System was used for genotyping of twenty-three most frequent Beta-gene mutations. In 340 subjects, the most common mutations were IVS II-I (with 11.7 % frequency), CD 36/37(with 9.7 % frequency) and IVS I-110(with 3.5 % frequency).*
Statistically, considerable difference was observed between Beta-thalassemia Major and Beta-thalassemia trait as far as MCH and MCV with respective P-value of (0.23 & 0.25), index of MCH between Hb Variants (P-value = 0.04) and Beta-thalassemia trait. However, no considerable changes were found between the remaining hemoglobinopathies and red cell indices.
Conclusion: *Alpha and Beta genes mutation are rampant. In order to avoid superfluous iron supplementation and diagnosis of mysterious microcytosis, molecular genotyping of Beta-thalassemia and Alpha-thalassemia is significant.*
Keywords: *Evaluation of anaemia, Alpha-gene deletions, Microcytic hypochromic anaemia, Beta-gene mutations*

Corresponding author:
Dr. Rashid Mehmood,
DHQ Hospital,
Faisalabad

QR code

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INTRODUCTION:

Like other countries in the region there are increasing number of thalassemia patients [1]. Distribution and frequency of Beta-globin mutations is required to be explained in various regions. The possibility of co-inheritance of Alpha and Beta thalassemia due to tremendous frequencies of Alpha and Beta globin mutation can pave the way to a great variety of phenotypes [2]. Beta thalassemia is rarely found. The frequency of gene, however, is greater and may vary from area to area. Its greatest ratio i.e. about 10 % is found in Persian Gulf and the Caspian Sea. Alpha-thalassemia detection through simple biochemical test is a difficult one. Data on Alpha and Beta genotyping is insufficient.

Preliminary haematological data is the key ingredient in detecting hemoglobinopathies such as thalassemia. Mean corpuscular haemoglobin (MCH) or low mean corpuscular volume (MCV) are indicators of thalassemia.

Various irregular haemoglobins can be the reasons for a potential anaemia. Generally speaking, anaemia is haemolytic as well as dysery-thropoietic. HbS, in some cases, the reason can be obvious since reduction in oxygen tension may pave the way to sickling of red cells. It can further be damaged by the spleen. In clinical practice, general haematological abnormality is microcytic hypochromic anaemia. Thalassemia and iron deficiency traits are responsible for it.

Type of mutation in thalassemia and the degree of microcytosis demonstrated profound alteration between MCV ranges. In literature, there is dearth of required data in terms of incidence of alpha-gene removal in the cases of microcytosis [3, 4]. α-gene deletion possess light microcytosis without or with anaemia. Since anaemia is missing but it is very significant for ß-thalassemia diagnosis to identify the microcytosis cause and to evade the time and again costly analysis. In ß-thalassemia patients, the co-existence of α-deletions brings changes to phenotype. In order to detect α-thalassemia carriers, no biochemical diagnostic test is yet available. Globin chain synthesis studies have other negative aspects such as expensiveness and wastage of time. For analysis, they are demand for radioactive amino acids. To diagnose α-thalassemia case, traditional methods such as molecular methods as sequencing. Nonetheless, molecular diagnosis by PCR has proved its worth in terms of its time, cost and results [5, 6]. Amplification Refractory Mutation System (ARMS) method is considered very common in ß-thalassemia mutation diagnosis method [7, 8]. Deletions are responsible for α-thalassemia which is contrary to ß-thalassemia. By using gap- PCR, the detection of alpha-thalassemia rearrangements and removal can be made [9 – 11]. The study at hand is conducted to determine the existence of haemoglobin and thalassemia mutation alternates in inexplicable cases of microcytic anaemia so that its utility can be validated in the practice of clinic with particular reference to genetic counselling.

METHODS:

From total 850 cases, blood samples of two milli litres was taken from every subject in Allied Hospital, Faisalabad (September, 2016 to November, 2017). All the subjects gave written consent prior to blood samples and measurement of red-cells was carried out. Measurement of Hb F and Hb A2 was done in Hb-Variant [USA, “Bio-Rad” system]. Kits (Span Diagnostics Ltd.) were utilised to measure total iron binding capacity and the serum iron. Saturation %age was measured accordingly. The subjects were termed as iron deficient who were having saturation less than 16 %.

When preliminary evaluation was over, 340 blood samples were identified as microcytic [MCV less than 80 fl] hypochromic [MCHless than 27 pg mL–1] anaemia. They were declared to undergo thalassemia research. They were 88 cases having the traits of Alpha-thalassemia, 42 cases having iron-deficiency anaemia, 171 cases possessing the traits of Beta-thalassemia, 13 cases were with thalassemia major and 11 subjects having haemoglobin variants (HbD, HbS and HbC). Definite aetiology was not established in 15 cases.

In order to have á and ß genotyping, standard phenol chloroform extraction method was used for genomic DNA preparation from peripheral blood. To detecy removal of $-\alpha^{3.7}$, the protocol was observed, $-\alpha^{4.2}$ removal was followed as Agarwal et al. proposed [6]. Liu et al, [12] method was employed for detection of other deletions. The products which were amplified electrophoresed on 1.50 % agarose gel. Ethidium bromide was used to stain them. Analysis of 88 suspected α-thalassemia patients was carried out with the help of α-globin gene direct mutation analysis through (gap-PCR), we also found the mutation type. Analysis of 171 suspected ß -thalassemia patients was carried out with ß -globin gene direct mutation analysis by ARMS (Amplification Refractory Mutation System). Mutation type was also found and reflected in the research outcomes. Results were documented on the gel documentation system.

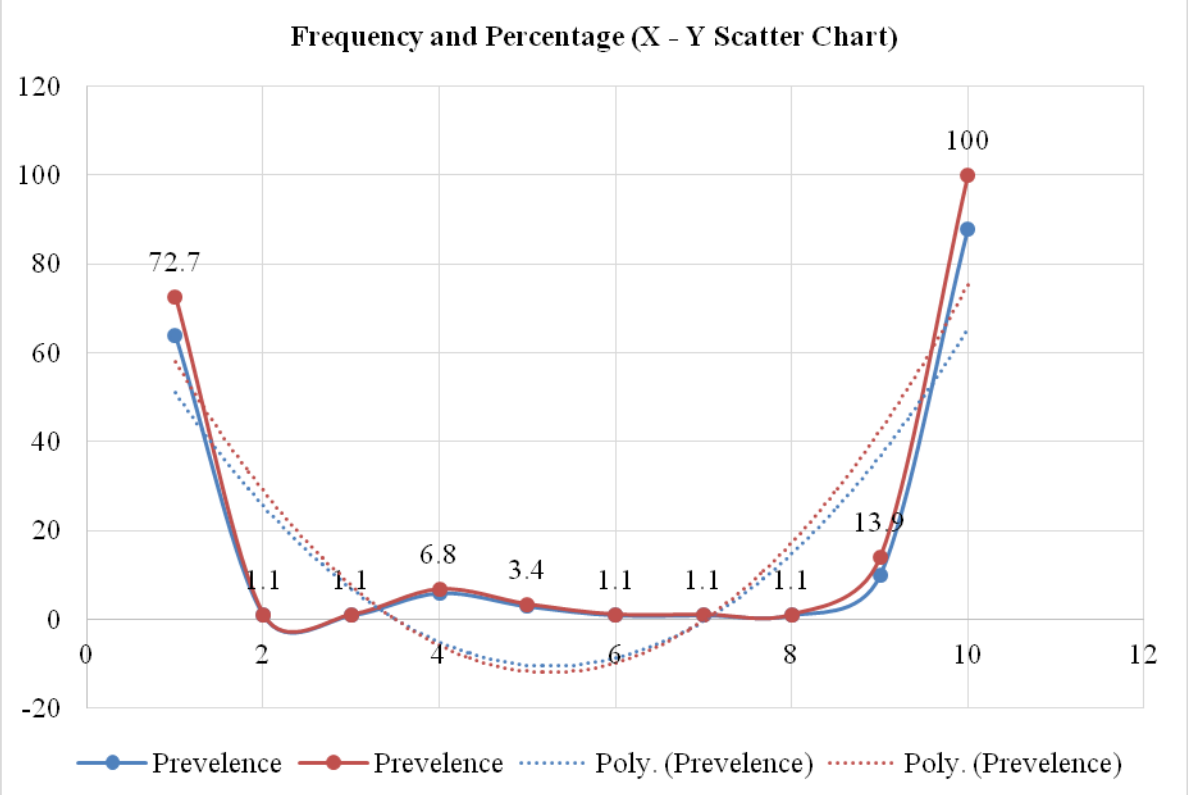
SPSS was used for the sake of statistical analysis. For contrast of haematological parameters, sample T-test was utilised with allelic and frequencies genotypic.

RESULTS:

On 850 samples, haematological parameters analysis was carried out initially. Identification of hypochromia [MCH less than 27 pg.] and microcytosis [MCV less than 80 fl] was done on 340 samples out of total 850 samples. 283 samples displayed hemoglobinopathy. Iron deficiency anaemia was diagnosed by %age of saturation technique in 42 samples in the rest of 57 samples. Fifteen cases exhibited no defined aetiology. GAP-PCR was used for α-gene number analysis in every 340 samples. Table – II has manifestations of the frequency of $-\alpha^{3.7}$ deletion in different groups.

Table – I: Prevalence of α –genotype in various á-thalassemia subgroups of microcytic hypochromic anaemia

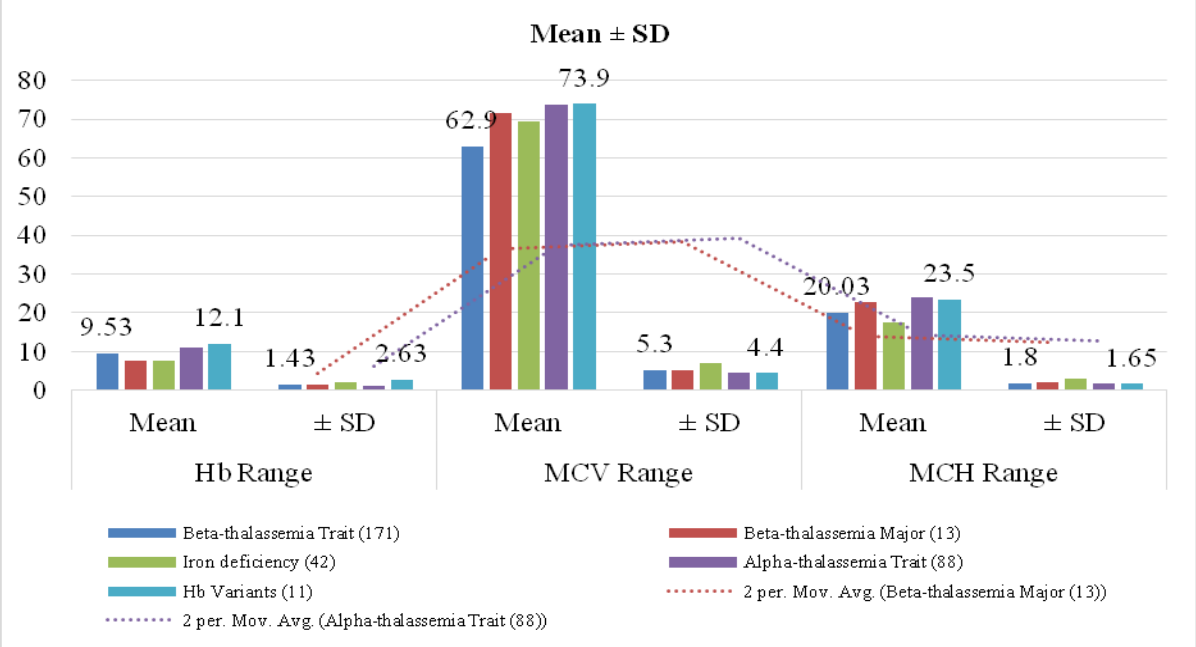
Group	Alpha - genotypes	Prevalence		Homozygote	Heterozygote	Compound heterozygote
		Numbers	Percentage			
1	αα /-$\alpha^{3.7}$	64	72.7	4	60	0
2	αα /-$\alpha^{4.2}$	1	1.1	0	1	0
3	αα /-α^{PA}	1	1.1	0	1	0
4	αα /- -^{MED}	6	6.8	0	6	0
5	αα /-α^{5NT}	3	3.4	1	2	0
6	HbD and αα /-$\alpha^{4.2}$	1	1.1	0	0	1
7	HbD and αα /-$\alpha^{3.7}$	1	1.1	0	0	1
8	HbC and αα /-$\alpha^{3.7}$	1	1.1	0	0	1
9	Unknown	10	13.9	4	6	0
10	Total Prevalence	88	100	9	76	3.5



The cases diagnosed with microcytic hypochromic anaemia amongst 340 patients, four patients possessed homozygous -α3.7 deletion [–α $^{3.7}$/– α $^{3.7}$] whereas heterozygous –α $^{3.7}$ deletion [–α $^{3.7}$ / αα] was observed in sixty cases. Twenty % was the carrier status amongst 340 patients. Calculation of Allele frequency for –α $^{3.7}$ deletions was done as 0.10 (seventy in six hundred and eighty chromosomes). Amplification Refractory Mutation System was used for ß-gene number analysis in all 340 cases. Table – III has shown the frequencies of various identified ß-gene mutations. In 340 subjects, the most common mutations were IVS II-I (with 11.7 % frequency), CD 36/37(with 9.7 % frequency) and IVS I-110 (with 3.5 % frequency). Table – I has manifested haematological parameters of cases in various groups.

Table – II: Haematological parameters in different groups with microcytic hypochromic anaemia

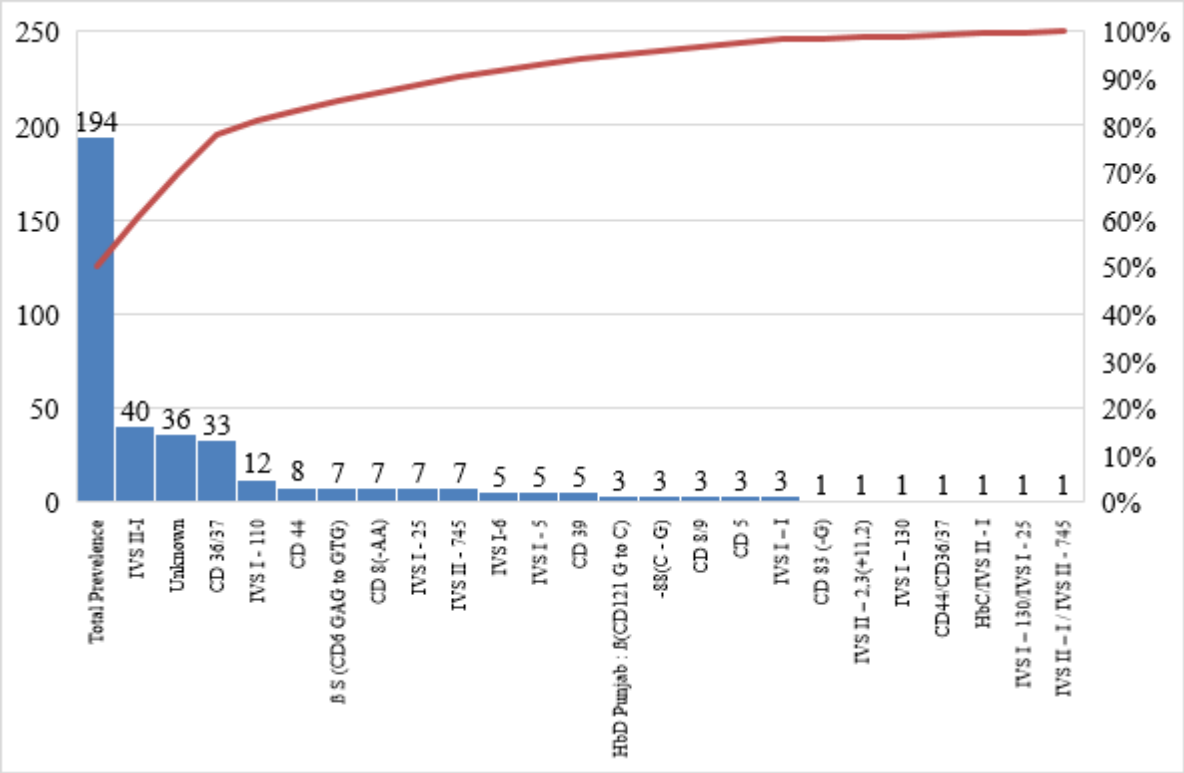
Group (No. of cases)		Beta-thalassemia Trait (171)	Beta-thalassemia Major (13)	Iron deficiency (42)	Alpha-thalassemia Trait (88)	Hb Variants (11)
Hb Range	Range	(5.60 – 12.10)	(5.20 – 9.28)	(4.3 – 12.95)	(9.20 – 12.25)	(7.8 – 15.20)
	Mean	9.53	7.5	7.75	11.1	12.1
	± SD	1.43	1.34	2.05	1.25	2.63
MCV Range	Range	(49 – 78.90)	(63 – 79)	(52.0 – 77.8)	(60 – 79)	(66 – 79)
	Mean	62.9	71.6	69.35	73.6	73.9
	± SD	5.3	5.2	6.95	4.67	4.4
MCH Range	Range	(15 – 26)	(20.9 – 25.6)	(12.10 – 23.12)	(19 – 26.20)	(21 – 26.5)
	Mean	20.03	22.9	17.52	23.9	23.5
	± SD	1.8	2.1	2.84	1.82	1.65



Statistically, considerable difference was observed between Beta-thalassemia Major and Beta-thalassemia trait as far as MCH (P –value =0.23) and MCV (p- value = 0.25) indices are concerned, and also MCH index between Hb Variants (P-value = 0.04) and Beta-thalassemia trait. However, no considerable changes were found between the remaining hemoglobinopathies and red cell indices.

Table – III: Prevalence of ß–thalassemia in various ß-thalassemia subgroups of microcytic hypochromic anaemia

Group	Beta - genotypes	Prevalence		Homozygote	Heterozygote	Compound heterozygote
		Number	Percentage			
1	CD 36/37	33	17	3	30	0
2	IVS II-I	40	20.6	3	37	0
3	ß ^{S} (CD6 GAG to GTG)	7	3.6	0	7	0
4	HbD Punjab: ß (CD121 G to C)	3	1.5	0	3	0
5	IVS I-6	5	2.5	5	0	0
6	CD 8(-AA)	7	3.6	1	6	0
7	IVS I - 25	7	3.6	0	7	0
8	-88(C - G)	3	1.5	0	3	0
9	CD 8/9	3	1.5	0	3	0
10	IVS I - 110	12	6.1	1	11	0
11	IVS II - 745	7	3.6	0	7	0
12	IVS I - 5	5	2.5	0	5	0
13	CD 83 (-G)	1	0.5	0	1	0
14	CD 44	8	4.1	0	8	0
15	IVS II – 2,3(+11,2)	1	0.5	0	1	0
16	CD 5	3	1.5	0	3	0
17	IVS I – I	3	1.5	0	3	0
18	CD 39	5	2.5	0	5	0
19	IVS I – 130	1	0.5	0	1	0
20	CD44/CD36/37	1	0.5	0	0	1
21	HbC/IVS II - I	1	0.5	0	0	1
22	IVS I – 130/IVS I - 25	1	0.5	0	0	1
23	IVS II – I / IVS II - 745	1	0.5	0	0	1
24	Unknown	36	20.8	10	26	0
25	Total Prevalence	194	100	23	167	4



DISCUSSION:

Globally, the most frequent single-gene haemoglobin disorder is Alpha and Beta-thalassemia [13-15]. The $-_{\alpha}^{3.7}$ deletion is widely known type of ß- thalassemia. The frequency of ß-thalassemia is found 57 % in microcytic patients. On the other hand, hypo-chromic anaemia patients have 25.8 % of the frequency of ß-thalassemia in our hospitals. Reports from Hadavi et al prove that the prevalence of $-_{\alpha}^{3.7}$ deletion is found as 30.2 % in research population [16]. Our study also revealed only two cases of $-_{\alpha}^{4.2}$ deletion. A prevalence (3.5%) of $-_{\alpha}^{4.2}$ deletion is recorded by Hadavi et al [16]. The prevalence of IVS-II-I beta-thalassemia mutation is reported by Najmabadi et al as 34 % [17,18]. In our study the commonest mutations were IVS II-I (with 11.7 % frequency), CD 36/37(with 9.7 % frequency) and IVS I-110 (with 3.5 % frequency). Comparison of $_{\alpha}$-thalassemia haematological parameters with iron-deficiency anaemia haematological parameters and ß-thalassemia haematological parameters was made. Patients having single-gene deletion ($-_{\alpha}^{3.7}$) possess lower levels of haemoglobin, MCH and MCV than normal controls. A mild microcytic hypochromic anaemia was observed in the carriers of Hb Variants and á-thalassemia. Despite this fact, their MCH and MCV were found much better than the iron-deficiency anaemia cases.

If we want the diagnosis of α -thalassemia, MCH is a superior discriminator than other indices of red-cell. It is normally lesser than 26 pg. As there is dearth of any exact haematological marker to diagnose α-thalassemia, molecular analysis stands the only marker in diagnosing hypochromic and microcytic cases. The findings of our study are in conformity with the erstwhile researches in which α-gene number was the basis for microcytosis details [3,19 – 21].

The detection of the carrier status of α and ß-thalassemia is of poignant significance as it can prevent the costly and faulty explorations in order to delineate the anaemia aetiology and undesired prolonged iron supplementation. In any given population there is a need of awareness about α and ß-gene number in α and ß-thalassemia traits since by changing the ratio of α and ß -chains of haemoglobin, it can change the phenotype of thalassemia. Therefore, in the process of genetic counselling of high-risk thalassemia couples, there should be consideration for thalassemia screening.

CONCLUSION:

By summing up all the facts and figures we concluded that Alpha and Beta genes mutation are rampant. In order to avoid superfluous iron supplementation and diagnosis of mysterious microcytosis, molecular genotyping of Beta-

thalassemia and Alpha-thalassemia is of poignant significance.

REFERENCES:

1. Najmabadi H, Pourfathollah AA, Neishabury M, Sahebjam F, Krugluger W, Oberkanins C. Rare and unexpected mutations b-thalassemia patients and prenatal samples discovered by reversehybridization and DNA sequencing. Haematologica 2002; 87:1113-4.
2. Bergeron J, Weng X, Robin L, Olney HJ, Soulieres D. Prevalence of alpha-globin gene deletions among patients with unexplained microcytosis in a north- American population. Hemoglobin 2005; 29:51-60.
3. Sivera P, Roetto A, Mazza U, Camaschella C. Feasibility of molecular diagnosis of α -thalassemia in the evaluation of microcytosis. Haematologica 1997; 82:592-3.
4. Mach-Pascual S, Darbelly R, Pilotto PA, Beris P. Investigation of microcytosis: A comprehensive approach. Eur J Haematol 1997; 57:54-61.
5. Borges E, Wenning MRSC, Kimura EM, Gervasio SA, Costa FF, Sonati MF. High prevalence of α -thalassemia among individuals with microcytosis and hypochromia without anemia. Braz J Med Biol Res 2001; 34:759-62.
6. Agarwal S, Sarwai S, Nigam N, Singhal P. Rapid detection of α + thalassemia deletion & ß-globin gene triplication by GAP-PCR in Indian subjects. Indian J Med Res 2002; 116:155-61.
7. Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res 1989; 17:2503-16.
8. Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of ß-thalassemia: Studies in Indian and Cypriot populations in the UK. Lancet 1990; 336:834-7.
9. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of alpha-thalassemia. Blood 2000; 95:360-2.
10. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of alpha-thalassemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000; 108:295-9.
11. Tan JA, Tay JS, Lin LI, Kham SK, Chia JN, Chin TM, et al. The amplification refractory mutation system (ARMS): A rapid and direct prenatal diagnostic technique for beta-thalassemia in Singapore. Prenat Diagn 1994; 14:1077-82.
12. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of á-thalassemia deletions and _-globin gene triplication by multiplex polymerase Chain reactions. Br J Haematol 2000; 108:295-9.
13. Weatherall DJ, Clegg TB. The thalassemia syndromes, 4th ed. Oxford: Blackwell Science 2001;267. Baysal E, Huisman THJ. Detection of common deletional á-thalassemia –2 determinants by PCR. Am J Hematol 1994; 46:208-13.
14. Shaji RV, Eunice SE, Baidya S, Srivastava A, Chandy M. Determination of the breakpoint and molecular diagnosis of a common -thalassemia – 1 deletion in the Indian population. Br J Haematol 2003; 123:942-7. 28 Pak J Med Sci 2008 Vol. 24 No. 1 www.pjms.com.pk
15. Hadavi V, Taromchi AH, Malekpour M, Gholami B, Law HY, Almadani N, et al. Elucidating the spectrum of á-thalassemia mutations. Haematologica 2007; 92:992-3.
16. Najmabadi H, Karimi-Nejad R, Sahebjam S, Pourfarzad F, Teimourian S, Sahebjam F, et al. The beta-thalassemia mutation spectrum. Hemoglobin 2001; 25:285-96.
17. Haghshenas M, Zamani J. [Thalassemia]. 1 st ed. Shiraz University of Medical Sciences Publishing Center: Shiraz; 1997. [Book in Persian]
18. Najmabadi H, Ghamari A, Sahebjam F, Kariminejad R, Hadavi V, Khatibi T. Fourteen-year experience of prenatal diagnosis of thalassemia in. Comm Gen 2006; 9:93-7.
19. Ko TM, Hwa HL, Liu CW, Li SF, Chu JY, Cheung YP. Prevalence study and molecular characterization of alpha thalassemia in Filipinos. Annals of Hematology 1999; 78:355-7.
20. Bergeron J, Weng X, Robin L, Olney HJ, Soulieres D. Prevalence of alpha-globin gene deletions among patients with unexplained microcytosis in a north-American population. Hemoglobin 2005; 29:51-60.
21. Nadkarni AH, Gorakshakar AC, Mohanty D, Colah RB. Alpha genotyping in a heterogeneous Indian population. Am J Hematol 1996; 53:149-50.