Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/7465
DOI URL: http://dx.doi.org/10.21474/IJAR01/7465
ISSN NO. 2320-5407

RESEARCH ARTICLE

STUDYING THE SOCIO-DEMOGRAPHIC PROFILE AND THE MOLECULAR BASES OF THALASSEMIA IN BASRAH/ IRAQ.

Dr. Khairallah A S Mohammed, Dr. Majid A. Maatook, Dr. Hussam S. Aziz, Dr. Ali M. Afat and Sarah Hani.
Department of Medical Laboratory Technology, College of Health and Medical Technology, Southern Technical University, Basrah / Iraq.

Manuscript Info

Manuscript History

Received: 22 May 2018
Final Accepted: 24 June 2018
Published: July 2018

Keywords:-
β-thalassemia; Mutation; ARMS-PCR; Basrah .

Abstract

The thalassemia is one of the most common inherited diseases worldwide with considerable frequencies in the Middle East region, including Iraq. As the disease requires long-term care, an establishment of an effective preventative program creates a major armament in management. As part of this effort, we established a primary study to determine the types of thalassemic patients based on family medical history, age, region, blood groups and consanguineous marriages. The results showed that the recruited patients (120) were distributed in nine different regions of Basrah province with high percentage (43.33%) in central city with age 8-12 years (30.7%), O+ blood group (40%) and 80.83 % of the investigated patients had consanguineous marriages. Based on the present findings, the molecular basis of fifty four thalassemic patients were investigated, using ARMS-PCR (Amplification Refractory Mutation System), to determine the most common types of thalassemia mutations in the region. Seven mutation types were detected (IVS1nt.5, Codon 8/9, Codon -88, Codon 8, Codon 15 Asia Indian, Codon 41/42, Codon 30). The results showed that codons 30, -88 and IVS Int 5.mutations were the highest frequent compared with other mutations studied in the thalassemic samples.

Introduction:-

Thalassemia is widely distributed throughout the world, with considerable frequencies in the Middle Eastern countries, including Iraq (1, 2). It is an inherited autosomal recessive blood disorder in which the synthesis of one of the globin subunits of the hemoglobin is decreased or absent (3). Based on the type of globin chain involved, two main types; α- and β - thalassemias can be distinguished (2, 4). In addition, other types of thalassemia can be a result from defective production of two to four different globin chains (δβ-, γδβ-, and εγδβ-thalassemia) (5). β-thalassemia major that is caused by homozygosity for β-thalassemia and Hb Bart's fetal hydrops syndrome resulting from deletion or dysfunction of all four α-globin genes are the major clinical types of thalassemias that are targets of prevention (6, 7).

There are about 200 different mutations which have been described in patients with β- thalassemia (8). Most β-thalassemia mutations involve changes (substitutions, deletions or insertions) of either a single or a few nucleotides. However, β-thalassemia mutations may involve the entire or multiple genes in the β globin gene cluster (9). All

Corresponding Author:- Khairallah A S Mohammed.
Address:- Department of Medical Laboratory Technology, College of Health and Medical Technology, Southern Technical University, Basrah / Iraq.

pathological mutations result either in absence (βº-thalassemia) or in reduction of β globin chains synthesis (β+ -thalassemia). The types of β-thalassemia mutations, in middle and northern parts of Iraq, have been investigated by numerous studies (10-15). These investigations showed the geographical and social distributions of thalassemia mutations in different regions of Iraq (10-15). However none of those studies fully covered the southern part of Iraq especially Basrah province. The objectives of this study were to study the socio-demographic profile of β-thalassemia and to investigate the molecular basis of this disease to determine the most common types of mutations in Basrah province, using Amplification Refractory Mutation System (ARMS), with the aim of establishing a proper preventive program.

.

Methods:-

Patients Group:-

One hundred twenty transfusion-dependent β-thalassemia patients from different Iraqi populations in Basra province were taken, representing different ages and all are receiving regular blood transfusion. Some patients had undergone splenectomy and their exhibited clinical complications by secondary iron overload. They were asked to fill in a questionnaire form during their visiting periods to the thalassaemic centre in Basra (Age, Gender, ABO- Rh blood group, Diagnosis of β-thalassemia, Major/Intermedia, Duration of transfusion, Is the patient receiving iron chelating drugs, Type, Regular, Irregular).

Control group:

Twenty of apparently healthy individuals (without Hemoglobinopathy disorders) from different locations in Basra city with age and gender matched with the patient population.

Blood Sampling:

Three millilitres of blood were collected by vein puncture in EDTA tubes from Fifty four of the recruited patients and control groups and transmitted within a few hours then stored in refrigerator (4ºC) for DNA extraction and molecular analysis.

Isolation of genomic DNA:

The genomic DNA isolated from the whole fresh blood collected in EDTA anticoagulant tubes by using Wizard genomic DNA purification kit following the manufacturer's instructions (Promega).

Principles of ARMS:

The ARMS primers are designed and corresponded to specific mutant sequences. They are designed in such a way that the nucleotide distinguishing the alleles is at 3' terminus of the primer. Thus, if the mutation present, only the mutant primer will bind and give PCR product; whereas in the presence of wild type sequence (WT), only the normal primer binds and gives PCR product. Originally, ARMS performed in two tubes; one corresponding to the WT sequence and the other corresponding to the mutation. This enabled homo / heterozygosity to be easily determined, as such heterozygous individuals will show products in both tubes (16, 17).

Primers Selection:

The primer sets which are selected for ARMS analysis of mutations are shown in Table (1).

PCR reaction:

Green master mix 2X [*Taq* DNA polymerase, dNTPs, MgCl2 and Rreaction buffers] (Promega):	12.5 µl
Common Primer C or D 10 picomols/ µl:	1 µl
Normal or Mutant Primer 10 picomols/µl:	1 µl
DNA Sample 0.1µg/ µl:	2 µl
Deionized Nuclease-free Water:	8.5 µl
Total Volume:	25µl

PCR programme:

Step	Temperature (°C)	Time (minutes)	No. of Cycles
Initial denaturation	94	2	1
Denaturation	94	1	
Annealing	65	1	25

Extension	72	1.5	
Final extension	72	3	1

The annealing temperature was changed according to the sequences of primers.

Table 1:-Sequences of the primers to detect β-Globin Gene Mutations. Normal and Mutant Primer (18, 19, 20, 21, 22, 23).

Mutation	Sequence(5'-3')	PCR Product bp
IVS - I-5 (G – C) Normal	5-CTCCTTAAACCTGTCTTGTAACCT TGTTAG-3 5-CTCCTTAAACCTGTCTTGTAACCTTGTTAC-3	285bp
Codon 8 \ 9(+ G) Normal	5-CCTTGCCCCACAGGGCAGTAACGGCACACC-3 5-CCTTGCCCCACAGGGCAGTAACGGCACACT-3	225 bp
3. 88 (C-T) Normal	5-TCACTTAGACCTCACCCTGTGGAGCCTCAT-3 5-TCACTTAGACCTCACCCTGTGGAGCCTCAC-3	684 bp
Codon 8 (-AA) Normal	5-ACACCATGGTGCACCTGACTCCTGAGCACG-3 5-ACACCATGGTGCACCTGACTC CTGAGCAGA-3	520 bp
Codon 15 (G- A) Normal	5-TGAGGAGAAGTCTGCCGTTACTGCCCAGTA-3 5-TGAGGAGAAGTCTGCCGTTACTGCCCAGTG-3	500 bp
Codon 41/42(-TCTT). Normal	5-GAGTGGACAGATCCCCAAAGGACTCAA CCT-3 5-GAGTGGACAGATCCCCAAAGGACTCAAAGA-3	439 bp
Codon 30 (G-C) Normal	5-TAA ACC TGT CTT GTA ACC TTG ATA CCT ACG-3 5-TAAACCTGTCTT GTA ACC TTG ATACCTACC-3	280bp
Common reverse primer C	5--ACC TCA CCC TGT GGA GCC AC 3'	
Common reverse primed D	5--CCC CTT CCT ATG ACA TGA ACT TAA 3-	

Analysis of ARMS-PCR Products:

The ARMS-PCR products and the ladder marker were resolved by electrophoresis. 7µl of the product were loaded on 1% agarose gel and run at 80 volt (23. Sambrook et al., 1989). After 45 mints, bands were visualized on UV transiluminator and photographed by using gel documentation system (Bio – Rad).

Results:-

Geographical distribution data showed that the information was collected from patients who have β-thalassemia and got regular blood transfusion from seven different regions that represent various parts of Basrah province (Fig. 1). The total number of patients was 120, distributed as follows: City centre: 43.33%, Al-Zubair: 15.83%, Al-Qurnah: 13.33%, Al-Medina: 10.83%, Abu-Alkasib: 7.5%, Shatt al-arab: 7.5%, Al-Fao:1.0% (Table. 2).

Figure 1:- Map of Basrah province, Iraq.

Table 2:-Distribution of thalassemic Patients over Basrah regions.

The Region	No. of Patient	Percentage %
City centre	52	43.33
Al-Zubair	19	15.83
Al-Qurnah	16	13.33
Al-Medina	13	10.83
Abu-Alkasib	9	7.5
Shatt al-arab	9	7.5
Al-Fao	2	1.0
Total	120	100%

The patients were from different age groups, the highest group (31.7%) was between 8-12 years, the lowest group (5%) was between 15 – 30 years and groups 0 – 4, 4-8, 12 – 15 were 14%, 29%, 15% respectively as shown in table 3. The results showed that most of the patients (40%) were from group O+, the second main group of the patients (21.67%) were from groups A+ and B+ whereas other groups AB+ (5.8%), AB- (1.67%) , O- (3.3%), A- (2.5%), B- (2.5%) showed low percentage (table 4). Most of the patients (80.83%) had consanguineous marriages (table 5).

Table 3:-Range of age among thalassemic patients

Age (year)	Male	Female	Total	Percentage %
0 – 4	9	8	17	14.2
> 4 – 8	19	16	35	29.2

> 8 – 12	22	16	38	31.7
> 12 – 15	7	11	18	15
> 15 – 18	2	4	6	5
> 18 – 30	3	3	6	5
Total	62	58	120	100%

Table 4:- Blood groups among thalassemic patients

Blood group	Male	Female	Total	Percentage %
A +	18	8	26	21.67
A -	2	1	3	2.5
B +	13	13	26	21.67
B -	1	2	3	2.5
AB+	4	3	7	5.83
AB -	1	1	2	1.67
O +	21	28	49	40.83
O -	2	2	4	3.33
Total	62	58	120	100%

Table 5:- Consanguineous marriages among thalassemic patients

Found	97	80.83%
Not found	23	19.16%
Total	120	100%

ARMS-PCR Screening:-

DNA was extracted from fifty four thalassemic patient's samples. ARMS-PCR technique was employed to screen seven studied types of β-thalassaemia mutations using a specific set of primers for each mutation.

In all successful ARMS-PCR reactions, products for normal, heterozygous and /or homozygous cases for each type of the studied mutations were observed. For normal cases, the ARMS-PCR products was found within the normal primer reactions while in positive diagnosed patients, the ARMS-PCR products was found within both normal and mutant reactions in heterozygous cases and only within mutant primer reactions in homozygous cases.

ARMS-PCR products were observed as shown in table 6 and the representative figures (1, 2, and 3).

Table 6:- The pattern of results of the studied types of β-thalassaemia mutations

Mutation	Positive results %	ARMS-PCR productbp
IVS - I-5 (G – C):	24.3%	285 bp
Codon 8 \ 9 (+ G):	7.1%	225 bp
88 (C-T):	24%	684 bp
Codon 8 (-AA):	9%	520 bp
Codon 15 (G- A):	8.69%	500 bp
Codon 41/42:	2.94%	493 bp
Codon 30 (G-C):	28.57%	280 bp

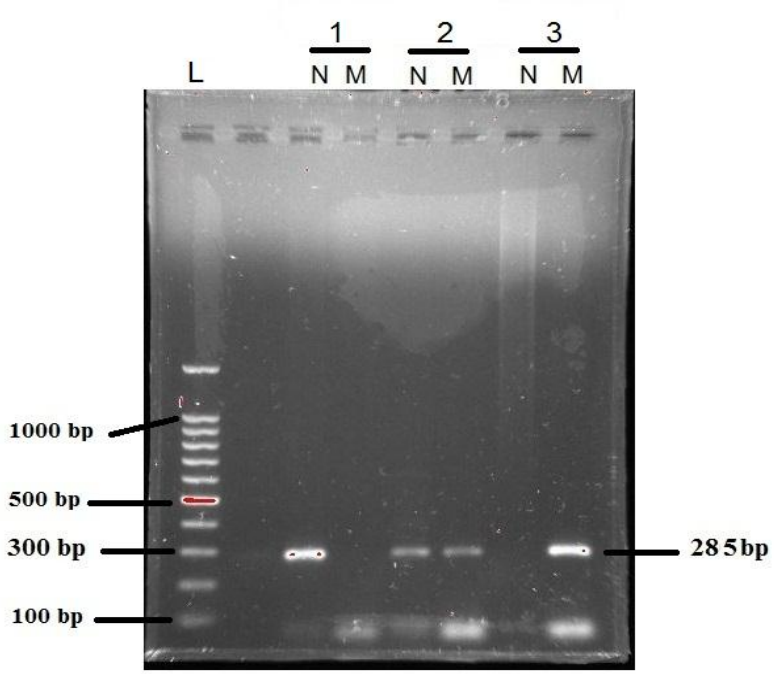

Figure 2p ARMS-PCR products of -IVS-I-5β-thalassaemia mutation.1: Heterozygous genotype 2:Normal genotype, 3: Homozygous genotype, L: ladder marker DNA 100bp .**N**: Normal; **M**: Mutant.

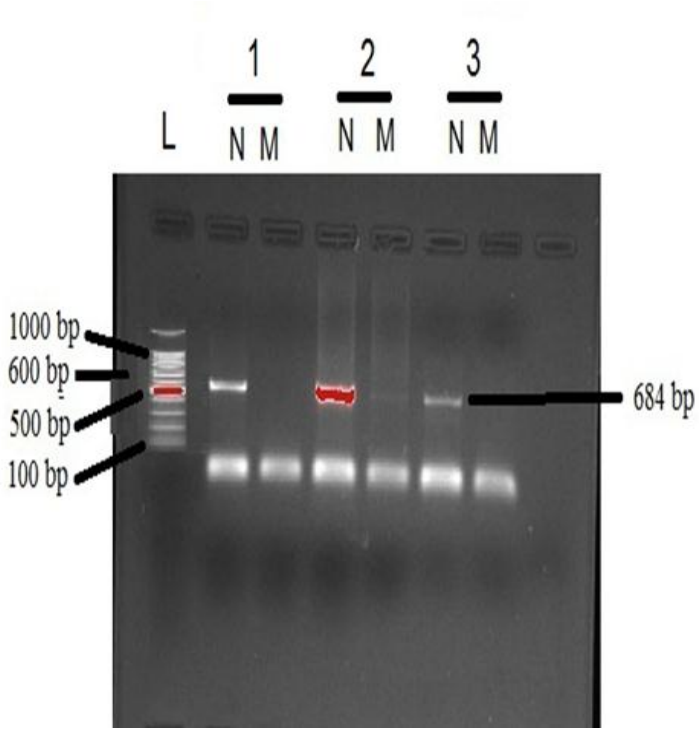

Figure 3:- ARMS-PCR products of 88 β-thalassemia mutation**.** 1 & 3: Normal genotype, 2: Heterozygous genotype , L: ladder marker DNA 100bp.**N**: Normal; **M**: Mutant.

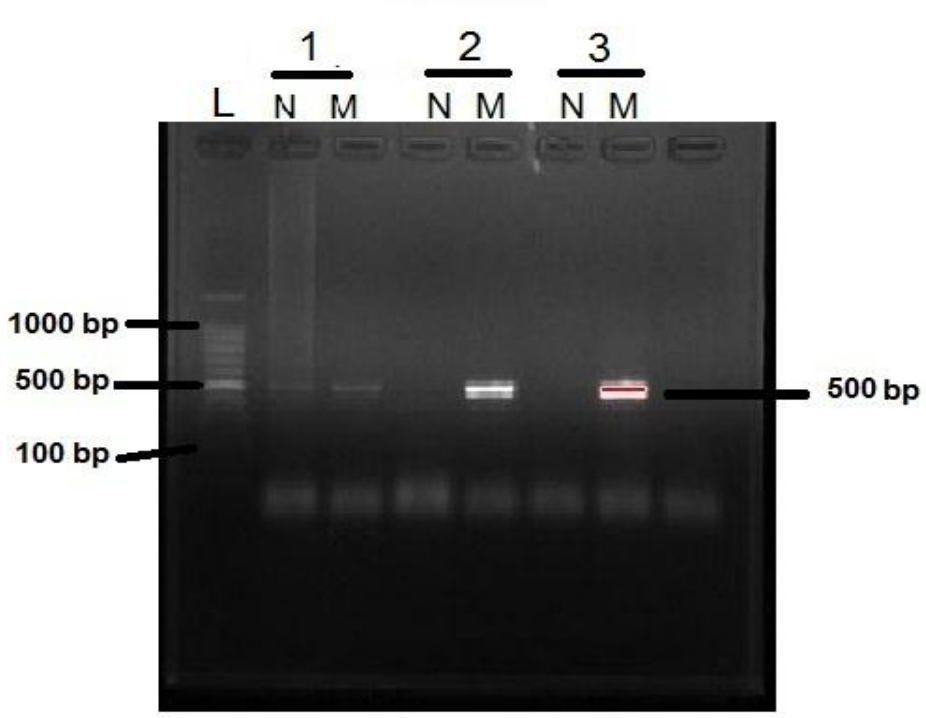

Figure 4:-ARMS-PCR products of codon 15 β-thalassaemia. 1: Heterozygous genotype 2 & 3: Homozygous genotype, L: ladder marker DNA 100bp. N: Normal, M: Mutant

Discussion:-

Thalassemia is a major health problem in Basra. However, the geographical and social distributions of thalassemia in this region has yet to be studied. The geographical distribution of the thalassemic patients in the present study reflects the presence of β-thalassaemia within different regions in Basrah province. The results indicate that β-thalassaemia can occur in all regions, races and ethnic groups. The highest incidence was in the city centre that may be due to the high population in this region. The results also showed that the consanguineous marriages can be considered as a main factor for the high prevalence of thalassemia in Basrah province.

β-thalassaemia was found in 60% of the patients under 12 years old (Table 3). Similar results have been reported by other studies (8).

Most of the patients (40%) were from group O+, which may be due to the majority of O+ blood group in Basrah.

Determining the precise spectrum of mutations in a given populations is vital to implement strategies for genetic counseling (Chan *et al*., 2010). In this study, we employed ARMS-PCR technique for molecular screening of seven studied types of β-thalassaemia mutations using a specific set of primers for each mutation. We found that the Codons 30 (G-C) 28.57%, IVS - I-5 (G – C) 24.3%, and -88 (C-T) 24% were the highest frequent in Basrah province. Whereas, Codon 8 (-AA) 9%, Codon 15 (G- A) 8.69%, Codon 8 \ 9 - 7.1% occurred in less frequency among the sample tested. On other hand, Codon 41/42- 2.94% was the lowest frequent compared with other mutations investigated in the present study. These findings reflect the distribution of mutations in various parts of Iraq as has been reported by other studies (10-15). The types of β-thalassemia mutations, in middle and northern part of Iraq, have been frequently investigated (10-15). Those studies showed the geographical and social distributions thalassemia mutations in the region. Al-Allawi et al. (2006) carried out a study to determine the molecular basis of β-thalassemia in Dohuk of northern Iraq and found that the eight most frequent mutations in order of frequency are: IVS-II-1 (G-to-A), codon.44 (-C), codon5 (-CT), IVS-I-1 (G-to-A), IVS-I-6 (T-to-C), codon 39 (C-to-T), codon 8/9 (+G), and IVS-I-5 (G-to-C) (11).

The four less frequent mutations are: codon 8 (- AA), IVS-I-110 (G-to-A), codon 22 (-7 bp), and codon 30 (G-to-C). These findings, partially, similar to our results except the mutation in codon 30 (G-to C) was the highest frequent in Basra province whereas this was the lowest in northern Iraq. Al-Assadi (2007) study showed that the frequency of IVS1 nt.5 has been 12.5% in thalassemic samples in Iraq (12). While in present study the frequency of this mutation in thalassemic patient's samples was 24.3%.

Saud et al (2012) showed that highest frequency of IVS-I-5 mutation in Iraq was in Basrah with 66.6% as a percentage from the total number of thalassemic samples studied in this city (25). Whereas our results showed that the frequency of this mutation in thalassemic patient's samples was 24.3% that may be because of the low number of samples used in Saud's study. Saud et al (2012) study showed that codon 8/9 mutation was higher in Sulaymaniyah in North Iraq (50%) while in present study the frequency of this mutation was 7% (25). Also, Saud (2012) found that codon 8 mutation was only observed in Sulaymaniyah in northern Iraq, on frequency 10% but not in other cities (25). Our results showed that the frequency of codon 8 mutation was 9% in Basra province. Saud (2012) stated that Babylon and Wasit, in the middle of Iraq, showed the highest frequency of codon 15 (25% for both cites), in addition, -88 mutation was higher in Anbar and Salahden, west of Iraq, (33.3% for both cites) (25). In the present study we found that the mutation of codon -88 (C-T) 24% was of higher frequency in Basrah province. Other study carried out in AL-Muthanna province showed three types of mutation in β-thalassemic patients (IVS- I-5, Codon 8\9, Codon15), the highest percent of β-thalassemic patients mutation is IVS-I-5 (53.8 %) followed by Codon 8\9 and Codon15 with percentage (27.6%) and (18.4 %) respectively (15). Whereas, our results showed the frequency of codon IVS-I-5, 15 and 8/9 were 24.3%, 8.69% and 7.1% respectively.

On the other hand, codon 30 mutation was not found in any of the thalassemic samples investigated by other studies, while it has the highest frequency (28.57%) in our study. This may be because of the small number of samples used by other studies which, of course, were not presenting the real number of the thalassemic patients in Iraq.

The comparison of the mutation frequencies observed in Basra province with other mutations in different regions of Iraq referred that the distribution of β-thalassaemia alleles was differed within each area. These findings supported by the previous observations which indicated that the frequency of several mutations varies from one region to another, β-thalassemia mutations were very heterogeneous, and finally there were nospecific distribution patterns that would aid in the identification of any ethnic background.

This difference in percentage may be due to each province having experienced admixtures from various populations throughout history. Furthermore, migration between cities has been common until the present times.

Conclusion:-

Seven different β-thalassemia mutations were detected. Codons 30, -88 and IVS - I-5 mutations were the highest frequent compared with other mutations studied in the present study. Codon 30 mutation was observed with high frequency for the first time in Basrah. These results can be added to other research findings to build up a database for β - thalassemia mutations types in Iraq, aiming to establish a proper preventive program.

References:-

1. Modell B &Darlison M (2008). Global epidemiology of hemoglobin Disorders and derived service indicators. *Bull World Hlth Org* 86; 480-487.
2. Weatherall, D.J. (2006). The Thalassemias: Disorders of Globine Synthesis. *Hematology.7th ed*. *New York: McGrow Hill*. 633-66.
3. Mok,S.; Imwong, M.and Mackinnon, M. J. (2011). Artemisinin resistance in *Plasmodium falciparum* is associated with an altered temporal pattern of transcription. *BMC Genomics*.12; 391
4. ChristiansonA, HowsonCP, ModellB (2006) .*March of Dimes Global Report on Birth Defects. New York, NY: March of Dimes Birth Defects Foundation.*
5. Chan, V.; Chan, V.W., Tang, M., Lau, K., Todd, D.,et al. (1997). Molecular defects in Hb H hydropsfetalis. *Br J Haematol*, 96 (2); 224-228.
6. Chui, D.H.K. & Waye, J.S. (1998). HydropsFetalis Caused by □-Thalassemia: An Emerging Health Care Problem. *Blood*, 91; 2213 - 2222.
7. Weatherall DJ and Clegg JB. 2001. The thalassaemia syndromes. *4th Ed.Blackwell Science, Oxford*

8. Thein, SL. (2004). Genetic insights into the clinical diversity of betathalassaemia. *Br J Haematol.* 124 (3); 264-74.
9. Li, Q., Li, LY. and Huang, SW. (2008). Rapid genotyping of knownmutations and polymorphisms in beta-globin gene based on theDHPLC profile patterns of homoduplexes and heteroduplexes. *Clin Biochem*.41; 681-687.
10. Al-Abboodi, M.J. (2011). Molecular Study for Some Beta-globinMutations in Sample of Iraqi Thalassemic Patients. MSc thesis,Institute of Genetic Engineering and Biotechnology for Post GraduateStudies, University of Baghdad, Iraq.
11. Al-Allawi,NA; Jubrael, JM. and Hughson, M.(2006). Molecularcharacterization of beta-thalassemia in the Dohuk region of Iraq. *Hemoglobin*. 30 (4); 479-486.
12. Al-Assadi, Z.M. (2007). Molecular detection of some mutationsassociated with beta-thalassemia in Iraq.Ph.D. thesis, Institute ofGenetic Engineering and Biotechnology for Post Graduate Studies,University of Baghdad, Iraq.
13. Al-Hadad, R.H. (2007). Molecular Genetic Study of β-thalassemiamajor syndrome in Baghdad. MS.c thesis, Al-Nahrain University,Iraq.
14. Al-Jasha'mi, J.N. (2008). Identification of Some Beta-Thalassemia Mutations in Iraqi Population MSc thesis, Institute of Genetic.
15. Khalid G .Al-Fartosi1 and Hanaa Ali Azez (2014). Mutation Which Causes β- thalassemia in AL-Muthanna Province. *International Journal of Advanced Research*, 2 (10); 321-329
16. Stephen, A. (2002). Mutation detection by ARMS/RFLP,pyrosequencing, oligohybridization, oligo ligation assay.*MRC pathself-help course (London).*
17. Talmaci, R.; Traeger, J.; Kanavakis, E.; Coriu, D., Colita D. andGavrila L. (2004). Scanning of β -globin gene for identification of β-thalassemia mutation in Romanian population. *J.Cell. Mol.Med*., 2; 232-240.
18. Baig, S.M.; Rabbi, F.; Hameed, U.;Qureshi, J.A.; Mahmood, Z.;Bokhari, S.H.; Kiani, A., Hassan, H.; Baig, J.M.;Azhar, A.andZamanT.(2005).
19. Molecular characterization of mutations causing β -thalassemia in Faisalabad Pakistan using the amplification refractorymutation system (ARMS-PCR). National Institute for Biotechnology and Genetic Engineering *(NIBGE).* 11 (2); 80-83.
20. Basak, A. N. (2007). The molecular pathology of β-thalassemia in Turkey. *Hemoglobin,* 31 (2); 233–241.
21. Mirasena, S.; Shimbhu, D.; Sanguansermsri, M. and Sanguansermsri,T. (2008). Detection of β-thalassemia mutations using a multiplexamplification refractory mutation system assay. *Hemoglobin*. 32 (4); 403-409.
22. Sarookhani,M. R.; Ahmadi, M.H.andAmirizade, N. (2009).Molecular Spectrum of Beta-Globin Mutations in TransfusionDependent Patients with Thalassemia in Qazvin Province, *Iran. I. J.Med. Sci* .34 (1); 17-22.
23. Saleh-Gohari,N**.**and Bazrafshani MR. (2010). Distribution of β-Globin Gene Mutations in Thalassemia Minor Population of KermanProvince, Iran.*Iranian J Publ Health*. 39 (2); 69-76.
24. Sambrook,J.; Fritsch, E.F. and Maniatis,T. (1989). Molecularcloning: *a laboratory manual. 2nd Ed., Cold spring harbor laboratorypress, cold spring harbor, New York*.
25. Chan, O.T.; Westover, K.D.; Dietz, L.; Zehnder, J.L. and Schrijver, I. (2010). Comprehensive and efficient HBB mutation analysis fordetection of beta-hemoglobinopathies in a pan-ethnic population. *Am.J. Clin. Pathol*. 133 (5); 700-707.
26. Saud, A .M.; Al-Azzaiwe, F .H. and Al-Kazaz, A.A.(2013). Molecular and Biochemical study on β-Thalassemia Patients In Iraq. PHD thesis. College of science .Baghdad University.