Türk Biyokimya Derneği
Turkish Biochemical Society

The results in two different provinces in Black Sea Region where thalassemia screening was implemented:a rare hemoglobin variant

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We aimed for assessing the results of a thalassemia test implemented for the purpose of screening in the provinces of Amasya and Tokat and for revealing the clinical features of a rare variant type of hemoglobin in our study.

☑ n=2258 patient samples (55.8% males and 44.2%) were screened for this study retrospectively

☑ Hemoglobin variant analysis was carried out through the method of HPLC (High Performance Liquid Chromatography) on Primus Ultra2 device (boronat afinity)

☑ The sample was also examined on a different system for the rare hemoglobin variant (Hb Pusan) and DNA chain analysis was implemented for substantiation for ***Hb Pusan*** variant.

Total 2258 patient samples

Normal resuls: 2170
(56.3% males and 43.7% females)

Suspected of beta thalassemia:
50 patients
(44% males, 56% females)

Suspected of alpha thalassemi = 37 patients
(40.6% male and 59.4 % female)

- 1 female patient with suspected of hemoglobin E variant
- *hemoglobin Pusan* variant was detected in n=1 male patient.

Case report

- A 25-year-old male patient of Turkish origin was admitted to Amasya Public Health Laboratory for thalassemia screening. Physical examination findings of the patient revealed no signs of anemia or other diseases. Liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin and direct bilirubin levels), iron, total iron-binding, B12, and folic acid levels were normal. Serology tests were negative.

In the hematology panel

Tests	Value	Referance range
Hemoglobin (Hb)	17 g/dL	13.2-16.6
Red blood cell (RBC)	5.95 M/µL	3.8
White blood cell (WBC)	6.21 K/µL	4.5-10.5
mean corpuscular volume (MCV)	84.5 fL	80-102
mean corpuscular hemoglobin (MCH)	28.6 pg	25.6-34
Hematoctit (HCT)	50.3%	36.9-49.1
P50 value	20 mmHg	26.5±1,3 mmHg

Erythrocytosis

P50 value was low (20 mmHg)

increased oxygen affinity

HPLC results of the case analyzed at Trinity Biotech Primus Ultra 2 hemoglobin variant analyzer

ultra2-215, Trinity Biotech Resolution Assay Version 5.2.0
Instrument Serial Number: 100695 - Page 2 -

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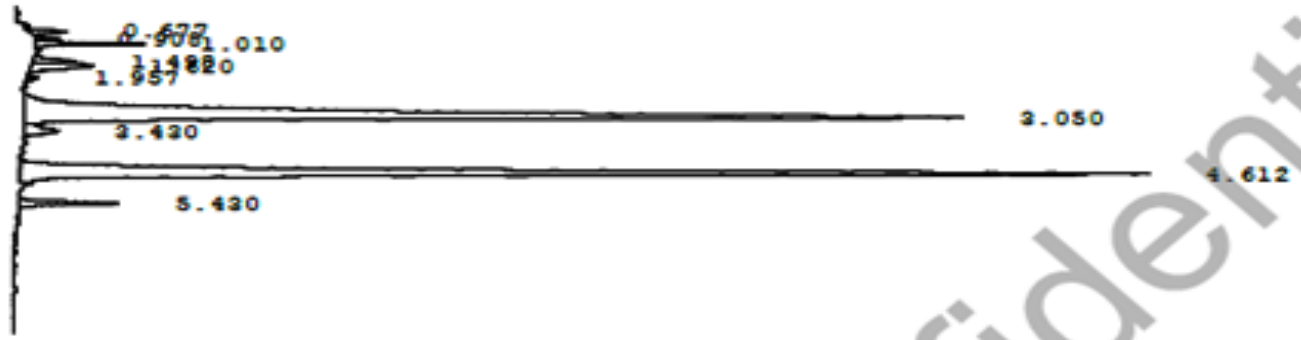
Batch 755, Rack A, Vial 001,
[C206E32D4E8D7E02] Jul 01, 2019 08:23:14 Pressure = 62 bar (62 to 62)



PEAK	RT		REL RT	% CONC	AREA	COMMENT
1	0.677	F	0.37	0.8%	9756	
2	0.908	F	0.50	0.5%	6387	
3	1.010	F	0.55	1.7%	20229	
4	1.498	F	0.82	0.9%	10650	
5	1.620	F	0.89	2.5%	30026	
6	1.957	F	1.07	0.3%	3372	
7	3.050	F	1.67	46.9%	573472	3
8	3.430	A	0.74	1.6%	19529	
9	4.612	A	1.00	42.6%	520357	2 A0 peak
10	5.430	S	0.88	2.4%	29046	A2 peak

Total Area: 1222824

Cation exchange HPLC results of the case analyzed at Bio-Rad Variant Turbo II hemoglobin variant analyzer and the graph showing the peaks

Bio-Rad CDM System
Bio-Rad Variant V-II Turbo Instrument #2

PATIENT REPORT
V2_BThal

Patient Data
Sample ID:
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

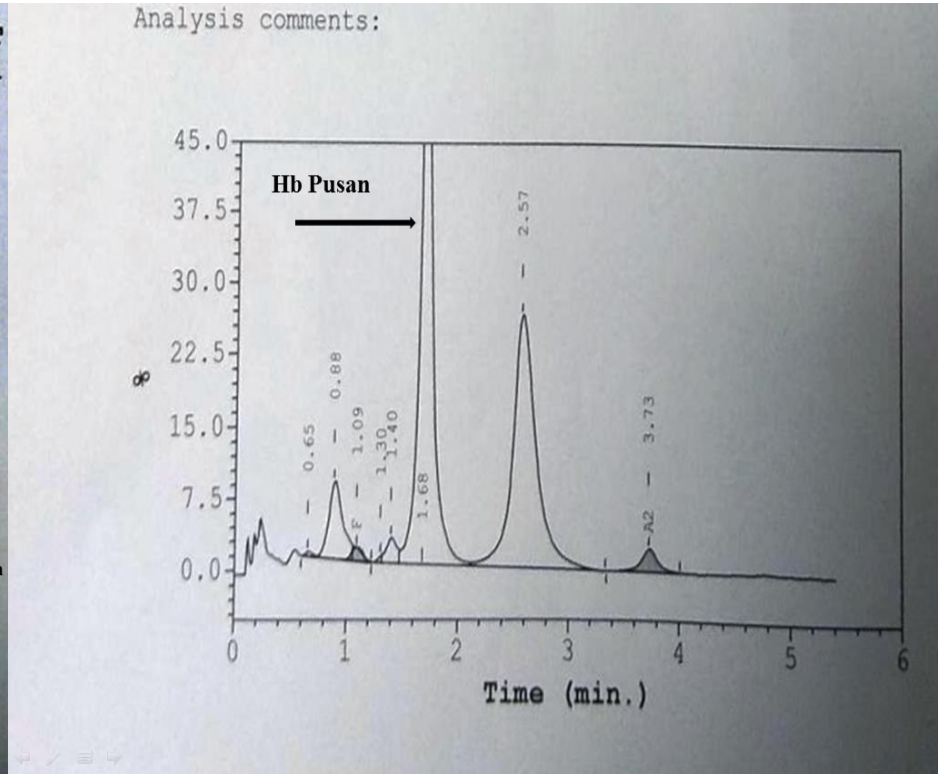
Analysis Data
Analysis Performed: 09/07/2019 12:41:42
Injection Number: 577U
Run Number: 94
Rack ID: 0001
Tube Number: 3
Report Generated: 09/07/2019 14:51:02
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.2	0.65	4220
P1	---	7.1	0.88	140184
F	1.0	---	1.09	18662
Unknown	---	0.2	1.30	4053
P2	---	1.7	1.40	32647
P3	---	48.3	1.68	948701
Ao	---	39.0	2.57	764806
A2	2.4	---	3.73	49679

Hb Pusan

Total Area: 1,962,951

F Concentration = 1.0 %
A2 Concentration = 2.4 %



Hastanın Adı Soyadı : [redacted]

Protokol / Dosya / İşlem No: 33151385 / 4062056

TC Kimlik: [redacted]

Doğum Tarihi , Cinsiyeti : 03.11.1993 / ERKEK / 25

Rapor Numarası: 177354.189.33151385.2019

[redacted]

Numune: /18243242

Tetkik İstem Zamanı : 10/07/2019 15:08

Num.Kabul Zamanı : 10/07/2019 15:59

Tetkiki İsteyen : [redacted]

Numune Alma Zamanı:

Uzman Onay Zamanı: 05/08/2019 15:49

Tetkik Adı	Sonuç
ÇALIŞILAN TEST:	HBB Geni Dizi Analizi
RAPOR NO:	2019-245
AÇIKLAMA:	Following the sequence analysis [5'UTR (-110), exon 1-3, IVS1-2, 3'UTR)] of DNA sample isolated from peripheral blood of the patient, the HBB gene (GRChg37: NM_000518) was identified as a heterozygous Hb Pusan variant (HBB: c.439C>A) (p.His146Asn).
SONUÇ:	
YORUM:	**

The genomic DNA of the sample was isolated according to the manufacturer's protocols. For the Sanger sequencing of HBB gene, the Polymerase chain reaction (PCR), purification (ExoSAP-IT®, Affymetrix) and cycle sequencing PCR (BigDye® Terminator v3.1, Thermo Scientific) reactions were carried out. Products were purified (ZR DNA Sequencing Clean-up Kit TM, Zymo Research) and run by capillary electrophoresis (3500 Genetic Analyzer, Thermo Scientific). The DNA sequences obtained were analysed in the Sequencing Analysis Program and compared with the reference sequences.

☑ There are no publications or case reports reporting this variant type, which was identified in a study carried out in the Korean population, in a Turkish patient.

☑ This is the first study in the literature in this regard.

Genetic mutations that have been encountered in the same region as Hb Pusan, and that have previously been identified by the DNA chain analysis, are as follows;

Hemoglobin Hiroshima (CAC>GAC, β146 histidine → aspartic acid),

Hemoglobin Bologna (CAC>TAC, β146 histidine → Tyrosine, HBB: c.439 C>T),

Hemoglobin York (CAC>CCC, β146 histidine → Proline, HBB: c.440 A>C),

Hemoglobin Cochin-Port Royal (CAC>CGC β146 histidine → Arginine, HBB: c.440 A>G),

Hemoglobin Cowntown (CAC>CTC, β146 histidine → Leucine, HBB: c.440 A>T),

Hemoglobin Kadaria I (CAC>CAA, β146 histidine → Glycine, HBB: c.441 C>A), and

Hemoglobin Kadaria II (CAC>CAG , β146 histidine → Glycine HBB: c.441 C>G)

****All the variants of the position 146 display a mild increased oxygen affinity leading to some degree of erythrocytosis.**


THANK YOU :)