Anatomy Physiology & Biochemistry
International Journal
ISSN: 2476-1400

Juniper Publishers
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Case Report
Volume 4 Issue 5– APRIL 2018
DOI: 10.19080/APBIJ.2018.04.555650

Anatomy Physiol Biochem Int J

Detection of a Rare Haemoglobin Variant- Hbj during Glycosylated Haemoglobin Analysis- A Rare Single Case Report

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Submission: March 12, 2018; **Published:** April 13, 2018

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Abstract

We report the case of a very rare haemoglobin variant, "Haemoglobin J", discovered while performing haemoglobin electrophoresis following low HbA1C value reported in pregnant female with Gestational diabetes mellitus. Haemoglobin J is an alpha chain variant and heterozygous group of fast moving Haemoglobin (FMH).

Abbreviations: FMH: Fast Moving Haemoglobin; Hb: Haemoglobin; HCT: Haemocrit; RBC: Red Blood Cell; RWD: Red Cell Distribution Width; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; IBCT: Iron Binding Capacity Total; GTT: Glucose Tolerance Test

Introduction

Glycosylated Haemoglobin is routinely used to maintain long term glycemic control in diabetic patients [1]. But sometimes HbA1C levels are formed contradicting with plasma glucose levels. This may be because of presence of abnormal and rare haemoglobin HbJ which was first reported in an American Negro family by Thorup et al. [2]. HbJ is fast moving hemoglobin (FMH). This alpha globulin gene variant resulting from substitution of a negatively charged amino acid residue is alpha, beta or gamma globulin chain [3]. HbJ can be differentiated and identified solely from its retention time. Prevalence of Fast Moving Haemoglobin Variant HbJ is very rare. Different authors during epidemiologic investigation in different population did not find any single case of HbJ showing it to be rare variant [4-6]. In a large study of 13,913 individuals of North Americans 524 had abnormal Hb variant but none of the patient had HbJ variant [7].

Similarly in a study of 9792 cases of Tunisian population only 228 cases (2.33%) were found to be having abnormal Hb Variant but none was having HbJ variant [8]. Although during a large retrospective study (65,779 cases) for screening, detection, and identification of Hb variants within a clinical laboratory setting of India only 46 cases (0.07%) were found to be having HbJ variant [9] and 10 yr study of hemoglobin variant on 1,19,336 cases from eastern Indian population only three cases were found to be having Hb J variant [10] which shows this variant to be very rare. Here we report our experience of detection of abnormal Hb variant (HbJ) during further investigation of pregnant female as low HbA1C value was contradicting with high glucose level. It was not a case of Sickle cell disease (SCD) nor had received any blood transfusion.

Case Report

Table 1: Pathological and Biochemical findings of patient.

Parameters	Value	Reference Range
RBC	3.66 L	3.8-4.8 ml/Ul
Hb	9.8 L	12-15g/dl
HCT	32.1	36.0-46.0%
RDW	17.2 L	11.6-14.0%
MCH	26.8 L	27-32 pg
MCHC	30.5 L	31.5-34.5 g/dl
Glycosylated Hemoglobin	4.5 L	<5.7%
GTT(2)		
Glucose (F)	84.0	mg/dl
Glucose 2hour (PP)	203 H	mg/dl

Iron	57	50-170 ug/dl
IBCT	462 H	250-450 ug/dl
% Saturation	12	13-45%
Ferritin	14.0	13.0-150 mg/dl

Abbreviations: Hb: Haemoglobin; HCT: Haemocrit; RBC: Red Blood Cell; RWD: Red Cell Distribution Width; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; IBCT: Iron Binding Capacity Total; GTT: Glucose Tolerance Test

A 29 years old four and a half months pregnant female with gestational diabetes mellitus presented to Gynae OPD of Fortis Hospital, Shalimar Bagh for routine ante natal check up. As a part of routine investigation Complete Blood Count, GTT, HbA1C and Iron Profile was advised. EDTA anticoagulated whole blood specimen collected in Becton Dickinson Vacutainer was analyzed for CBC, HbA1C and 2 ml blood sample was collected in fluoride vial for Blood glucose levels. After 2 hours of taking 75gms glucose her blood glucose levels were on the higher side (blood glucose- 203mg/dl). Hb (9.8%) was on the lower side with low haemocrit (32.1), low MCV and MCHC (Table 1). Complete Blood Count was done on Sysmex XN 550 (L Series). Blood glucose was performed on Dimension RxL Max series.

Glycosylated hemoglobin was performed on Bio Rad D10 HPLC instrument as per standard procedures which come out to be low (HbA1C - 4.5%). Our patient was diabetic but her HbA1C came out to be very low (4.5%) indicating it to be falsely reduced as evidenced by the level of elevated blood glucose (203mg/dl). HPLC Chromatogram showed an unknown peak of Retention Time (1.40min) indicating the possibility of abnormal variant (Figure 1). So Haemoglobin Electrophoresis was done with two different laboratories. Both the laboratories evidenced the rare variant HbJ in the patient sample (Figures 2 & 3). Our patient was not a case of sickle cell disease (SCD) in which abnormal variant is a usual phenomenon and also patient had not received any blood transfusion. Hb Variant analysis of both the laboratories showed an unknown peak of 20.6% and 20.8% with Retention Time of 1.96 and 1.99 min respectively suggestive of presence of alpha/beta chain variant (HbJ) as HbJ is fast moving variant. Both the laboratories detected almost same findings(Table 2-4).

Table 2: Peak values with their retention time during HbA1C analysis.

Peak	Retention Time	Height	Area	Area %
A1a	0.19	2853	16254	1.1
A1b	0.28	2379	10596	0.7
F	0.43	1382	8502	0.6
LA1C/CHb	0.65	2015	27515	1.9
A1C	0.88	3076	47093	4.5
P3	1.37	12343	46581	3.2
Unknown	1.40	48501	127273	8.6
A0	1.46	263415	1189578	80.7
Total Area	1473393			
HbA1C	4.5%			

Table 3: Hemoglobinopathy evaluations by HPLC (Lab-1).

Test	Within range	Out of range	Biological ref. Range	Units
HbAO+Other Normal Hb Peak		76.2 L	>96.0	
HbA2	2.0		1.8-3.5	%
HBF	1.1		<2.0	%
HbE	0.00			%
HbD	0.00			%
HbS	0.00			
Other ABN Hb Variant	There is an abnormal peak seen with RT of 1.96 and concentration of 20.6%			

Table 4: Peak values with their retention time during Hemoglobin Electrophoresis (Lab-1).

Peak name	Calibrated area	Area %	Biological ref. Range	Peak area
Unknown	---	0.3	0.96	5052
1*	1.1	---	1.04	16407
Unknown	---	1.6	1.22	23368

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P2	---	2.9	1.35	50212
P3	---	3.4	1.71	51896
Unknown	---	20.0	1.96	310109
Ac	---	47.0	2.44	1010684
A2	2.0*	---	3.62	31684
Total area	1,507,009			

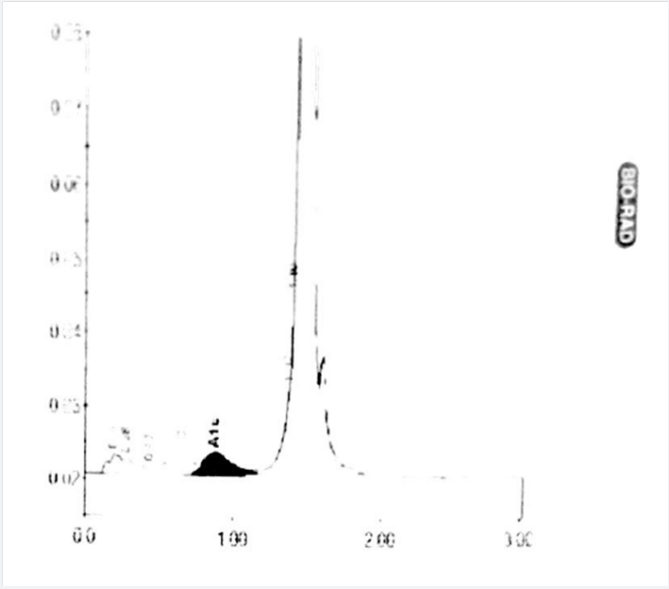

Figure 1: Chromatogram showing HbA1C values without unknown peak at RT 1.40min.

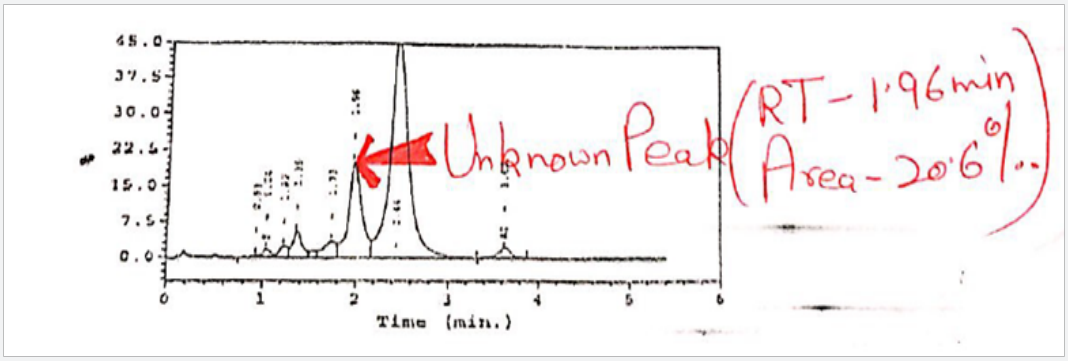

Figure 2: HPLC variant analysis showing abnormal hemoglobin peak of HbJ (Lab-I).

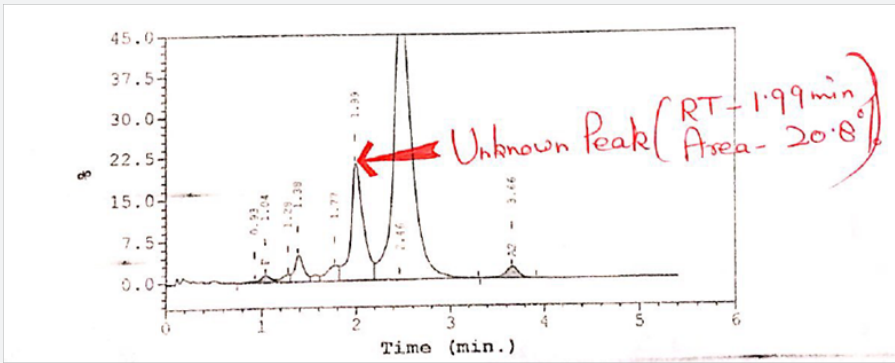

Figure 3: : HPLC Variant analysis showing abnormal hemoglobin peak of HbJ (Lab-II).

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Treatment And Follow Ups

Family studies, DNA studies and genetic counselling were advised for the patient from both the laboratories. But as HbJ is rather clinically silent and our patient had no adverse symptoms related to abnormal variant. Further characterization of this haemoglobin variant was not indicated clinically, so molecular studies were not pursued (Table 5).

Table 5: Hemoglobinopathy evaluation by HPLC (Lab-2).

Test	Within range	Out of range	Biological ref. Range	Units
HbA		76.0 L	95-98	%
HbA2	2.1		2.0-3.7	%
HbF	1.0		0.1-1.2	%
HbS	0.0		0.00-0.00	%
HbD	0.0		0.00-0.00	%
HbC	0.0			%
Unknown Unidentified Peak		20.8H	0.00-2.0	%

Discussion

HbA1C is used for long term management of patients with DM type-1 and 2 [11] but wherever the value of HbA1C is discrepant there is always possibility of abnormal fast moving haemoglobin [12]. In our patient HbA1C came out to be low in spite of High glucose levels during antenatal screening indicating the possibility of abnormal variant. Abnormal haemoglobin like Hb J which produces no haematological symptoms is rarely detected. Similarly a case of HbJ-Meerut was detected when pregnant female came for routine antenatal check up showing falsely low HbA1C levels not corresponding to their Blood glucose levels. HPLC analysis for patient's mother and two younger siblings showed the presence of same abnormal haemoglobins seen in patient herself. The mean percentage of this abnormal Hb variant in these four family members was 19.025 eluted at a retention time of 1.87-1.90min [3]. During study of abnormal haemoglobin variant in large Indian Population other authors [13-15] found out only one case of HbJ Meerut among 232 cases, 543 cases, 290 cases of abnormal hemoglobin variant respectively showing it to be very rare (Table 6).

Table 6: Peak values with their retention time during HbA1C analysis (Lab-2).

Peak name	Calibrated area %	Area %	Retention time (min)	Peak area
Unknown	---	0.2	0.93	3400
F	1.0	---	1.04	17478
Unknown	---	1.0	1.28	17974
P2	---	3.4	1.38	61151
P3	---	2.7	1.77	48315
Unknown	---	20.8	1.99	372983
Ao	---	68.7	2.46	1231491
A2	2.1*	---	3.66	39272
Total area	1,792,069			

HbJ variant which is an alpha chain variant found in heterozygous state may also be detected via using different techniques - mass spectrometry. In a study Bhatt, et al. [16] identified Variant HbJ - Rajappen using mass spectrometry technique. Patient being diabetic had blood sugar levels 232 mg/dl but HbA1C level 4.4% indicating presence of abnormal variant. Upon electrophoresis a p3 Peak with a retention time of 1.3 min and a percentage of 23.4% was found out. This finding is very much similar to our findings [16].

Even though hemoglobin variants are prevalent in general population, reports of transfusion acquired Hb Variants are rare. Soumya P et al [17] performed a retrospective analysis on SCD patients who underwent blood transfusion. 66 patients were found to be having abnormal Hb Variant and only on patient had HbJ variant. There are more than 40 HbJ variants described in the literature. They all have an electrophoretic mobility faster than the Hb variants. All are classified under variants of the alpha or beta chains [18-20]. In sickle cell disease patients due to number of blood transfusion, it is very common to found abnormal variant. N Sweden et al. [21] discovered HbJ variant while performing Hemoglobin electrophoresis following exchange transfusion of SCD patient after red cell exchange. The clinical impact of rare hemoglobin variant such as HbJ when combined with hemoglobin SCD (or trait) is not clear in the medical literature. Clinical follow up of the patient showed no complications and the clinical cause was consistent with the patient's underlying medical condition. Five units of RBC's

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were donated by two sisters. When hemoglobin electrophoresis was performed on samples of two sisters they were found to be having HbJ variant [21].

Take Home Message

1. Wherever there is falsely low HbA1C with Unknown peak in case of known chronic diabetic one should raise alarm to look for Unusual Variant affecting HbA1C.

2. HbJ is a rare Hemoglobin Variant which is heterozygous alpha globulin gene variant.

3. Unknown peak on HbA1C chromatogram and retention time are very useful information for detection and identification of rare hemoglobin variant.

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