ORIGINAL ARTICLE

Chromatogram characteristics of seven Hb J variants on capillary zone electophoresis

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ABSTRACT

Background: Historically, Hb J variants have been identified by high performance liquid chromatography, though capillary zone electrophoresis is increasingly being used as a first line technique for Hb variant identification. However, there is a lack of Hb J capillary zone electrophoresis electrophoretic chromatograms in the existing literature. The objective of this report is to describe chromatogram characteristics of seven Hb J variants on capillary zone electrophoresis.

Methods: A routine diagnostic in-house laboratory haemoglobinopathy database was searched for Hb J variants. Capillary zone electrophoresis characteristics of each case were documented, including the zone, x-axis position, and Hb variant percentage. Clinical characteristics, presenting red blood cell parameters, and serum ferritin levels were recorded.

Results: 14 heterozygous cases for Hb J comprising seven different Hb J variants were identified: four *HBA* and three *HBB* variants. *HBA:* Hb J-Cape Town (four cases) with 35.0-37.7% variant migrated in zone 11 at x-axis 121, 0.8-0.9% of Hb J-Cape TownA_2 migrated in the S zone at x-axis 217, and a further minor aberrant peak of 0.5% migrated in zone 12 at x-axis 84; Hb J-Tongariki (two cases) 34.3-36.2% variant migrated in zone 12 at x-axis 106 and 0.6-0.8% of Hb J-TongarikiA_2 migrated in the D zone at x-axis 203; Hb J-Toronto (one case) 23.1% variant migrated in zone 12 at x-axis 97 and 0.4% of Hb J-TorontoA_2 migrated in the D zone at x-axis 208; Hb J-Paris-I (one case) 27.8% variant migrated at x-axis 81 and 0.3% of Hb J-Paris-IA_2 migrated in the D zone at x-axis 198; *HBB*: Hb J-Baltimore (four cases) 50.3-54.1% variant migrated in zone 12 at x-axis 81; Hb J-Bangkok (one case) 51.9% variant migrated in zone 12 at x-axis 93; Hb J-Kaohsiung (one case) 47.3% variant migrated in zone 13 with an x-axis 66.

Conclusions: This article describes characteristic capillary zone electrophoresis chromatogram patterns, zone, x-axis and Hb variant percentage, for seven Hb J variants, five of which have not been previously described on capillary zone electrophoresis. This will be of value in expanding the capillary zone electrophoresis chromatogram library and aid in the provisional identification of Hb J variants.

Keywords: -α3.7 (rightward) deletion, capillary zone electrophoresis, chromatogram, Hb J variants, x-axis.

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INTRODUCTION

Hb J are haemoglobin variants that migrate anodally to Hb A on alkaline gel electrophoresis. Over 50 different Hb J variants have been reported with mutations described on the *HBA1, HBA2* or *HBB* genes (1,2). The frequency of different Hb J variants varies considerably with geographical location and ethnicity (3-6). Hb J heterozygosity is usually associated with normal clinical and haematological findings (7). Though, some Hb J variants are commonly co-inherited with α-thalassemia which causes microcytic red blood cell indices (4), while some Hb J variants have abnormal properties which affect the haematological indices (8).

The majority of Hb J cases reported to date have been identified by alkaline Hb electrophoresis or cation exchange HPLC, with definitive identification by DNA molecular analysis. Consequently, there is considerable published literature detailing expected findings of HPLC retention times, percent of total haemoglobin, and characteristic elution peak shape by these methods for Hb J variants (9).

Capillary zone electrophoresis is an alternative first line technique that is emerging as a reliable method for Hb variant detection (10). However, there is a paucity of existing Hb J variant chromatograms using this methodology. Similar to HPLC retention times, capillary zone electrophoresis utilizes a specific x-axis migration position which along with the migration zone, Hb variant percentage, and characteristic capillary zone electrophoresis chromatogram pattern can aid in the provisional identification of Hb variants, including Hb J (11).

In our laboratory, where capillary zone electrophoresis is used for routine haemoglobinopathy screening, 14 heterozygous cases for Hb J were identified over a two-year period. These 14 cases comprised seven different Hb J variants: Hb J-Cape Town, Hb J-Tongariki, Hb J-Toronto, Hb J-Paris-I, Hb J-Baltimore, Hb J-Bangkok, and Hb J-Kaohsiung. This paper describes the chromatogram characteristics of these seven Hb J variants, five of which have not been previously described. The capillary zone electrophoresis migration patterns, zone, X-axis, and Hb variant percentage are reported to aid in provisional identification of Hb J variants and expand the library of variants. All Hb variants were confirmed by subsequent DNA molecular analysis.

MATERIALS AND METHODS

All subjects investigated had full blood counts analysed on an electronic Sysmex XN900 analyser *(Sysmex* Corporation, Kobe, Japan) and serum ferritin levels on a Cobas 6000 instrument (Roche Diagnostics, Indianapolis, Indiana, USA. Identification and quantification of abnormal haemoglobins, Hb A_2 and Hb F levels, were measured by capillary zone electrophoresis using the Hb E programme on a Sebia Capillarys 2 Flex Piercing analyser (Sebia, Lisses, France), and the x-axis migration position was recorded for each Hb variant peak. Alkaline (cellulose acetate, pH 8.5) Hb electrophoresis was performed using the Sebia Hydragel (E) Hb kit (Sebia, Lisses, France) and stained with amidoblack.

α-Thalassemia screening was performed using the immunochromatographic strip test (i+Med Laboratories, Bangkok, Thailand). Confirmatory testing was undertaken by assessing the *HBA* gene copy number using multiplex ligation-dependent probe amplification SALSA probe mix P140 *HBA* assay (MRC-Holland, Amsterdam, Netherlands), followed by quantification on an ABI PRISM® *3130xl* genetic analyser (Applied Biosystems).

Examination of haemolysate and tryptic peptides by electrospray ionization mass spectrometry was undertaken on an Agilent 6230 time-of-flight instrument operating under MassHunter software and connected to a 1260 Infinity binary pump (Agilent Technologies, Santa Clara, CA, USA) by the method of Brennan *et al.*(12).

DNA was extracted from peripheral blood and studies for mutation in *HBA1*, *HBA2* and *HBB* undertaken. Mutation analysis was determined by direct sequencing of overlapping PCR products spanning the entire α- and β-globin genes. Sanger sequencing methodology using the BigDye terminator v3.1 cycle sequencing chemistry (ThermoFisher, Waltham, MA, USA) was performed. The products were then separated by capillary electrophoresis on an ABI PRISM® *3130xl* genetic analyser (Applied Biosystems, Foster City, CA, USA). Reference sequences used were: NM_000558.4 for HBA1, NM_000517.4 for HBA2, and HBB NM_000518.4.

RESULTS

HBA Hb J variants

Four different Hb J *HBA* variants were detected with all cases heterozygous for Hb J. Hb J-Cape Town HBA1:c.278G>A (four cases); Hb J-Tongariki HBA1:c.347C>A (two cases); Hb J-Toronto HBA1:c.17C>A and Hb J-Paris-I HBA2:c.38C>A (one case each). Table 1 provides a summary of results.

All four Hb J-Cape Town cases had normal Hb, mean cell volume (MCV) and mean cell haemoglobin (MCH) with mild erythrocytosis and normal ferritin. Two cases were from European subjects and two were from a mother and daughter of African ethnicity. The Hb J-Cape Town variant levels were between 35.0-37.7% and migrated in zone 11 at x-axis 121. The corresponding minor Hb J-Cape TownA_2 variant levels of 0.8-0.9% were clearly observed in the S zone at x-axis 217. In addition, Hb J-Cape Town had a minor aberrant peak in zone 12 at x-axis 84 comprising approximately 0.5%. All cases were confirmed with –a3.7 (rightward) deletion (Figure 1A).

Hb J-Tongariki was discovered in a mother and daughter of New Zealand European descent. Both had microcytic red blood cell indices, normal Hb with a reduced MCV and MCH, and normal ferritin. Hb J-Tongariki variant levels of 34.3% and 36.2% migrated in zone 12 at x-axis 106. The corresponding minor Hb J-TongarikiA_2 variant levels of 0.6% and 0.8% were clearly observed in the D zone at x-axis 203. Both cases were confirmed with –a3.7 (rightward) deletion (Figure 1B).

Hb J-Toronto was identified in a European subject with normal RBC parameters and ferritin. The Hb J-Toronto variant level of 23.1% migrated in zone 12 at x-axis 97. The corresponding minor Hb J-TorontoA_2 variant level of 0.4% was clearly observed in the D zone at x-axis 208 (Figure 1C). There was a full complement of normal α-globin genes.

Hb J-Paris-I was identified in an Indian subject with normal RBC parameters and ferritin. The Hb J-Paris-I variant level of 27.8% migrated in zone 12 at x-axis 81. The corresponding minor Hb J-Paris-IA_2 variant level of 0.3% was clearly observed in the D zone at x-axis 198 (Figure 1D). There was a full complement of normal α-globin genes.

HBB Hb J variants

Three *HBB* Hb J variants were detected, with all cases heterozygous for Hb J. Hb J-Baltimore *HBB:* c.50G>A (four cases), Hb J-Bangkok *HBB:* c.170G>A and Hb J-Kaohsiung *HBB:* c.179A>C (one case each). All were associated with no abnormal clinical findings and normal haematology parameters. Table 1 provides a summary of results.

Hb J-Baltimore, identified in four New Zealand Europeans with variant levels of 50.3%, 52.1%, 52.2%, and 54.1%, migrated in zone 12 at x-axis 81 (Figure 2A). Hb J-Bangkok, identified in a Chinese subject, had a variant level of 51.9% and migrated in zone 12 with an x-axis of 93 (Figure 2B). Hb J-Kaohsiung, identified in an Asian subject, had a variant level of 47.3% and migrated in zone 13 with an x-axis of 66 (Figure 2C).

Table 1. Summary of data for Hb J cases identified in our laboratory.

Hb J-variant	HGVS[a]	Ethnicity[b]	CBC[c]	α-genotype	Alkaline Ep[d]	CE[e] zone Hb J (HbA_2 variant)	CE[e] x-axis Hb J (HbA_2 variant)	% Hb J (HbA_2 variant)
J-Cape Town	HBA1:c278G>A	European	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	35.0 (0.8)
J-Cape Town	HBA1:c278G>A	European	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	35.5 (0.9)
J-Cape Town	HBA1:c278G>A	African	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	36.7 (0.9)
J-Cape Town	HBA1:c278G>A	African	Mild erythrocytosis	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	11 (S)	121 (217)	37.7 (0.9)
J-Tongariki	HBA1:c.347C>A	NZ[f] European	Microcytic indices	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	12 (D)	106 (203)	34.3 (0.6)
J-Tongariki	HBA1:c.347C>A	NZ European	Microcytic indices	$-\alpha^{3.7}/\alpha\alpha$	HbA + Fast band	12 (D)	106 (203)	36.2 (0.8)
J-Toronto	HBA1:c.17C>A	European	Normal	αα /αα	HbA + Fast band	12 (D)	97 (208)	23.1 (0.4)
J-Paris-I	HBA2:c.38C>A	Indian	Normal	αα /αα	HbA + Fast band	12 (D)	81 (198)	27.8 (0.3)
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	50.3
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	52.1
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	52.2
J-Baltimore	HBB:c.50G>A	NZ European	Normal	αα /αα	HbA + Fast band	12	81	54.1
J-Bangkok	HBB:c.170G>A	Chinese	Normal	αα /αα	HbA + Fast band	12	93	51.9
J-Kaohsiung	HBB:c.179A>C	Asian	Normal	αα /αα	HbA + Fast band	13	66	47.3

[a]HGVS: Human Genome Variation Society. [b]Ethnicity classified according to Ethnicity New Zealand Standard Classification 2017 protocol (13). [c]CBC: complete blood count. [d]Ep: Electrophoresis. [e]CE: capillary zone electrophoresis. [f]NZ: New Zealand.

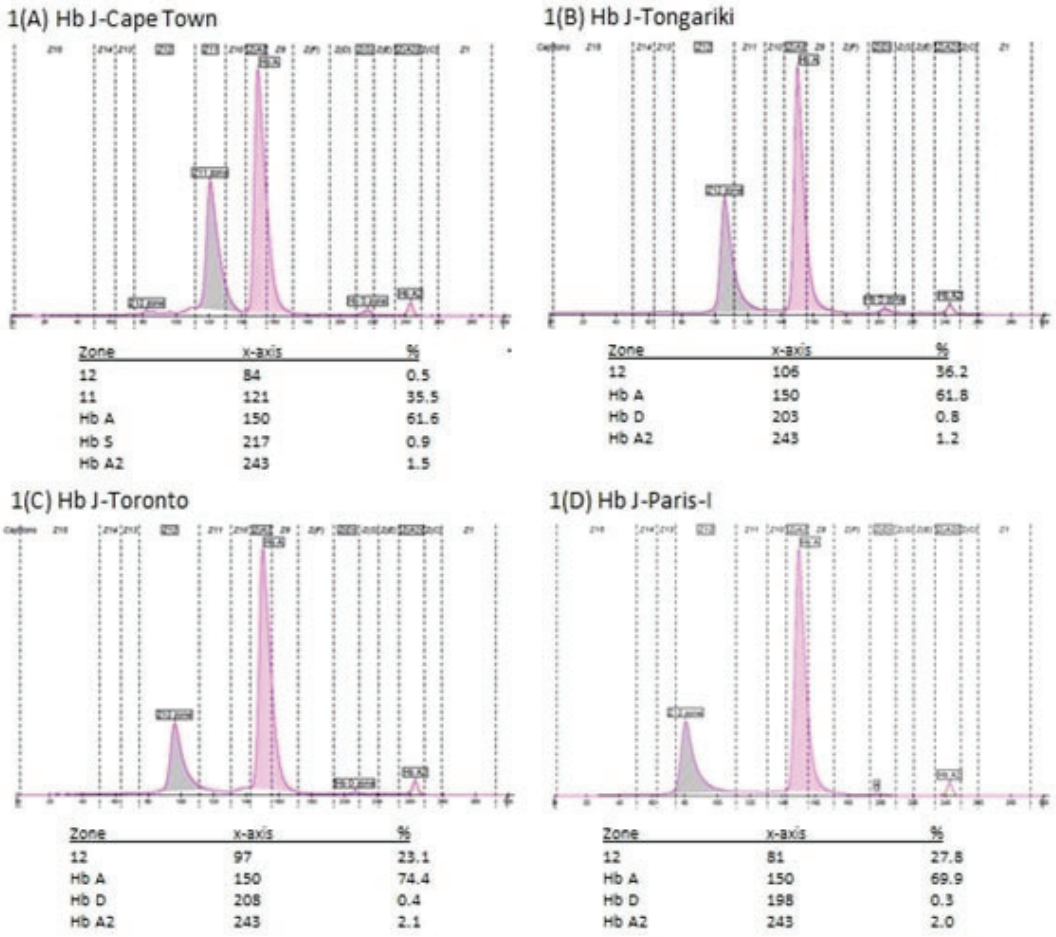

Figure 1. Capillary zone chromatogram migration patterns, zone, x-axis, and Hb variant percentage of *HBA* Hb J variants. (A): Hb J-Cape Town. (B): Hb J-Tongariki. (C): Hb J-Toronto. (D) Hb J-Paris-I.

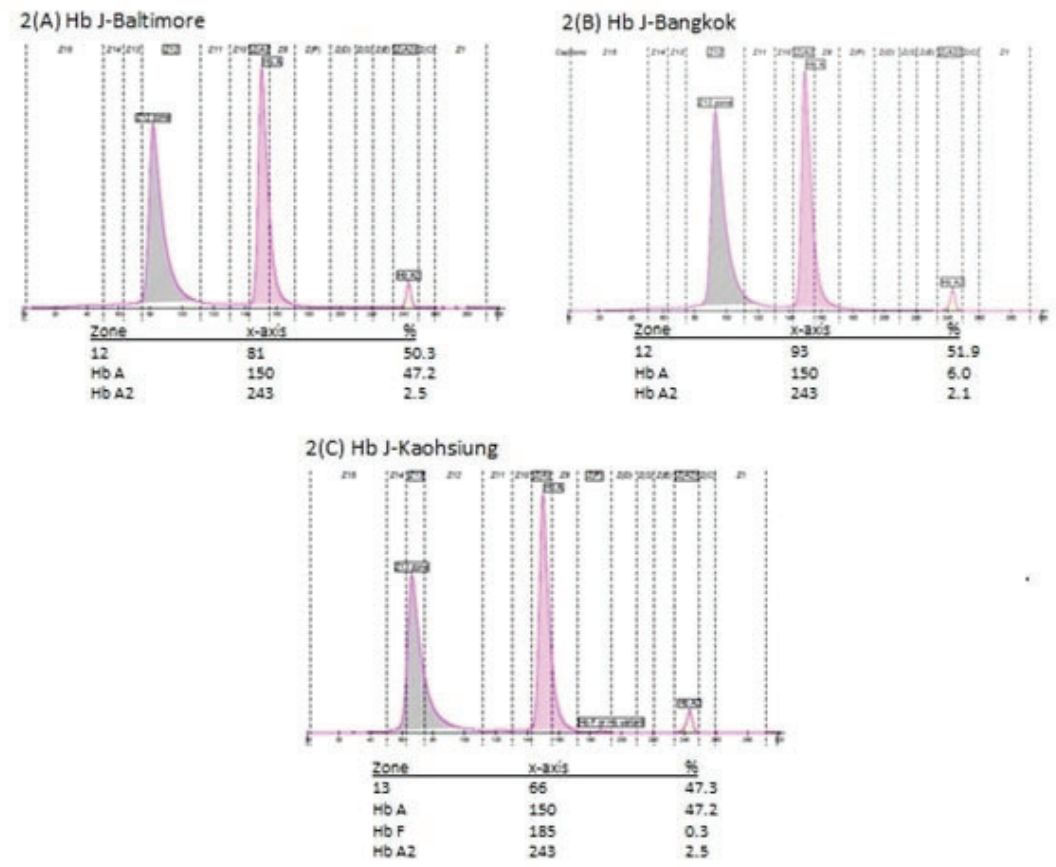

Figure 2: Capillary zone chromatogram migration patterns, zone, x-axis, and Hb variant level of *HBB* Hb J variants. (A): Hb J-Baltimore. (B) Hb J-Bangkok. C): Hb J-Kaohsiung.

DISCUSSION

There is sparse information on capillary zone electrophoresis characteristics of Hb J in the literature. This article details specific x-axis and zone migration position, and characteristic capillary zone electrophoresis chromatogram migration patterns on 14 heterozygous cases of Hb J covering seven different Hb J variants; Hb J-Cape Town, Hb J-Tongariki, Hb J-Toronto, Hb J-Paris-I, Hb J-Baltimore, Hb J-Bangkok, and Hb J-Kaohsiung. This appears to be the first report of the variants Hb J- Cape Town, Hb J- Tongariki, Hb J-Toronto, Hb J-Paris-I, and Hb J-Baltimore detected on capillary zone electrophoresis. This data will add to the body of knowledge and aid in the presumptive identification of Hb J variants.

A search of the literature and Sebia website customer extranet, Hemoglobin Atlas (14) uncovered nine reported capillary zone electrophoresis occurrences of Hb J from six separate publications: three *HBA* variants; Hb J-Abidjan HBA2:c.155G>A (or HBA1) (14), Hb J-Broussais (Tagawa-I) HBA2:c.273G>T (or HBA1) (11,14), Hb J- Sardegna HBA2:c.151C>G (15) and four *HBB* variants; J-Bangkok HBB:c.170 G >A (16,17), Hb J-Europa HBB:c.188C>A (14), Hb J-Kaohsiung HBB:c.179 A > C (6,17), Hb J-Lome HBB:c.180 G >C (15). All publications reported Hb J capillary zone electrophoresis migration zone and Hb variant percentage, but few gave x-axis information.

The ethnic distribution of the Hb J variants described here is consistent with that as reported by others (1,2). Similarly, most of the Hb J heterozygote cases had normal clinical and haematological findings, as described previously (1,2,7). Typically, described features of Hb J-Tongariki with microcytic RBC indices, normal Hb with a reduced MCV and MCH (4), were seen in both of our Hb J-Tongariki cases. Likewise, the characteristic findings of Hb J-Cape Town, a high oxygen affinity variant which is associated with a mild erythrocytosis and normal Hb levels, were observed in all four Hb J-Cape Town cases (8).

The Hb variant percentage is a differentiating feature between *HBA* and *HBB* variants. *HBA* variants have approximately 25%, and *HBB* variants approximately 40-50%. As anticipated, the expected Hb variant percentages were observed in our Hb J heterozygous cases, the *HBA* variants had between 23.1-27.8%, whilst the *HBB* variants had between 47.3-54.1%. However, when an *HBA* variant is co-inherited with an abnormal α-globin genotype, the Hb variant percentage increases (18). The relatively elevated percentage of Hb J-Tongariki (34.3% and 36.2%) and Hb J-Cape Town heterozygote cases (35.0%, 35.5%, 36.7%, and 37.7%), were attributed to co-inheritance of the –a3.7 (rightward) deletion. As described in previous reports, both Hb J-Tongariki and Hb J-Cape Town are commonly co-inherited with α-thalassemia (4,8).

Consistent with previous capillary zone electrophoresis reports of *HBA* Hb J variants, (11,14,15), there were two aberrant peaks on the capillary zone electrophoresis chromatogram, a major peak with a corresponding slow Hb A_2 peak (minor peak). Fucharoen *et al.* had observed this previously with *HBA* variants on capillary zone electrophoresis (16). They reported that the capillary zone electrophoresis system could clearly demonstrate this small second Hb A_2 peak for stable *HBA* variants. Conversely, these Hb A_2 variants were not observed on the HPLC formats (19,20). Hb J- Cape Town had an additional small peak in the Hb Bart's zone 12. As expected, only one aberrant peak was observed for the *HBB* Hb J variants on capillary zone electrophoresis, similar to previous reports (6,14,16,17).

Hb J variants have an electrophoretic mobility faster than Hb A, thus on capillary zone electrophoresis migrate ahead of Hb A. Like previous documented Hb J cases (11,14-17), the majority of our cases migrated in zone 12. The exceptions were Hb J-Cape Town and Hb J-Kaohsiung, which migrated in zone 11 and 13, respectively. Similarly, apart from Hb J-Cape TownA_2, which migrated in the S zone, all other slow *HBA* Hb A_2 peaks migrated in the D zone, which concurres with previous reports (11,14,15).

Although many of the Hb J variants in this study migrated in the same zone, when compared to published Hb variants, including Hb J variants, most had distinctive capillary zone electrophoresis chromatograms. Hb J-Tongariki could be distinguished from other zone 12 Hb variants by the variant level, 34.3% and 36.2%, x-axis migration position of the major peak at 106, and corresponding minor Hb J-TongarikiA_2 peak in the D zone at 203 on the capillary zone electrophoresis chromatogram. Likewise, Hb J-Bangkok with a variant level of 51.9% migrated in zone 12 with an x-axis migration position at 93 on the capillary zone electrophoresis chromatogram, whilst Hb J-Kaohsiung migrated in zone 13 with an x-axis migration at 66 on the capillary zone electrophoresis chromatogram and variant level of 47.3%.

Hb J-Paris-I and Hb J-Baltimore both migrated in zone 12 at migration x-axis position 81, however, they were easily distinguishable by the Hb variant level and the presence/absence of a slow Hb A_2 variant. Hb J-Paris-I had a variant level of 27.8% with a slow Hb A_2 variant peak in the D zone, whilst Hb J-Baltimore had variant levels of 50.3%, 52.1% 52.2%, and 54.1% and no slow Hb A_2 variant peak. Similarly, Hb J-Cape Town and a published case of Hb Fannin-Lubbock I (HBB:c.359G>A) (11,14) both migrated in zone 11 with migration x-axis position 121. Again, they were distinguishable by the Hb variant level and the presence/absence of a slow Hb A_2 variant. Hb J-Cape Town had a variant level of 35.0-37.7% with a slow HbA_2 variant peak in the S zone and a second minor Hb variant peak in zone 12, whilst Hb Fannin-Lubbock I had a variant level of 44.7% and no slow Hb A_2 variant peak (14).

Only one Hb J variant in this study did not appear to have a unique x-axis or differing Hb variant level when compared to published capillary zone electrophoresis data. Hb J-Toronto shared an x-axis migration position with Hb J-Broussais (11,14). Both are *HBA* variants, with the major Hb variant peak eluting in zone 12 at migration x-axis position 97. The Hb variant level was similar, Hb J-Toronto 23.1% and Hb J-Broussais 20.4%. The slow Hb A_2 variant peaks both migrated in the D zone with an identical variant level of 0.4%, but with marginally different x-axis. Hb J-TorontoA_2 migrated at x-axis 208 whilst Hb J-BroussaisA_2 migrated at an x-axis of 207 (14).

Generally, the capillary zone electrophoresis migration patterns, zone, and x-axis, along with the Hb variant percentage and clinical findings, can lead to an adequate presumptive identification of the Hb J variants. However, for those Hb J variants with an overlap of capillary zone electrophoresis migration patterns, definitive identification can only be achieved by DNA molecular analysis.

CONCLUSIONS

This article documents characteristic capillary zone electrophoresis chromatogram migration patterns, zone, x-axis and Hb variant percentage, for seven Hb J variants. For five of these, this is the first time that capillary zone electrophoresis features have been demonstrated. The characterisation of these Hb J cases will be of value to diagnostic laboratories in expanding the capillary zone electrophoresis reference library of Hb variants and facilitate provisional Hb J variant identification.

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