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# The profile of free amino acids in latent fingerprint of healthy and beta-thalassemic volunteers

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ABSTRACT

The aim of the present work is to apply a non-invasive test, using thumb fingerprint residue analysis, for detection of beta-thalassemia (β-Thal). The relative percentages of free amino acids (AA) in the latent fingerprint of β-Thal patients and healthy subjects were compared. The sample included 24 β-Thal patient and 24 healthy subjects, aged 5–10 years old. Twenty-three AA plus ammonia were analyzed by a sensitive high-performance liquid chromatographic method with fluorescence detection. The profile of AA was calculated based on the percentage of relative amount of each AA to serine (Ser) as it found to be the predominant peak. The statistical and chromatographic profiles of β-Thal patients were characterized by significant decrease of ornithine, lysine, and zero tyrosine, with significant increase of ammonia, and proline. Other amino acids that exist in low ratios were estimated statistically for significance changes. The relative percentages of each AA of healthy subjects were approximately constant. For this reason, these mentioned AA were assigned as major fingerprint markers of β-Thal.



## 1. Introduction

The chemical composition of latent fingerprints has been investigated by several authors [1–3]. Hamilton [1], Oro and Skewes [2], and Hadorn et al. [3], using ultra-micro methods, found the relative concentrations of amino acids (AA) on the surface of the fingers of 10 subjects to be essentially constant. This included aspartic acid, glutamic acid, histidine, serine, threonine, glycine, alanine, valine, isoleucine, leucine, tryptophane, ornithine, lysine, and tyrosine, in addition to ammonia and smaller amounts of citrulline, methionine and arginine. Lee and Gaensslen reported that the total amounts of AA present in fingerprints were in the range of 0.3–2.59 mg $L^{-1}$ [4]. Also, it has been concluded that serine, glycine and alanine were the most abundant amino acids [1–4]. Quantitatively, amino acid concentrations can vary as much as 2–20 times depending on the collection method and the location of the sample collected from (on the body) [4]. The AA content, collectively or separately, has been investigated in plasma or urine as biomarkers for some diseases or as an indication of various metabolic disorder, for example: cases of colon carcinoma [5], Parkinsonism [6], oxidative stress of AA [7], and hemoglobinopathy [8]. β-Thalassemia is a form of blood disorder due to mutations in the β-globin (HBB) gene on chromosome 11, inherited in an autosomal recessive fashion and the severity of the disease depends on the nature of the mutation [8]. This genetic defect results in a reduced rate of synthesis of one of the globin chains that make up hemoglobin. Reduced synthesis of one of the globin chains can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of the thalassemia. The genetic defect may be due to substitution of one amino acid for another [9]. Fucharoen and Winichagoon [10] reported that β-Thal is a heterogeneous disorder, caused by various defects in the β-globin gene. Hemoglobin (Hb) E arises from a mutation of the β-globin chain which replaces glutamic acid with lysine. Abdulrazzaq et al. [11] described the relation between amino acids metabolism and thalassemia major. He concluded that lower plasma values of essential amino acids and a decrease in urinary amino acids are the most important characteristic features in thalassemic patients. Growth impairment both in height and weight also occurs in thalassemic patients compared to a control population. Several authors illustrated the correlation between amino acid content, as markers, in plasma or urine and β-Thal [12,13]. A comprehensive review article has been compiled by Molnar-Perl [14] discussing the separation and simultaneous quantitation of amino acids and amines in the same matrix by high-performance liquid chromatography (HPLC). The same review cited the most applied HPLC methods for the analysis of amino acids and some amines using different derivatization reagents and detection methods. The most convenient reagent was dansyl chloride (Dns-Cl), as it gave less or no reaction by-products, in addition to its derivative stability [15]. However, the publications that described the use of Dns-Cl, showed uncompleted separation of some AA due to overlap or bad resolu-

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tion of certain AA. This was in addition to the extensive sample pretreatment requirements.

In this work we described and validated a new HPLC analytical method of AA using Dns-Cl as derivatizating reagent. The developed method was applied to the analysis of AA in the latent fingerprints of healthy and β-Thal people. The profiles of AA of both healthy and β-Thal subjects were also described.

## 2. Experimental

### 2.1. Chemicals and reagents

All solvents were of HPLC grade, Merck (Darmstadt, Germany). All amino acid reference standards were purchased from Permed Scientific Chemicals Ltd. (Bedford, England). Amino acids used were; L-aspartic acid (Asp), L-glutamic acid (Glu), L-asparagine $H_2O$ (Asn), DL-histidine HCl (His), DL-citrulline (Cit), DL-serine (Ser), DL-threonine (Thr), glycine (Gly), L-alanine (Ala), L-arginine (Arg), L-proline (Pro), DL-valine (Val), DL-norvaline (Nva), DL-methionine (Met), DL-isoleucine (Ile), L-leucine (Leu), DL-norleucine (Nle), L-B-phenylalanine (Phe), L-tryptophan (Trp), L-cystine (Cys), DL-ornithine HCl (Orn), DL-lysine HCl (Lys), DL-tyrosine (Tyr). 1-Hexanesulfonic acid sodium salt (HSA-Na, >98%), and dansyl chloride (≥99.0% HPLC for fluorescence BioChemika) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other materials were of analytical grade.

### 2.2. Equipment

High-performance liquid chromatographic system used was consisting of an Alliance Waters Separations Module 2695, and Waters 2475 multi wavelength fluorescence detector (Milford, MA, USA). The HPLC system control and data processing were performed by Waters Empower software Build 1154 (Milford, MA, USA) run on an IBM-compatible PC. Screw capped autosampler vials (flat-bottom 1.8 mL and V-shaped 300-μL) were from Alltech GmbH (Unterhaching, Germany). Heraeus heating oven (Kendro, Hanau, Germany) was adjusted at 60 °C. Calibrated digital microtransfer pipettes 5–250 μL, Brand (Wertheim, Germany), were used. Kenwood Microwave model MW450, 17 L capacity—800 W, adjusted at the second power level equivalent to 264 W and 416 MHz (Kenwood Electronics Co. Ltd., Songjiang, Shanghai, China).

### 2.3. Chromatographic conditions

Analytes were separated on the following two columns in sequence; analytical column 1, Zorbax Eclipse XDB C18, 250 mm × 4.6 mm, 80 Å, 5 μm, connected to analytical column 2, Zorbax Extend C18, 150 mm × 4.6 mm, 80 Å, 5 μm. Pre-column used was, Zorbax Eclipse XDB C18, 12.5 mm × 4.6 mm, 80 Å, 5 μm. All columns and pre-column were from Agilent (Agilent Technologies, Palo Alto, CA, USA). The fluorescence detector was set at 340 and 515 nm, as excitation and emission wavelengths, respectively. The mobile phase was prepared by dissolving 600 mg of HSA-Na in 100 mL water, from this solution a volume of 50 mL was mixed with 950 mL acetonitrile (mobile phase A) and the remaining 50 mL was mixed with 950 mL water containing 1.36 g sodium acetate trihydrate, filtered through a 4.5 Å Nylon membrane filter and adjusted to pH 7.6 with few drops of 0.01 M NaOH (mobile phase B). The mobile phase flow rate was adjusted to 1 mL $min^{-1}$. The HPLC pump was programmed to deliver the mobile phase as follows; from 0 to 5 min; isocratic elution of 10.0% mobile phase A, 90.0% mobile phase B; from 5 to 100 min; gradient elution of mobile phase A (10.0–43.5%) and mobile phase B (90.0–56.5%); from 100 to 110 min; gradient elution of mobile phase A (43.5–66.0%) and mobile phase B (56.5–34.0%). A sample volume of 10 μL was injected.

### 2.4. Standard solutions and quality control samples

Separate solutions of each AA and ammonium chloride were prepared in water to get a stock solution of 1.0 mg $mL^{-1}$. Appropriate dilutions in water were prepared from these stock solutions to obtain calibration standards that contain 23 AA and ammonium chloride (6 concentration levels). The first quality control samples (QC1) were prepared in the concentration range of 25, 50, and 100% of the upper linear limit of each substance. The second quality control sample (QC2) was a real sample (200 μL) of latent fingerprint of healthy subject. (Sample collection and preparation is described in Section 2.7.) The QC samples were divided to small aliquots and stored in borosilicate glass vials at −20 °C until use. The samples were thawed and a volume of 50 μL of each level was derivatized and analyzed at time intervals of; 0, 10 and 30 days. The mass concentrations of each substance were calculated from the corresponding calibration curve using peak area. The calibration curves were constructed using the least-square method for the calculation of slope, intercept and correlation coefficient.

### 2.5. Derivatization reagent and bicarbonate solution

One hundred milligrams of dansyl chloride was accurately weighed into 10-mL volumetric flask, dissolved in a solvent mixture of acetonitrile: acetone (9:1, v/v), and completed to volume with the same solvent. Sodium bicarbonate (0.1 M) solution was prepared in water and adjusted to pH 9.7 ± 0.1 with 0.1 M sodium hydroxide.

### 2.6. Derivatization preparation

A volume of 50 μL Dns-Cl solution, 50 μL aqueous standard solutions of AA or sample solution, and 200 μL 0.1 M bicarbonate solution were transferred to autosampler vial (1.8-mL capacity). The vial was capped well, swirled, and left to stand inside the microwave oven over the rotating glass platform. A plastic container (250-mL, microwave-safe) was inverted over the vial just for safety reason, the power was switched on for 5 min (at the 2nd energy level, 264 W), vial allowed to cool at room temperature for 2 min, irradiated again for another 5 min, cooled, and the reaction mixture was transferred to a 300-μL V-shaped autosampler and a volume of 10 μL was injected for HPLC analysis. Alternatively, the derivatization reaction was also conducted at 60 °C for 60 min in hot-air oven.

### 2.7. Sample collection and preparation

Twenty-four β-Thal volunteers and 24 healthy volunteers, aged 5–10 years old, were enrolled in this study. Volunteers were advised to wash hand by tap water and wait about 2 min to air-dry not to touch anything before sampling. The thumb finger was introduced into a clean 25-mL glass beaker containing 200 μL distilled water and keep touching the wetted inner surface for 2 min. From this solution, a volume of 50 μL was used for derivatization with Dns-Cl and analyzed as described above. Samples were immediately labeled with the corresponding clinical case report number. The collected samples were either derivatized and analyzed immediately or stored in the freezer at −20 °C until derivatization and analysis.

**Table 1**
Chromatographic performance parameters of investigated amino acids as dansyl-derivative ($n = 6$).

| Name | $t_R$[a], min | Area | Concentration, ng μL$^{-1}$ | ($W_{0.05}$)[a] s | $k$[a] | $\alpha$[a] | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|---|---|
| Asp | 9.677 | 1,773,739 | 7.75 | 51 | 2.87 | | | 1.04 | 13,393 |
| Glu | 11.899 | 2,030,862 | 7.58 | 77 | 3.76 | 1.31 | 5.07 | 0.99 | 8135 |
| Asn | 29.869 | 1,810,582 | 8.33 | 91 | 10.95 | 2.91 | 30.33 | 1.03 | 33,319 |
| His | 32.772 | 2,271,373 | 8.5 | 87 | 12.11 | 1.11 | 4.75 | 1.01 | 53,275 |
| Cit | 33.740 | 3,303,265 | 7.33 | 48 | 12.50 | 1.03 | 1.61 | 1.06 | 45,195 |
| Ser | 35.021 | 3,462,813 | 7.5 | 74 | 13.01 | 1.04 | 1.96 | 1.04 | 42,913 |
| Thr | 37.433 | 3,401,703 | 7.75 | 73 | 13.97 | 1.07 | 3.57 | 1.05 | 48,752 |
| Gly | 39.324 | 6,895,482 | 5.42 | 100 | 14.73 | 1.05 | 2.77 | 1.05 | 51,745 |
| Ala | 40.944 | 3,653,204 | 4.42 | 78 | 15.38 | 1.04 | 2.36 | 1.04 | 57,610 |
| Arg | 45.296 | 6,307,521 | 9.25 | 56 | 17.12 | 1.11 | 6.34 | 1.12 | 69,284 |
| Pro | 46.882 | 4,682,917 | 5.83 | 70 | 17.75 | 1.04 | 2.31 | 1.02 | 74,582 |
| Val | 51.018 | 7,775,680 | 6.33 | 79 | 19.41 | 1.09 | 6.10 | 1.05 | 91,895 |
| Nva | 54.315 | 8,077,646 | 6 | 79 | 20.73 | 1.07 | 4.90 | 1.07 | 104,238 |
| Met | 55.617 | 5,663,887 | 6.67 | 61 | 21.25 | 1.03 | 1.96 | 1.07 | 116,353 |
| Ile | 58.789 | 9,747,877 | 7.25 | 72 | 22.52 | 1.06 | 4.91 | 1.08 | 134,433 |
| Leu | 59.947 | 12,914,151 | 8.17 | 88 | 22.98 | 1.02 | 1.79 | 1.09 | 134,933 |
| Nle | 63.273 | 9,542,986 | 5.75 | 79 | 24.31 | 1.06 | 5.16 | 1.07 | 157,544 |
| Phe | 64.590 | 7,305,762 | 8.33 | 51 | 24.84 | 1.02 | 2.11 | 0.97 | 175,065 |
| Trp | 65.340 | 4,682,132 | 6.5 | 71 | 25.14 | 1.01 | 1.22 | 1.15 | 178,846 |
| Cys | 67.214 | 3,126,191 | 7.25 | 80 | 25.89 | 1.03 | 3.21 | 1.06 | 238,960 |
| NH3 | 77.495 | 9,943,871 | 11.17 | 99 | 30.00 | 1.16 | 12.31 | 1.06 | 75,644 |
| Orn | 90.668 | 12,776,612 | 6.5 | 58 | 35.27 | 1.18 | 14.89 | 1.06 | 307,645 |
| Lys | 91.599 | 15,308,308 | 5.58 | 52 | 35.64 | 1.01 | 1.42 | 1.15 | 304,740 |
| Tyr | 105.599 | 4,225,280 | 8.58 | 70 | 41.24 | 1.16 | 23.65 | 1.04 | 645,780 |

[a] $t_R$, retention time; $W_{0.05}$, peak width at 95% height from peak apex; $k$, retention factor; and $\alpha$, selectivity coefficient.

## 3. Results and discussion

### 3.1. Optimization and validation of the chromatographic method

The developed HPLC analytical method was optimized and validated using Dns-Cl as derivatizing reagent with fluorescence detection. The use of microwave helped the reaction to be completion within 10 min instead of 60 min at 60 °C. The HPLC methods reported in the literature for separation of dansylated amino acids (Dns-AA) have shown some peak overlap [14,16]. The use of two analytical columns was necessary to separate overlapping or closely eluted peaks like the ornithine–lysine, leucine–isoleucine and phenylalanine–tryptophane peak-pairs. Also, peaks retention times precision was optimized by maintaining the strength of hexane sulphonic acid constant throughout the entire run time of gradient elution. The completeness of the derivatization reaction using microwave was investigated by preparation of standard solution containing 23 AA plus $NH_3$ (mass concentrations as per Table 1) mixed with Dns-Cl and sodium bicarbonate solution. This solution was prepared 12 times in well capped autosampler vials (1.8-mL). Samples were exposed to microwave irradiation for different time intervals applying different irradiation power levels (132, 264, and 396 W). The same solution was prepared and derivatized using the conventional heating method (hot-air oven, at 60 °C for 60 min). The peak areas of Dns-AA obtained under both conditions were matched. The % differences of peak areas [(peak area of Dns-AA prepared in microwave-peak area of Dns-AA)/(peak area of Dns-AA prepared at 60 °C) × 100] were monitored. The completeness of the reaction was achieved upon irradiation for 5 min, twice at 264 W. Microwave-assisted derivatization reaction showed an average % difference in peak area of not more than ±0.10% (RSD ≤ 0.02%).

The method was fully validated as required by International Committee of Harmonization (ICH) including; linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, selectivity, robustness and ruggedness [17]. This chromatographic method showed that all investigated AA were separated selectively and completely from each other and from any unknown extracted materials (Fig. 1). Peaks at 19–27 min are corresponding to excess Dns-Cl reagent. The average relative standard deviation (RSD) values of 6 readings of each parameter were not more than 1.00%. The chromatographic performance parameters are listed in Table 1. For the evaluation of method robustness, one chromatographic parameter was changed while all other parameters were kept unchanged. The chromatographic parameters (including retention factor, $k$, retention time, $t_R$, peak asymmetry, $A_s$, resolution, Rs, and USP width) were calculated and compared with the system suitability (Table 1). The method robustness was tested after changing the pH of the acetate solution (7.0–8.0), gradient profile, and upon using different amounts of counter-ion (HSA-Na, 260–320 mg L$^{-1}$). The results revealed that the method was robust and unaffected with these small changes in gradient profile and the amount of counter-ion, but the pH should be within 7.4–7.8. Any change of the pH outside this range will leads to overlap of some peaks, including His-Cit, Ile-Leu, and Phe-Trp. The method was also robust upon using end capped C18 HPLC column from (Zorbax Eclipse XDB); however, unacceptable resolution with shorter retention time for all separated peaks was observed upon using the non-end capped C18 column (Zorbax Extend) as column 1. Furthermore, the method robustness toward changing the reaction conditions was tested. No extra peaks or varied responses were observed upon using reagent amount in the range of 8–12 mg mL$^{-1}$, or heating in the range of 55–65 °C for 60 min.

Linear HPLC peak areas for all AA were observed over the mass concentration range listed in Table 2. The calculated calibration parameters include: response factor (RF), squared regression coefficient ($r^2$), mass concentration range (ng μL$^{-1}$), limit of quantification, and limit of detection in pg μL$^{-1}$. The RSD values of RF ($n = 6$) LOD ($n = 5$) and LOD ($n = 5$) did not exceed 1.5%. The method was sensitive and precise enough for qualitative and quantitative analysis of AA in human fingerprint secretions.

Intra- and inter-day precision and accuracy were evaluated for each AA by analyzing six replicates of quality control samples at three concentration levels of AA mixtures. Precision, of mass concentration of each AA, was expressed as the relative standard deviation, though accuracy was presented as a percent error (relative error), {[(observed concentration − nominal concentration)/nominal concentration] × 100 (%)}. Intra- and inter-day relative standard deviations were less than 1.21%. Accuracies were within 0.20% when compared with nominal concentrations. The

**Table 2**
Calibration parameters of dansylated standard solution of amino acids.

| Amino acid | Response factor[a] (6 levels, including zero point) | $r^2$[b] | Range, ng μL$^{-1}$ | LOD[c] (pg μL$^{-1}$) | LOQ[c] (pg μL$^{-1}$) |
|---|---|---|---|---|---|
| Asp | 228,867 | 0.9825 | 0.06–7.75 | 20 | 60 |
| Glu | 267,925 | 0.9895 | 0.05–8.00 | 16 | 48 |
| Asn | 217,358 | 0.9790 | 0.06–10.00 | 20 | 60 |
| His | 267,218 | 0.9834 | 0.05–9.00 | 16 | 48 |
| Cit | 450,646 | 0.9958 | 0.04–7.50 | 12 | 36 |
| Ser | 461,711 | 0.9968 | 0.04–7.50 | 12 | 36 |
| Thr | 438,932 | 0.9897 | 0.04–7.75 | 12 | 36 |
| Gly | 1,272,231 | 0.9980 | 0.02–5.50 | 6 | 18 |
| Ala | 826,519 | 0.9899 | 0.03–5.50 | 8 | 24 |
| Arg | 681,892 | 0.9977 | 0.03–9.30 | 10 | 30 |
| Pro | 803,243 | 0.9881 | 0.03–6.00 | 8 | 24 |
| Val | 1,228,387 | 0.9965 | 0.02–7.00 | 6 | 18 |
| Nva | 1,346,276 | 0.9968 | 0.02–6.00 | 6 | 18 |
| Met | 849,160 | 0.9964 | 0.03–7.00 | 8 | 24 |
| Ile | 1,344,532 | 0.9978 | 0.02–8.00 | 6 | 18 |
| Leu | 1,580,677 | 0.9972 | 0.02–9.00 | 5 | 15 |
| Nle | 1,659,648 | 0.9973 | 0.02–5.80 | 5 | 15 |
| Phe | 877,041 | 0.9971 | 0.03–6.50 | 8 | 24 |
| Trp | 720,328 | 0.9984 | 0.03–9.00 | 10 | 30 |
| Cys | 431,196 | 0.9835 | 0.04–8.00 | 12 | 36 |
| $NH_3$ | 890,232 | 0.9592 | 0.03–12.00 | 8 | 24 |
| Orn | 1,965,631 | 0.9973 | 0.02–6.50 | 5 | 15 |
| Lys | 2,743,428 | 0.9968 | 0.02–6.00 | 4 | 12 |
| Tyr | 492,458 | 0.9841 | 0.03–9.00 | 10 | 30 |

[a] $n = 3$ for each level.
[b] Squared regression coefficient.
[c] $n = 5$ and the RSD values were not more than 1.5% of each amino acid.

results indicate that the method is reliable, reproducible, and accurate.

The stability of AA in aqueous solution mixture was investigated through three freeze–thaw cycles of the QC samples during a storing period of one month at −20 °C. Amino acids investigated were considered stable in aqueous solution after three freeze–thaw cycles at the concentration levels of 25, 50, and 100% (of the linear range). The average RSD of the recovered amounts of three levels of all AA were not more than 2.5%, except His and Cys. The QC samples left at −20 °C for one month showed that the RSD values of

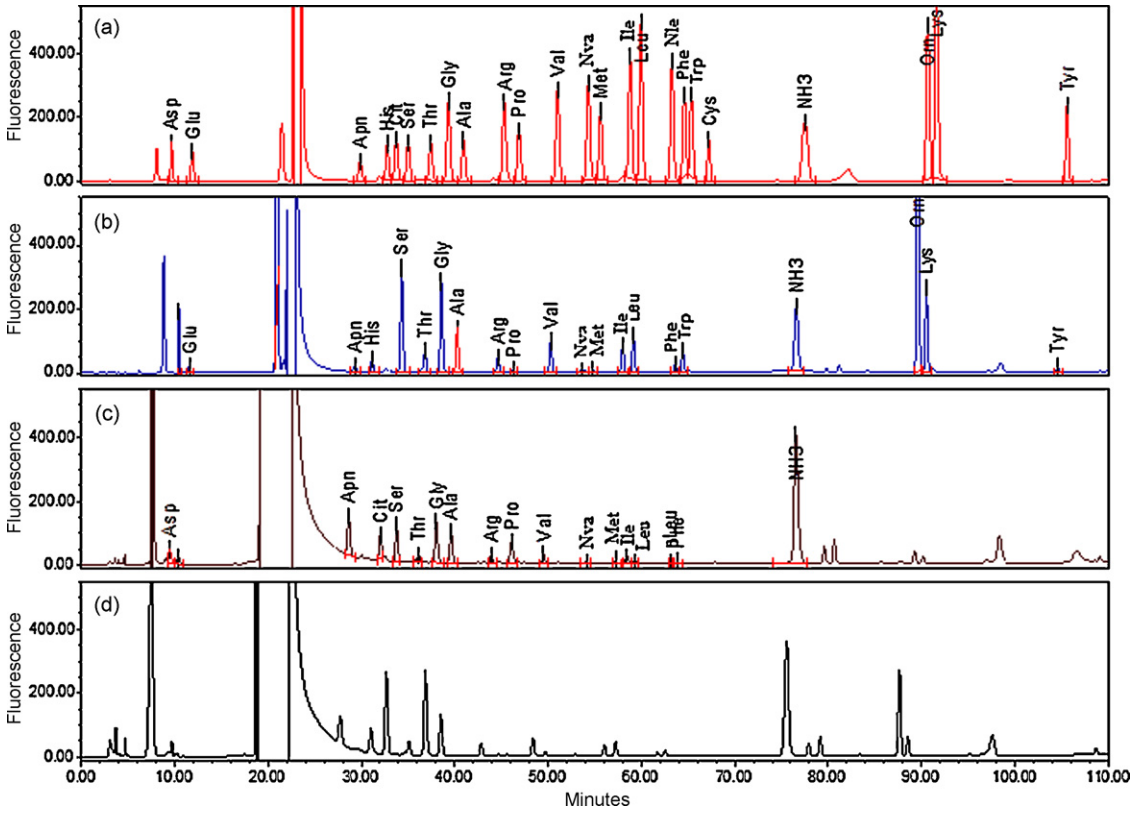


**Fig. 1.** Representative chromatograms of dansyl-derivative of amino acids; (a) standard AA solution, (b) extracted from human fingerprint residue of healthy volunteers and (c) β-Thal major volunteer and (d) β-Thal minor volunteer.

**Table 3**
Statistical data of relative percentages of each amino acid to serine in the fingerprint residue of both healthy and β-thalassemic volunteers ($n1 = n2 = 24$, $t$ df = 46, tabulated p 0.05% = 2.00).

| Name | Healthy volunteers | | | Thalassemic volunteers | | | $F$-value | $P(F \leq f)$ one-tail | $t$-Value | $P(T \leq t)$ two-tail |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Variance | SD | Mean | Variance | SD | | | | |
| Asp | 23.29 | 9.22 | 3.04 | 11.29 | 47.23 | 6.87 | 0.201 | 4.80E−03 | 5.418 | 1.17E−04[a] |
| Glu | 4.64 | 0.87 | 0.93 | 18.22 | 77.32 | 8.79 | 0.014 | 1.30E−09 | −5.100 | 4.64E−04[a] |
| Asn | 17.78 | 6.24 | 2.50 | 66.18 | 233.90 | 15.29 | 0.004 | 4.26E−13 | −10.395 | 1.11E−06[a] |
| His | 7.31 | 5.34 | 2.31 | 6.49 | 7.60 | 2.76 | 0.678 | 2.54E−01 | 0.824 | 4.18E−01[b] |
| Cit | 3.02 | 1.93 | 1.39 | 16.26 | 93.69 | 9.68 | 0.019 | 9.53E−09 | −4.502 | 1.14E−03[a] |
| Ser[c] | 196.3 | 108.05 | 10.39 | 228.18 | 791.01 | 28.12 | 0.135 | 7.53E−04 | −3.588 | 3.73E−03[a] |
| Thr | 20.44 | 4.85 | 2.20 | 17.45 | 24.88 | 4.99 | 0.198 | 4.55E−03 | 1.860 | 8.57E−02[a] |
| Gly | 51.08 | 7.17 | 2.68 | 44.41 | 27.21 | 5.22 | 0.259 | 1.40E−02 | 3.886 | 1.65E−03[a] |
| Ala | 28.43 | 3.18 | 1.78 | 27.57 | 3.04 | 1.74 | 0.991 | 4.79E−01 | 1.234 | 2.29E−01[b] |
| Arg | 5.95 | 0.73 | 0.85 | 14.17 | 5.39 | 2.32 | 0.133 | 6.90E−04 | −11.203 | 1.04E−07[a] |
| Pro | 1.33 | 0.49 | 0.70 | 15.37 | 42.80 | 6.54 | 0.011 | 3.41E−10 | −7.086 | 3.35E−05[a] |
| Val | 9.43 | 0.60 | 0.78 | 8.41 | 20.69 | 4.55 | 0.028 | 1.04E−07 | 0.732 | 4.81E−01[a] |
| Nva | 0.81 | 0.09 | 0.30 | 0.62 | 0.50 | 0.70 | 0.234 | 9.26E−03 | 0.858 | 4.06E−01[a] |
| Met | 0.80 | 0.07 | 0.26 | 0.75 | 1.86 | 1.37 | 0.037 | 5.08E−07 | 0.129 | 9.00E−01[a] |
| Ile | 6.07 | 0.67 | 0.82 | 4.46 | 4.98 | 2.23 | 0.137 | 7.92E−04 | 2.290 | 4.09E−02[a] |
| Leu | 6.91 | 1.17 | 1.08 | 5.12 | 8.45 | 2.91 | 0.138 | 8.48E−04 | 1.948 | 7.51E−02[a] |
| Nle | 1.41 | 0.04 | 0.19 | 1.59 | 0.18 | 0.42 | 0.183 | 3.17E−03 | −1.364 | 1.96E−01[a] |
| Phe | 4.95 | 0.86 | 0.93 | 2.51 | 1.05 | 1.02 | 0.859 | 3.90E−01 | 6.346 | 1.47E−06[b] |
| Trp | 0.12 | 0.03 | 0.18 | 4.33 | 2.62 | 1.62 | 0.014 | 1.35E−09 | −8.593 | 6.26E−06[a] |
| Cys | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | – | – | – | – |
| NH3 | 37.82 | 6.98 | 2.64 | 210.13 | 1095.24 | 33.09 | 0.004 | 3.13E−13 | −17.229 | 9.18E−09[a] |
| Orn | 30.73 | 3.73 | 1.93 | 12.37 | 98.88 | 9.94 | 0.026 | 5.80E−08 | 4.360 | 3.77E−04[a] |
| Lys | 5.52 | 2.15 | 1.47 | 2.51 | 3.66 | 1.91 | 0.555 | 1.62E−01 | 4.546 | 1.32E−04[b] |
| Tyr | 3.16 | 0.41 | 0.64 | 0.00 | 0.00 | 0.00 | – | – | – | – |

[a] $t$-Test: two-samples assuming unequal variances.
[b] $t$-Test: two-samples assuming equal variances.
[c] Calculations made relative to glycine.

mass concentration of His and Cys were exceeding 4.5%. However, all other AA investigated showed an average RSD values of not more than 1.1%.

These results illustrated that all investigated AA and ammonium chloride were stable for three freeze and thaw cycles stored for one month, except His and Cys were stable only for 10 days. Based on these results, the collected samples were analyzed either immediately or within 10 days (kept at −20 °C).

### 3.2. Sample analysis

The dansylated samples were analyzed either immediately or within 16 h, after that time samples have shown deterioration. The profile of AA was calculated based on the percentage of relative amount of each AA to serine (Ser) as it found to be the predominant peak. The relative % of each AA to serine (w/w%) of healthy volunteers was almost the same, and showed an acceptable SD values for

**Table 4**
Relative percentages of each amino acid to serine in the fingerprint residue of healthy and β-thalassemic volunteers compared with the reported values.

| Name | Healthy | | β-Thal (%) | % Error of β-Thal relative to measure % | Statistical significance and status (CI 0.05%, df 46)[a] |
|---|---|---|---|---|---|
| | Reported[b] (%) | Measured (%) | | | |
| Asp | 11–22 | 23.29 | 11.29 | 51.5 | Significant, decreased |
| Glu | 5–12 | 4.64 | 18.22 | 292.7 | Significant, increased |
| Asn | – | 17.78 | 66.18 | 272.2 | Significant, increased |
| His | 13–17 | 7.31 | 6.49 | 11.2 | Insignificant |
| Cit | – | 3.02 | 16.26 | 438.4 | Significant, increased |
| Ser | 100 | 196.3[c] | 228.18[c] | 16.2 | Significant, increased |
| Thr | 9–18 | 20.44 | 17.45 | 14.6 | Insignificant |
| Gly | 54–67 | 51.08 | 44.41 | 13.1 | Significant, decreased |
| Ala | 27–35 | 28.43 | 27.57 | 3.0 | Insignificant |
| Arg | – | 5.95 | 14.17 | 138.2 | Significant, increased |
| Pro | – | 1.33 | 15.37 | 1055.6 | Significant, increased |
| Val | 9–12 | 9.43 | 8.41 | 10.8 | Insignificant |
| Nva | – | 0.81 | 0.62 | 23.5 | Insignificant |
| Met | – | 0.80 | 0.75 | 6.3 | Insignificant |
| Ile | 6–8 | 6.07 | 4.46 | 26.5 | Significant, decreased |
| Leu | 7–10 | 6.91 | 5.12 | 25.9 | Insignificant |
| Nle | – | 1.41 | 1.59 | 12.8 | Insignificant |
| Phe | 5–7 | 4.95 | 2.51 | 49.3 | Significant, decreased |
| Trp | – | 0.12 | 4.33 | 3508.3 | Significant, increased |
| Cys | – | 0.00 | 0.00 | 0.0 | Not detected |
| NH3 | – | 37.82 | 210.13 | 455.6 | Significant, increased |
| Orn | 32–45 | 30.73 | 12.37 | 59.7 | Significant, decreased |
| Lys | 5–10 | 5.52 | 2.51 | 54.5 | Significant, decreased |
| Tyr | 3–6 | 3.16 | 0.00 | 100.0 | Not calculated |

[a] Statistical significance of mean difference between β-Thal and healthy.
[b] Range as per cited reports (1–3).
[c] The measured value was relative % to glycine.

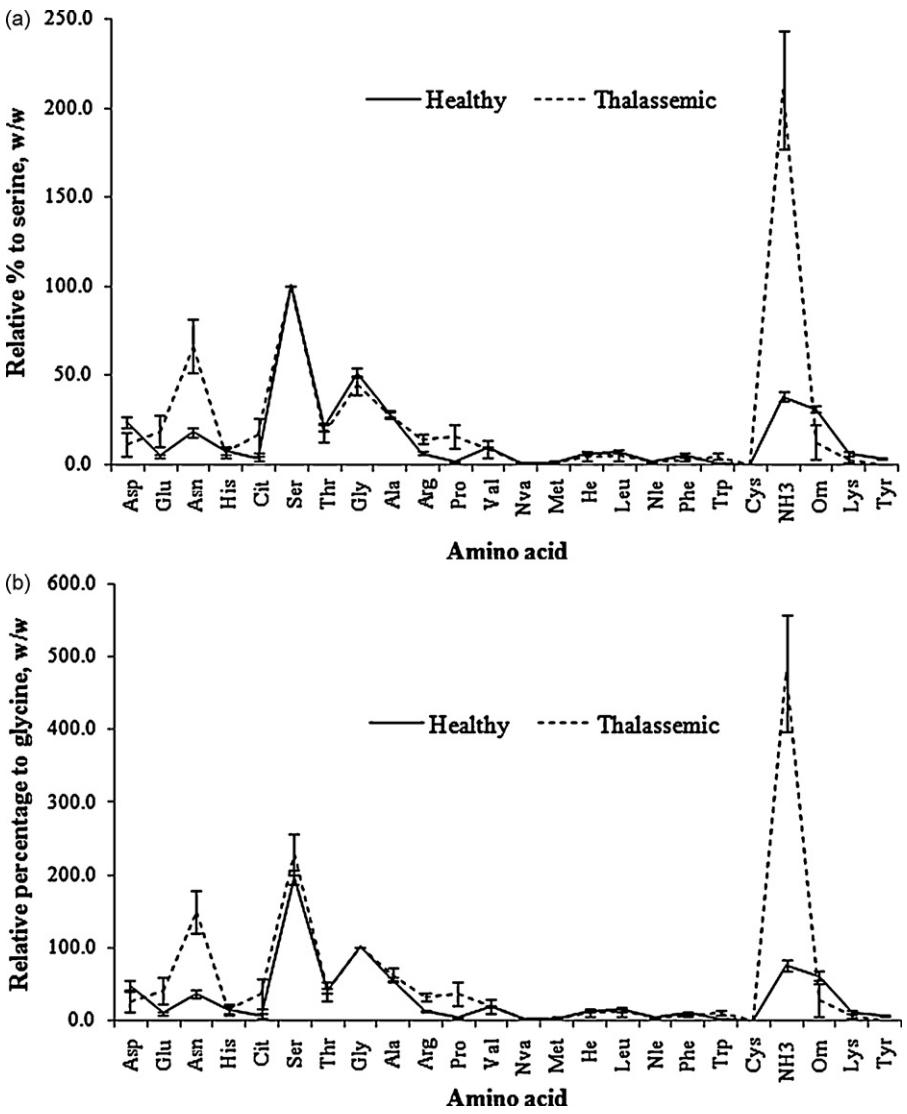


**Fig. 2.** Point-to-point graph of mean values of relative % of each AA to serine (a) or glycine (b) in latent fingerprint excretion, in both normal and thalassemic volunteers, respectively.

24 volunteers (Table 3). However, the chromatographic profile of AA of β-Thal volunteers was noticeably different from that obtained from healthy subjects (Fig. 1). *F*-Test for the equality of variances indicates that there is no significant difference between the variances of the two groups (β-Thal and healthy) for His, Ala, Phe, and lys, while for the rest of the listed AA, a significantly difference do exist (Table 1). Therefore, a two-sample *t*-test assume equal variances was performed for His, Ala, Phe, and lys, and *t*-test assume unequal variances was performed for the rest of the listed AA. The mean values of Asp, Gly, Ile, Phe, Orn, and Lys of β-Thal group were significantly smaller than the values for the corresponding AA of healthy volunteers. However, the mean values of Glu, Asn, Cit, Ser, Arg, Pro, Trp and $NH_3$ of β-Thal group, were significantly higher than those for the corresponding AA of healthy volunteers. The rest of the listed AA including His, Thr, Ala, Val, Nva, Meth, Leu, and Nle have all shown insignificant differences in mean values between the two groups.

The statistically significant increased or decreased percentages were considered as major markers for detection of β-Thal people. Table 4 shows the relative percentages of each amino acid to serine in the fingerprint residue of healthy and β-thalassemic volunteers compared to the reported values. The case severity of the enrolled β-Thal volunteers varied, this explains the high standard deviation (SD) values of certain AA. The statistical and chromatographic profile of β-Thal people was characterized by significant decrease of Orn, Lys, and zero Tyr, with significant increase of $NH_3$, and Pro. Other amino acids that exist in low ratios were estimated statistically for significance changes (Fig. 1). The direct visual inspection of chromatographic profile could also confirm β-Thal case, looking to Orn ($\leq$12.5%), Lys ($\leq$3.0%) and $NH_3$ (>80%). In the present work we did not statistically differentiate between β-Thal major and β-Thal minor. However, Orn and Lys were not detected in some β-Thal major cases, as shown in Fig. 1c, and the mild cases have shown low percentages as shown in Fig. 1d. Cystine was not detected in both groups, and tyrosine was detected only in healthy volunteers. Therefore, both Cys and Tyr were excluded from statistical calculations. The absence of Tyr could also, be considered as an additional marker of β-Thal cases.

Many reports and textbooks have illustrated the role and function of each essential and non-essential amino acid. Upon translating our data in term of clinical impact of each marker we conclude a typical β-Thal case. It has been reported that L-ornithine is one of the products of the action of the enzyme arginase on L-arginine, creating urea, and its deficiency leads to accumulation of ammonia [18]. Our results are in agreement with this point, since we found no or low ornithine, and much more ammo-

nia in fingerprint residue of β-Thal patients. Another metabolic dysfunction symptom in thalassemic people is that low L-lysine which plays a major role in calcium absorption for building muscle protein [19]. Thus the deficiency in lysine shall lead to bone mineral density deficits [20]. This is another correlation, as per our results, that β-Thal patients have shown no or very low percentages of lysine. We could not find any reports that estimate the blood levels of Orn and Lys β-Thal patients. To solve this particular problem, since lysine is an essential amino acid, which means that humans cannot biosynthesize it, in my humble opinion, instead of blood transfusion, as recommended by Leung et al. [20], lysine needs to be investigated as a supplementary treatment for such cases. The literatures also showed that proline plays an important role in the biosynthesis and stability of collagen [21] and its high blood level leads to a common feature of pulmonary dysfunction in thalassemia [22]. This is additional correlation, as per our results, the β-Thal patients have shown much higher ratios of proline. In addition, the relative percentage of each evaluated marker shall leads to identification of certain specific metabolic or physiological problem. The averages of data of healthy and thalassemic volunteers were drawn in form of point to point graph as shown in Fig. 2a, labeled with the corresponding ±SD. In most cases, healthy and β-Thal, the relative amount of serine to glycine was about 1 to 0.4, respectively. Some β-Thal samples have shown a higher amount of Ser. For this reason, Fig. 2b was constructed using glycine as reference peak to show the deviation of Ser peak.

## 4. Conclusions

The applied procedure was successful for complete separation of amino acids as their dansyl-derivative. The thalassemic people could accurately be identified through the analysis of latent fingerprint residue and defining the profile of amino acids qualitatively and quantitatively. The relative percentages of ornithine, lysine, ammonia and proline, found in latent fingerprint residues, were considered as major markers for β-Thal. The absence of tyrosine in β-Thal people is considered as an additional major marker for thalassemic cases. Generally the latent fingerprint residues could mirror the health status and highlights certain diseases, especially metabolic dysfunctions and liver or kidney related physiological abnormalities. The methodology could also draw the attention toward making use of human fingerprint residue as a non-invasive method for early detection of certain diseases.

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