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RESEARCH ARTICLE OPEN ACCESS

Exploring Raman Spectroscopy as a Detection Tool for Cardiovascular Diseases and Diabetes Mellitus in Fingernails

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ABSTRACT

The last decade has witnessed the rapid incline of cardiovascular diseases (CVDs) and diabetes mellitus (DM) cases globally. In many cases, the aforementioned diseases have been associated with diagnostic errors, which refer to the late or misdiagnosis of disease/medical condition and lead to further medical complications. The vast majority of diagnostic errors are seen in low- and middle-income countries (LMICs), where medical expenses are limited in terms of equipment/technologies, staffing and training. Traditional diagnostic methods like blood tests, cardiac catheterization and electrocardiograms (ECGs) are costly, invasive and dependent on sophisticated equipment and expert interpretation, making them less suitable for LMICs. This urges for alternative detection tools such as Raman spectroscopy, which offers rapid, non-intrusive/invasive and non-destructive analysis. Therefore, this work aimed to explore the feasibility of Raman spectroscopy for the detection of CVDs and/or DM in fingernails. Spectral interpretation revealed peaks corresponding to disease-related biomarkers such as cytochrome B and C in CVD fingernails and glycated proteins in diabetic fingernails. Additional endogenous compounds were also identified within the fingernails, including amino acids (cysteine, cystine, tyrosine), lipids (cholesterol) and proteins (keratin). While the aforementioned compounds were detected within all fingernails despite the absence/presence of disease, their spectral signature varied based on the participants' age, biological sex and ethnicity. This demonstrated the feasibility of Raman spectroscopy as a detection tool for CVDs and DM, while also removing the invasive and intrusive nature of traditional detection techniques and matrices (blood and urine).

1 | Introduction

Raman spectroscopy has emerged in recent years as a powerful analytical technique and has shown great success in the chemical analysis of complex biological matrices including blood, fingernails, saliva, urine and other tissues [1–4]. As a vibrational technique, Raman spectroscopy demonstrates the ability to detect biochemical changes at a molecular level. Conventional Raman spectroscopy is characterised by light scattering.

Through the application of a monochromatic light source, photons collide with the sample's molecules and cause irradiation. The monochromatic radiation is then transmitted, absorbed or scattered.

Spatially offset Raman spectroscopy (SORS), a phenomenon first described by Matousek et al. [5], allows for through-barrier examination. Through the application of SORS, Raman spectra are collected from spatially offset regions that vary in degrees

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from the point of incidence. Hence, the produced spectra contain different relative Raman contributions that are reflective of the surface and subsurface layers [5].

Traditionally, CVDs are detected through the application of ECGs. However, ECG findings should be approached with caution, especially in LMICs, where the sophistication of medical equipment and training is limited. Automated ECG interpretations are often unreliable; therefore, for accurate ECG interpretation, a well-trained ECG expert is required [6]. Moreover, previous studies have identified the low sensitivity of ECGs, especially in stable and/or asymptomatic patients [7]. A similar trend was observed in the diagnosis of DM through blood work focusing on fasting plasma (FPG) measurements and oral glucose tolerance tests (OGTT). While the aforementioned tests are considered the 'gold standard' for several clinical practices, they are limited based on their often-poor reproducibility, intrusive/invasive nature and high costs [8]. Thus, it is of the highest importance that alternative detection tools be explored for improved detection accuracy and decreased medical expenses. Given these limitations, it is crucial to explore alternative detection tools that offer improved accuracy and reduced medical expenses. This study aims to explore the feasibility of Raman spectroscopy as a detection tool for CVDs and DM in fingernails.

2 | Procedure

2.1 | Materials

Participants were recruited through the Liverpool John Moores University (LJMU) Forensic Science community site. Prior to the collection of fingernails, ethical approval was granted by LJMU (PBS/2023/09). The announcement placed on the community site was open to all participants that have access to this site. Included participants were adults (> 18 years old) who were willing to provide fingernail clippings. There were no limitations placed on disease status for participation in the study.

After expressing interest in participation, participants were given a brief introduction regarding the research experiments, collection of samples/clinical data and dissemination of findings. Moreover, participants were made aware that they could withdraw prior to the donation of fingernails. Those who came forward received a validated questionnaire and consent form. The questionnaire collected sociodemographic, clinical and lifestyle data impacting endogenous compounds' deposition in fingernails. Fingernails were donated in clear zip-locked bags and were given an anonymous code such as MWS1.

A total of 72 (47 female, 25 male) participants were recruited for this work and were aged between 18 and 85 years old. Participants were of White, Arab, Asian or Lebanese Arab ethnicity.

2.2 | Instrumentation

Raman spectra were collected using the Agilent Resolve Raman spectrometer (Agilent Technologies Inc., Santa Clara, CA), equipped with an 830 nm laser excitation wavelength and a maximum laser power of 475 mW [9]. While this instrument possessed

three modes of operation: (1) through barrier scan, (2) surface scan and (3) vial holder mode, the through barrier scan was utilised for this work [9]. Furthermore, the mode of choice utilised SORS and allowed for the non-invasive analysis of fingernail clippings through the zip-lock packaging. To ensure direct contact with the fingernail clippings, the pointer tool was employed.

2.3 | Method

Fingernail clippings were examined for the presence of nail polish and/or dirt. In cases where the highlighted contaminants were observed, fingernails were soaked in acetone for 2 h as per the protocols within the literature [10, 11]. To explore the influence of acetone, a comparison was made of a set of fingernails that was measured before and after soaking of fingernails in acetone. Soaking in acetone was found to not affect the Raman spectra of fingernails. Therefore, it was adopted for the remaining sets of fingernails.

To protect the samples from humidity, fingernails were stored in glass vials and transferred to clear zip-locked bags just before analysis. The fingernails were then pressed into the pointer tool and made direct contact with the Raman light source. To prevent environmental light from impacting the quality of Raman spectra, the sample and light source were covered using a black cloth supplied by Agilent Technologies. A total of 30 spectra were taken per fingernail set to maximise spectral data collection/value.

The employed Raman spectrometer utilised an in-built library identification mode [12]. Thus, measured materials were matched against the in-built library, and a percentage match was determined. Raman spectra were also exported for offline interpretation, as well as application to machine learning algorithms (MLAs).

2.4 | Data Analysis

To explore the feasibility of Raman spectroscopy as a detection tool, several MLAs were employed, including correlation in wavenumber space (CWS), principal component analysis (PCA) and self-organising maps (SOMs). CWS was employed to match the correlation coefficient (r) values of the test spectrum against the reference spectrum [13]. A calculated r value of -1 indicated that spectra were completely dissimilar, and an r value of $+1$ indicated identical spectra [14]. As the difficulty of obtaining an r value of $+1$ was high due to sample noise and light interference, a threshold of 0.95 was used to indicate a match. PCA was then explored and reduced the original matrix's dimensionality into two subspaces (scores and loading) and allowed for the classification of spectral data.

3 | Results

3.1 | Raman Signature of Fingernails

To determine the Raman signature of fingernails, spectral interpretation was carried out on fingernails of healthy, CVD, diabetic and CVD-diabetic fingernails.

3.2 | Spectral Quality of Raman Spectra

Spectral quality assessments were performed to determine the Raman scattering of healthy, CVD, diabetic and CVD-diabetes. Parameters included the number of peaks, maximum peak intensity/position, range and signal-to-noise (S/N) ratio. The number of peaks ranged from 12 to 21 (median = 17, IQR = 16–18). Fingernail set MWS57 displayed the highest number of peaks, and MWS58 demonstrated the smallest number of peaks. Additional peaks detected within MWS57 were attributed to phenylalanine (C–C₆H₅ stretching) and tyrosine (aliphatic CCH bending, in-plane C–H bending and C–C stretching) [3, 14]. During the collection of clinical data, participants in MWS57 reported the previous diagnosis of coronary artery disease (CAD). Therefore, the presence of additional peaks associated with phenylalanine and tyrosine lends itself to the presence of CVDs. This finding was supported by Jauhianinen et al. [15], who successfully identified a relationship between CAD, phenylalanine and tyrosine. Patients with CVDs such as CAD showed high Raman scattering for amino acids in comparison to healthy controls, such as in the case of set MWS58, which showed weaker Raman scattering in relation to phenylalanine and tyrosine.

The peak intensities of fingernails ranged from 734.959 to 22,993.46 arbitrary units. Despite the varying maximum peak intensities, fingernails shared similar maximum peak positions between 1445 and 1451 arbitrary units. This area was attributed to methylene CH₂ deformation, which is associated with the presence of disulphide bonds within the fingernails [16, 17]. An outlier was identified from set MWS5, which displayed a peak position of 1297 cm⁻¹. This area was associated with C–H deformation and amide III [18]. Furthermore, peaks present within the region 1206–1305 cm⁻¹ have been utilised as evidence of tissue degradation and the autoimmune disease, psoriasis [18]. The occurrence of psoriasis in fingernails lends itself to risk factors and habits including alcohol use, diet, drugs, infections, stress and smoking [19]. The habit of smoking was reported by participant MWS5, who declared smoking 2–8 cigarettes a day. However, as participant MWS5 did not report the presence of psoriasis, additional explanations were sought. Previous work highlighted that cigarette smoke altered the chemical and physical structure of fingernails. Such changes include yellow pigmentation and nail clubbing and are commonly referred to as Harlequin nails [19–22].

The presence of additional peaks was also attributed to the detection of disease-related biomarkers. Fingernail abnormalities and structural changes provide key insight into the onset and prognosis of systemic diseases. In several cases, defects to the fingernail's unit anatomical components, such as nail matrix, nail plate and nail bed/vasculature, have been the first presenting feature of systemic disease [23]. Singal and Arora [24] demonstrated the relationship between structural changes of fingernails in relation to renal autoimmune diseases, CVDs, central and peripheral nervous system disease, endocrine disease, gastrointestinal infection, renal disease and pulmonary disease. Through circulating molecules and passive diffusion, endogenous compounds and diagnostic biomarkers are incorporated into the fingernail [25].

Spectral interpretation made evident the presence of biomarkers employed for the diagnosis of CVDs, including cytochrome B and C. At peak 1128 cm⁻¹, cytochrome B was detected and attributed

to skeletal C–C trans conformation [26]. Through the mutation of the mitochondrial cytochrome B gene, this biomarker has been associated with hypertrophic cardiomyopathy [27]. Furthermore, at peaks 1337 (CH₃CH₂ twisting) and 1590 (C=C stretching) cm⁻¹, cytochrome C was encountered [28, 29]. The presence of cytochrome C is not unexpected for CVD patients, particularly for individuals with coronary heart disease (CHD). Many patients with CHD suffer from ischemia, which results in myocardium damage. Through the oxidation of cytochrome C, the rate of myocardial respiration and overall mitochondrial respiratory chain is impaired [30]. Despite being unclear, a relationship between cardiac failure and compromised cellular respiration of the heart has been identified. This relationship is reliant on the link between adenosine triphosphate (ATP) supply and the production of mitochondria [30]. For the production of ATP via mitochondria, cytochrome C-oxidase (COX4) is crucial. Low expression levels of COX4 can impair cytochrome C oxidase activity [31, 32]. Thus, it is hypothesised that mitochondrial dysfunction is related to increased reactive oxygen species and increased levels of ATP. Therefore, it contributes to myocardial insufficiency and cytochrome C presence [32]. The aforementioned relationship was also supported by Zhen-Bing et al. [33], who showed a relationship between cytochrome C, myocardial ischemia and myocardial infarction.

Additional Raman peaks were identified and accredited to the presence of diabetic biomarkers. The first indicator of diabetes was identified at 1128 cm⁻¹ (C–C trans conformation) and was attributed to the presence of glucose. Amino acids such as isoleucine and leucine were also detected at 1250 (amide III), 1585 (olefinic C–C stretching) and 1242 (C–C stretching) cm⁻¹, respectively. The branched-chain amino acids were linked to future risk of hyperglycaemia and overt DM.

Finally, the calculated S/N ratio ranged from 12.64 to 129 (median = 46.05, IQR = 32.56–60.13). Variability of S/N ratios can be explained through the hydration of fingernails prior to sampling. For example, conditions including hypertension and hyperthyroidism have been linked to dry, brittle nails [34, 35]. The relationship between the aforementioned conditions and dry fingernails was observed in set MWS5. Thus, the reduced presence of water within the fingernails ensured less noise and a high Raman signal [36].

Overall, fingernails produced strong ($n=33$), medium ($n=36$) and weak ($n=3$) Raman scattering.

3.3 | Impact of Age, Biological Sex and Ethnicity on Disease Diagnosis

To understand the relationship between age, biological sex, ethnicity and disease diagnosis, measures of variability were utilised. Furthermore, the Raman intensity of peaks could provide an indication of the presence of key endogenous compounds.

3.3.1 | Age

Participants were separated into one of five groups, those being: 18–24 (Group one), 25–34 (Group two), 35–39 (Group three), 40–65 (Group four) and 66–85 years old (Group five).

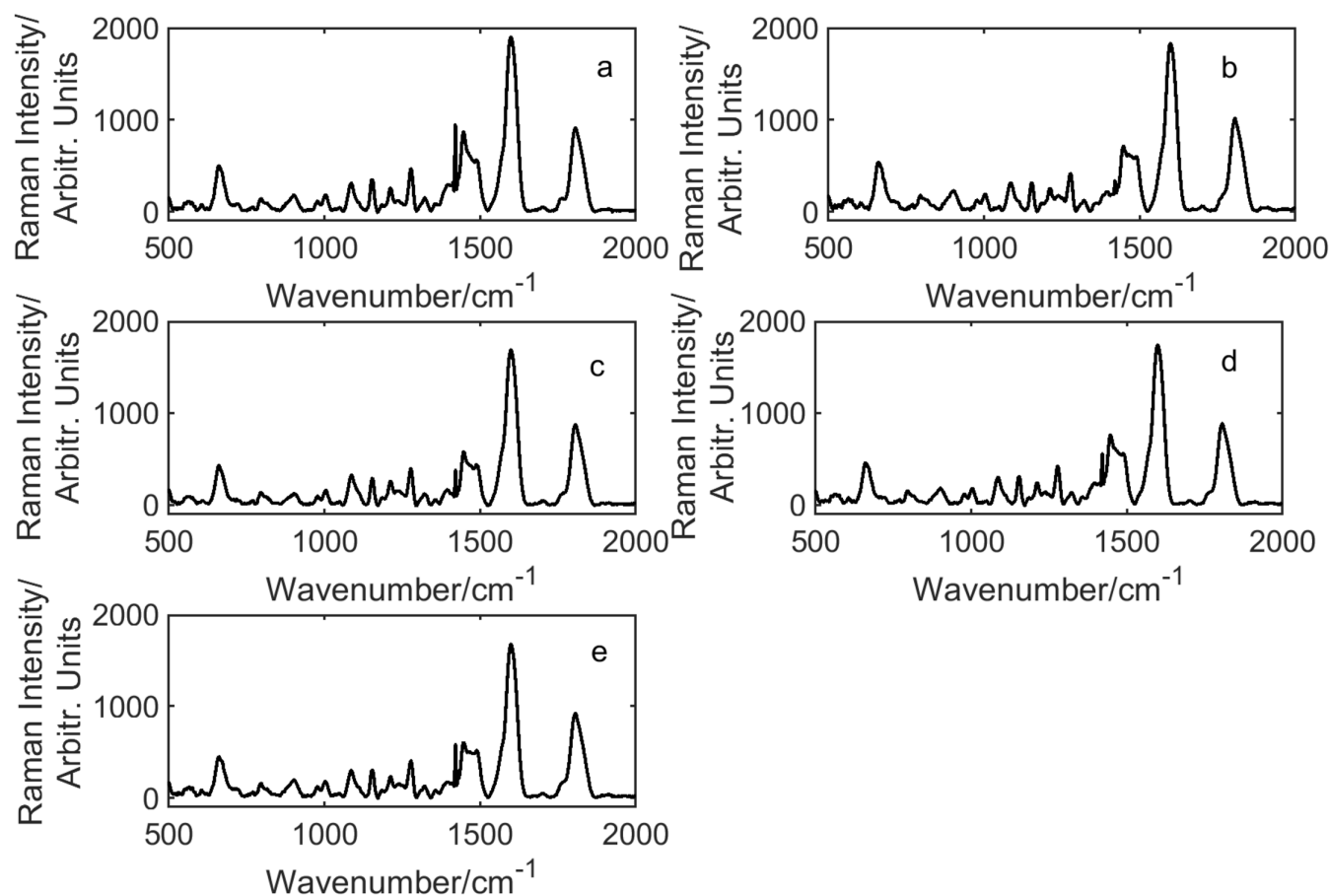


FIGURE 1 | Raman spectra of fingernails donated by participants aged 18–24 (black), 25–35 (red), 35–39 (green), 40–65 (blue) and 66–85 (magenta) years old measured using the Agilent Resolve Raman spectrometer, equipped with an 830 nm laser wavelength.

On average, Group one demonstrated the lowest Raman scattering of cysteine, cystine, tyrosine, tryptophan and proteins (Figure 1). While it was expected that Group five would display the highest Raman scattering of endogenous compounds across the five groups, this was not the case. For example, cysteine and cystine residues were detected at 647 cm^{-1} (C–S stretching). As predicted, Group one displayed the lowest Raman scattering of cysteine and cystine residues (mean = 34.53 arbitrary units, SD = 89.65 arbitrary units). This finding was supported by previous work which made apparent the significant oxidation shift of cysteine and cystine between the third decade of life (18–24 years old) and the ninth (> 70 years old) [37–39]. Nonetheless, Group two showed the highest Raman scattering of the highlighted amino acids (mean = 138.1 arbitrary units, SD = 63.14 arbitrary units). The increased presence of amino acids in participants aged 25–34 years old lends itself to the presence of additional medical conditions including irritable bowel syndrome (IBS) and high cholesterol [40]. Both of which were reported by participants MWS41 and MWS69. The oxidation of cysteine and cystine has been closely associated with high cholesterol [37–39]. Likewise, cysteine and cystine have been associated with IBS [40].

A similar trend was observed at 750 cm^{-1} (symmetric breathing) and corresponded to the presence of tryptophan. At the highlighted peak, participants aged 25–34 years showed the highest peak intensity of 22.58 arbitrary units and in turn the highest Raman scattering. This essential amino acid is

generally found at low concentrations in tissues [41]. Thus, the presence of tryptophan is reliant on the consumption of high-protein food products such as beans, chicken, eggs and fish [42]. The collected clinical data supported the spectral data and identified that several participants aged 25–34 practised high-protein diets.

Nonetheless, Group five showed the highest Raman scattering of cystine (mean = 44.18 arbitrary units, SD = 53.65 arbitrary units) at 512 cm^{-1} (S–S stretching) [3]. The group with the lowest Raman scattering of cysteine was Group one (mean = 32.74 arbitrary units, SD = 49.89 arbitrary units). The increased Raman scattering of cystine was expected in elderly participants as the amino acid, particularly in its predominant form (cysteine), plays an imperative role in catalysis, trafficking and mediating oxidative stress responses [43]. Furthermore, research suggested that cysteine and cystine oxidation was an imperative determinant of systematic inflammation and stress [44]. Thus, it contributed to ageing and was not unexpected in participants aged 66–85 years old.

3.3.2 | Biological Sex

Participants were then separated based on biological sex. Overall, female participants demonstrated the highest Raman scattering of the endogenous compounds found in Table 1 (Figure S1).

TABLE 1 | Spectral interpretation of fingernails taken from healthy, CVD, diabetic and CVD-diabetic participants measured using the Agilent Resolve Raman spectrometer, equipped with an 830 nm laser wavelength.

Peak (cm ⁻¹)	Functional group	Association	References
512	S–S stretching	Cystine	[3, 16]
647	C–S stretching	Cystine and cysteine residues	[3]
750	Symmetric breathing	Tryptophan	[14]
828	Aliphatic CCH bending	Tyrosine	[3]
855	C–C stretching CCH bending	Proteins Tryptophan	[3]
939	Skeletal C–C stretching	Proteins	[14]
1002	Aromatic C–C stretching	Phenylalanine	[3]
1034	In-plane C–H bending	Phenylalanine	[14]
1178	C–C stretching	Tyrosine	[3]
1448	Methylene CH ₂ deformation	Lipids and proteins	[16, 45]
1656	C=O bending	Amide I, collagen type I and protein	[45]

For example, at 512 cm⁻¹, females showed the highest Raman scattering of cysteine with an average peak intensity of 475.21 arbitrary units. In contrast, male participants displayed an average peak intensity of 444.51 arbitrary units.

Females also displayed increased Raman scattering of lipids, proteins, phenylalanine, tryptophan and tyrosine in comparison to male participants. The presence of the aforementioned endogenous compounds can be attributed to sex-specific energy metabolisms. In females, energy metabolism is utilised for gestation and lactation, while males require energy for a default state including food metabolism, respiration and water/body temperature regulation [46]. Furthermore, fat (lipids) storage is highly dependent on biological sex. For example, females are more likely to store lipids, whereas males frequently oxidise lipids [47]. This provides explainability for the higher Raman scattering of lipids seen in fingernail spectra taken from female participants.

Similarly, research has identified a relationship between protein metabolism and biological sex [48]. Muscle hypertrophy results revealed that males possess a greater net muscle protein synthesis in comparison to females [48]. Furthermore, testosterone makes a significant contribution to muscle protein synthesis and, as a result, increases muscle protein balance and overall muscle mass [49]. Therefore, rather than being deposited into

the fingernails, such as the case with females, proteins are utilised at a faster rate and higher degree in males, resulting in a limited number of proteins depositing into the fingernail matrix.

Research also suggested that biological sex impacted the regulation of amino acids including tyrosine [50]. These amino acids showed high Raman scattering in female versus male participants and coincided with the high Raman scattering of phenylalanine seen in females [51]. The relationship between the highlighted amino acids is attributed to the conversion of phenylalanine to tyrosine through phenylalanine hydroxylase enzymes [52].

3.3.3 | Ethnicity

The Health Survey for England revealed a key relationship between ethnicity and health, with Black and minority (BME) groups showing higher levels of poor health than White groups [53]. Individuals in BME groups were also more likely to experience poor health at a young age [53].

As an umbrella term, ethnicity captures several factors including culture, race, religion and nationality. Collectively, the aforementioned terms make a significant impact on the individual's identity and biochemical profile [53]. Research has also pointed out the variation between ethnic groups and health/disease status in response to socio-economic factors such as education, employment and income [54].

Ethnic variation was also identified with disease prevalence. For example, male individuals from South Asia are 50% more likely to experience angina or a heart attack than males in the general world population [55]. CVDs are most prevalent in Sri Lanka and Bangladesh, followed closely by Pakistan, India and South Asia [56, 57]. The prevalence of CVDs and DM in the highlighted countries lends itself to a combination of risk factors and socio-economic factors. There is also some suggestion that disease variation in relation to ethnicity is attributed to biological differences [58, 59]. Nonetheless, this concept is theorised and requires further evidence to reach conclusive decisions.

For this work, participants were separated into four groups, those being Arab, Asian, Lebanese Arab and White. Despite all groups sharing the same set of endogenous compounds, the Raman scattering of endogenous compounds varied between ethnic groups (Figure S2).

Variation between the four groups was first identified at peak 512 cm⁻¹. On average, White participants showed the highest Raman scattering of cysteine, with a peak intensity of 555.82 arbitrary units. In contrast, Asian participants demonstrated the lowest Raman scattering and peak intensity of 395.23 arbitrary units. The presence of cysteine is regulated through the consumption of protein [60–62]. Based on cultural and religious reasons, many individuals of Arab or Asian ethnicity carry out a halal diet/lifestyle. This was noted for several of the recruited participants. While halal meat does provide a source of protein, those that practice it are limited to certain meat types. Thus, the amount of proteins consumed is lower than that of individuals who do not practice a halal diet.

Variation in the presence of tryptophan was also observed between the four ethnic groups at peaks 750 and 1550 cm^{-1} . White participants showed the highest Raman scattering, while Asian participants demonstrated the overall weakest Raman scattering of tryptophan. Similarly, White participants possessed the highest Raman scattering of lipids at 1449 and 1580 cm^{-1} [16, 45]. The stronger Raman scattering of lipids observed in Asian participants can be attributed to race-ethnic differences in lipid profiles [6, 24]. Work by Wiley et al. [63] looked at the lipid profiles of White, Black and Hispanic individuals. This research made apparent that Hispanic subjects had the lowest level of lipids across the investigated groups, and this was attributed to socio-economic factors. For example, employment and income play a significant role in the creation of poverty and severely impact diet quality [64].

3.4 | Application of MLAs to Raman Spectra

To determine the feasibility of Raman spectroscopy as a detection tool, MLAs were employed. CWS was first utilised to evaluate the Raman spectrometer's ability to differentiate between healthy and diseased fingernails.

Fingernails produced an r value of 1 against themselves and represented a match (Figure 2). Overlap, however, was experienced between r values of different fingernail sets and, therefore, suggested a match/similarity. Mismatches between fingernails of different characteristics, e.g., healthy versus diabetic, can be attributed to a type I error. Moreover, a high number of matches

can be attributed to all fingernails sharing the same basic biochemical profile, despite the presence of disease.

Nonetheless, CWS and Raman spectroscopy showed some ability to differentiate between fingernails of different classes. For example, the healthy set MWS5 produced an r value of 0.75 against the diabetic set MWS58. CWS and Raman spectroscopy also demonstrated the ability to differentiate between CVD and diabetic fingernails, such as the sets MWS48 (CVD) and MWS59 (diabetic).

To explore the clustering amongst scores of healthy and diseased fingernails, PCA was employed. The PC scores plot of 72 fingernails comprised 85.52% of the variance amongst the data (Figure 3a). Nonetheless, the model was unsuccessful at independently differentiating between healthy, CVD, diabetic and CVD-diabetic fingernails. Figure 3 demonstrates the overlap between the groups of interest. This overlap lends itself to the fingernails sharing the same set of amino acids, lipids and proteins.

Type I and II errors were also shown. Frequently, type I errors were observed in scores associated with healthy and CVD fingernails. A type II error was encountered for a set of diabetic fingernails, which were clustered away from the remaining diabetic scores.

To further understand the significance of Raman peaks and their related endogenous compounds and biomarkers, PC loading plots were examined. Within PC1 (Figure 3b, 1), key Raman peaks were associated with cystine, cystine/cysteine residues,

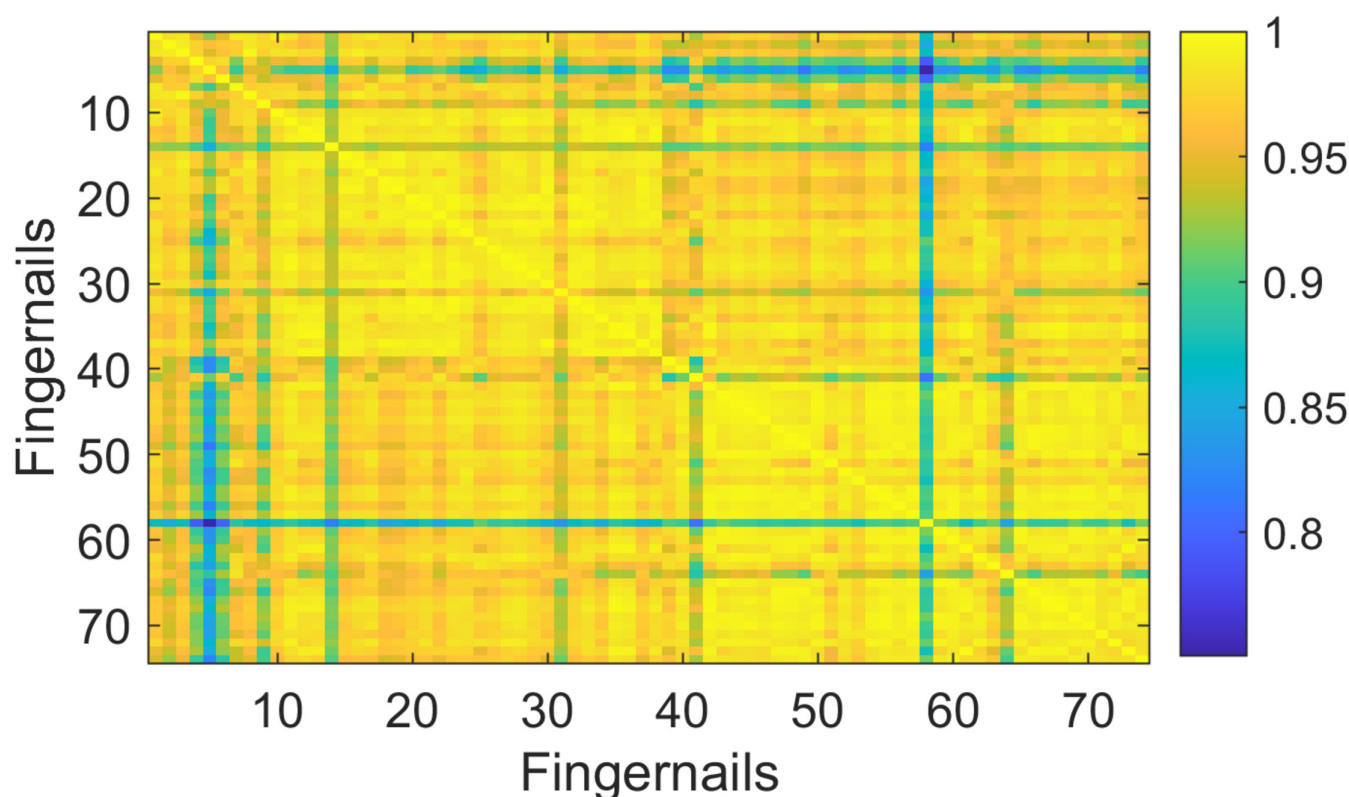


FIGURE 2 | CWS of Raman spectra taken from (1–55) healthy, (56–64) cardiovascular and (65–72) diabetic participants. A dark blue colour represents a minimum r value of 0.75 and a dark yellow colour represents a maximum r value of 0.9999. In between the r values 0.77–0.82 correspond to light blue colour, r value of 0.83–0.87 correspond to cyan colour, 0.88–0.92 correspond to green, 0.93–0.97 correspond to greenish yellow colour and 0.98–0.99 correspond to orange colour.

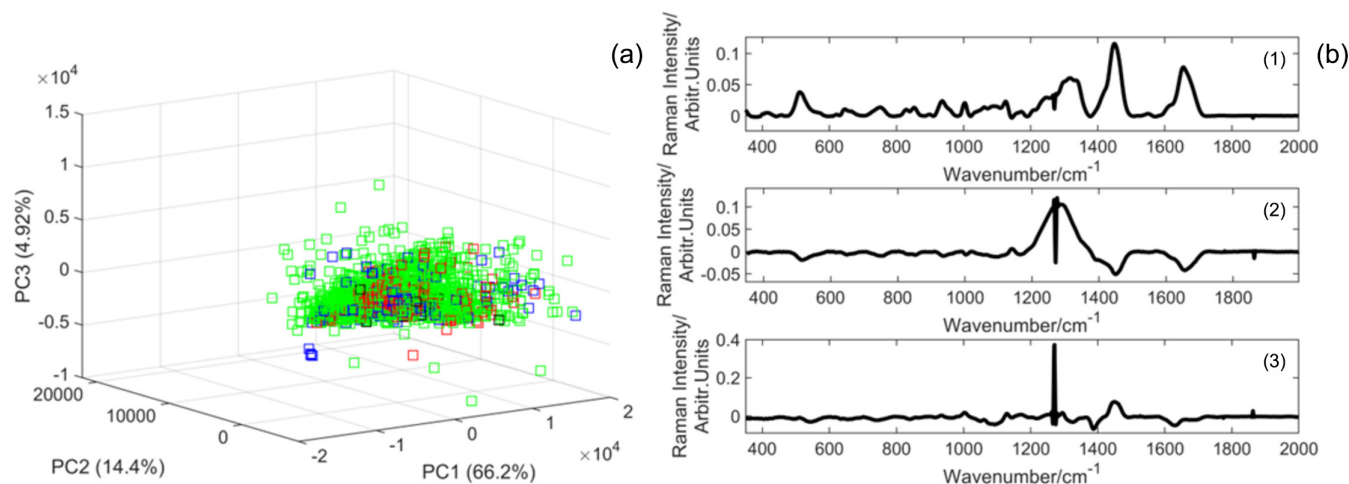


FIGURE 3 | (a) PCA plot of fingernails from healthy (green), CVD (red), diabetic (blue) and CVD-diabetic (black) participants and (b) PCA loading plot of PC1 (1), PC2 (2) and PC3 (3) loading plots of fingernails taken from healthy, CVD, diabetic and CVD-diabetic participants measured using the Agilent Resolve Raman spectrometer, equipped with an 830 nm laser wavelength.

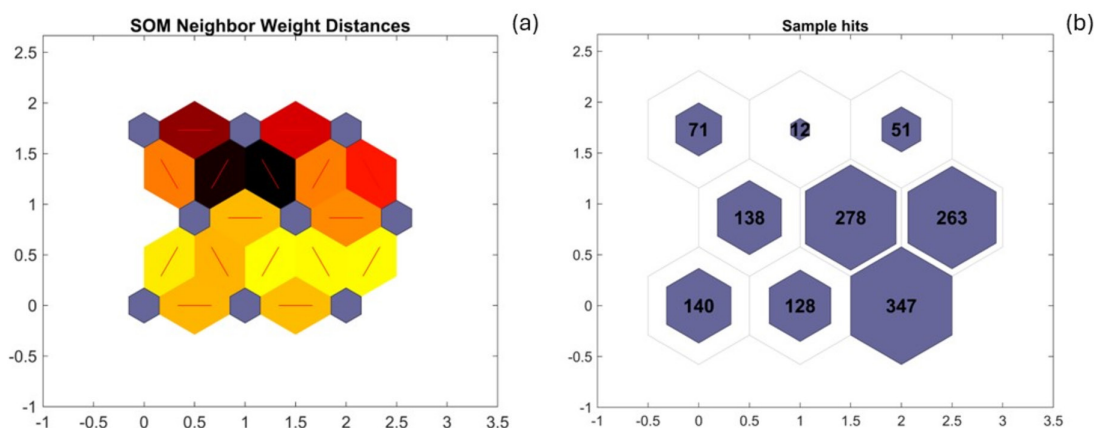


FIGURE 4 | (a) U-Matrix and (b) sample hits of SOM applied to Raman spectra of fingernails taken from healthy, CVD, diabetic and CVD-diabetic participants measured using the Agilent Resolve Raman spectrometer, equipped with an 830 nm laser wavelength.

tryptophan, tyrosine, proteins, phenylalanine and lipids and were encountered at 512 (S–S stretching), 645 (C–S stretching), 752 (symmetric breathing), 828 (aliphatic CCH bending), 854 (C–C stretching, CCH bending), 936 (skeletal C–C stretching), 1004 (in-plane C–H bending) and 1451 (methylene CH₂ deformation) cm^{−1}, respectively [3, 14, 16, 54]. PC2, capturing 4.92% of the variance, was related to cystine at 514 and 1656 cm^{−1} and lipids and proteins at 1449 cm^{−1}. The aforementioned peaks were associated with S–S stretching, C=O bending and methylene CH deformation, respectively [3, 54].

The presence of endogenous compounds identified within the PCA loading plots signifies their importance in relation to the prediction of disease. Therefore, the highlighted endogenous compounds should be considered in future CVD and DM diagnoses.

As an additional unsupervised clustering approach, SOMs were employed and confirmed the overlap seen within the PCA model. The U-Matrix showed the weighted distances between neighbouring neurons. The connection between neurons is

demonstrated through the red lines located in Figure 4. Larger distances between neurons possessed lower densities (lighter colours), and smaller distances between neurons had larger densities (darker colours) [65].

Sample hits complimented and confirmed PCA findings. The overlap seen between fingernails of varying characteristics can be attributed to the presence of additional medical conditions such as cancer, cholesterol and IBS. The presence of the highlighted medical conditions can impact the presence of endogenous compounds seen in fingernails. Therefore, future work will look at adding an additional group to the classification of participants. This group will consist of participants with a previous diagnosis of disease and/or medical conditions that are unrelated to CVDs and/or DM.

Nonetheless, nine groups were observed. The first group ($n = 71$) consisted of fingernails taken from diabetic participants. Groups two ($n = 12$) and three ($n = 51$) were also representative of diabetic fingernails. Overlap was observed within Group four ($n = 138$), which related to CVD, diabetic and CVD-diabetic fingernails.

Groups five ($n=140$) and six ($n=128$) consisted of fingernails taken from CVD and healthy participants. Finally, groups seven ($n=279$), eight ($n=263$) and nine ($n=347$) were composed of healthy fingernails. Despite the overlap between several spectra of varying characteristics, the sample hits model implied that Raman spectroscopy showed some capabilities with regard to the differentiation of healthy, CVD, diabetic and CVD-diabetic fingernails.

4 | Conclusions

Over the last two decades, Raman spectroscopy has emerged as a powerful analytical technique and has shown the ability to non-invasively identify disease (CVDs and/or DM) in fingernails.

Fingernails demonstrated strong ($n=33$), medium ($n=36$) and weak ($n=3$) Raman scattering. Raman spectra of fingernails possessed a high number of Raman peaks ($n=12$ – 21) and maximised spectral interpretation value. A range of endogenous compounds was successfully identified within the fingernails, including cysteine, cystine, tryptophan, tyrosine, lipids, phenylalanine and proteins. Disease-related biomarkers were also successfully detected, including cytochrome B and C for the diagnosis of CVDs and glucose, isoleucine and leucine for the diagnosis of diabetes.

Raman spectroscopy successfully demonstrated the relationship between the Raman scattering of endogenous compounds and factors including age, biological sex and ethnicity. For instance, the highest Raman scattering of endogenous compounds was observed in female participants, participants aged 66–85 years old and/or White ethnic groups. In contrast, the lowest Raman scattering of endogenous compounds was witnessed in males, participants aged 18–24 years old and/or of Asian and Arab ethnicity. As a result, clinical practices should consider these confounding factors during the diagnosis process and establish biological thresholds of endogenous compounds based on the patient's individual characteristics.

Raman spectroscopy combined with MLAs showed some capability to detect differences between healthy and diseased fingernails. CWS presented differences between healthy fingernails and fingernails taken from CVD or diabetic individuals. Despite the unsuccessful classification of the four groups of interest, PC loadings highlighted the significance of key Raman peaks associated with cystine, cysteine/cystine residues, tryptophan, tyrosine, phenylalanine, proteins and lipids. The aforementioned endogenous compounds are imperative not only to the growth, structural integrity and chemical make-up of the fingernail but also to the development and identification of CVDs and/or DM. Nonetheless, the poor clustering of the PCA model lends itself to the data imbalance between healthy ($n=55$) and diseased ($n=17$) fingernail sets. Future work will look at expanding the dataset, ensuring that all groups are equally distributed.

Few limitations were encountered in the study. The first was related to the sample size in terms of healthy and diseased participants. As a pragmatic approach was used, it was not possible to recruit more participants that have CVDs and/or DM. The second limitation was related to the position of the fingernails

to the instrument. This limitation was overcome by taking numerous spectra ($n=30$) from each fingernail set to ensure the robustness of the Raman signatures. Moreover, soaking the fingernails in acetone could have affected the Raman signature in denaturing the proteins in the fingernail samples. However, in this specific case, soaking was done for only 2 h and was not sufficient to denature the proteins as it did not influence the Raman spectra of fingernails. Furthermore, this limitation was systematic as it was applied to healthy and diseased fingernails consistently. Further research is also needed to explore the broader applications of the methodology, potentially improving diagnostic accuracy and reducing medical costs.

Ethics Statement

Ethical approval for the completion of this work was provided by two institutes, those being: Liverpool John Moores University (23/PBS/009A) in the United Kingdom and the Lebanese University (2022-0104) in Lebanon.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Raman spectra of fingernails taken from (red) female and (blue) male participants measured using the Agilent Resolve Raman spectrometer, equipped with an 830nm laser wavelength. **Figure S2:** Raman spectra of fingernails taken from (a) Arab, (b) Asian, (c) Lebanese Arab and (d) White participants measured using the Agilent Resolve Raman spectrometer, equipped with an 830nm laser wavelength.