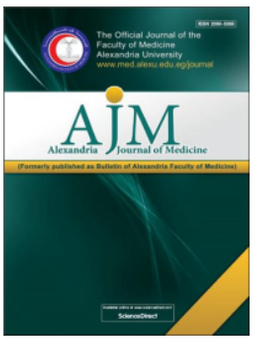

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Identification of urinary proteomic profile of patients with chronic allograft nephropathy

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ABSTRACT

Background and objectives: Chronic allograft nephropathy (CAN) is a major complication that occurs post-transplantation. At present, the diagnosis of CAN is based on renal biopsy. Therefore, there is an ultimate need to identify more specific and sensitive noninvasive methods for the early diagnosis of CAN. Recently, proteomic-based modalities have been developed to discover biomarkers of CAN.

Methods: Urine samples from 75 participants were collected. Participants were divided into three groups: Group I: 25 patients with CAN, Group II: 25 transplanted patients with stable renal functions, and Group III: 25 healthy control subjects matched for age and sex. Each group was divided into training set and test set. Specimens were purified with magnetic beads-based weak cation exchange chromatography and analyzed using MALDI-TOF MS.

Results: A Genetic Algorithm (GA) was used to set up the classification models. Five peaks represented the proteomic profile that differentiates between the CAN patients and the control group with sensitivity of 100%, specificity of 100%, recognition capability of 100%, and cross-validation of 91.7% and five peaks differentiate between the transplant patients with normal renal functions and the control groups with sensitivity of 96.8%, specificity of 95.5%, recognition capability of 98%, and cross-validation of 100%.

Interpretation and conclusions: We identified a pattern for CAN and transplant patients with normal renal functions by proteomic profiling using MALDI-TOF-MS and magnetic beads.

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KEYWORDS
ClinPro Tools; magnetic bead; MALDI-TOF MS; CAN; urinary proteome

1. Introduction

End-stage renal disease (ESRD) is considered the point of irreversible renal functional deterioration beyond which life cannot be maintained without renal replacement therapy in the form of hemodialysis, peritoneal dialysis, or transplantation [1,2].

Renal transplantation is widely accepted as the best form of renal replacement therapy as it prolongs survival of the patients, reduces mortality and morbidity, cost-effective, improves quality of life, releases from the tedium of dialysis, and facilitates social rehabilitation of patients with ESRD. Therefore, it is considered to be the treatment of choice for most of ESRD patients [3].

Renal graft rejection can be defined as a series of events in which the body start to recognize the graft as non self. The process involves the participation of both local and systemic immune responses involving CD_4 T-lymphocytes, CD_8 T-lymphocytes, B cells, natural killer (NK) cells, macrophages, and cytokines; the establishment of a local inflammatory injury; major histocompatibility complex (MHC) expression; and graft necrosis [4–6].

Proteomics is the systematic analysis of proteins for their identity, quantity, and function. Proteomics has played a significant role in the study of CAN since 2003. Consequently, a significant number of promising biomarkers may be used for diagnosis and prediction of rejections has been identified; however, the information obtained from these studies is not closed to clinical application [7].

Very recently, proteomic-based modalities to discover urine or serum biomarkers of CAN have been widely studied. Profiling methods using MALDI TOF and magnetic beads, one of the available proteomic approaches, are promising successful tools for the identification of new reliable biomarkers in transplantation field [8,9].

Numerous studies have been conducted to outline serum proteomic profile in patients with the field of renal transplantation [9–11]. However, there are few data on the urinary proteomics in this field that invited us to conduct the current research [12,13].

2. Subjects

This study was conducted on 75 subjects and divided into three groups: Group I: 25 patients with chronic allograft nephropathy (CAN)-proven biopsy, Group II:

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25 transplant (TN) patients with normal renal functions, and Group III: 25 healthy control (C) subjects matched for age and sex. Each group was divided equally into training set and test set. Cases were recruited from the nephrology and transplantation outpatient clinic in Alexandria Main University Hospital, Alexandria, Egypt. Approval was obtained from ethics committee of Alexandria University, and written informed consent was obtained from each participant.

3. Methods

All patients were subjected to full history taking and complete physical examination and laboratory investigations. The urine was put for proteomic analysis. Within a maximum of 1 h, the tube was then separated in a cooling centrifuge at 4°C for 15 min at 3700 RCF. Then, the urine sample was aliquoted where 50 µL of it were placed in 1.5 safe-lock Eppendorf tubes from Eppendorf (Hamburg, Germany) and stored immediately at −80°C till further analysis. During aliquoting, Eppendorf tips (Hamburg, Germany) were used. This high-quality plastic ware was used to avoid polymer contamination and protein proteolysis.

3.1. Proteome separation

Magnetic silica beads coated with a weak cation exchange surface (MagSi-WCX) (Magna Medics) was used for proteome separation of samples following the standard protocol by the manufacturer. A sample containing approximately 10 µg protein or peptide was added to the washed MagSi-WCX beads and adsorption solution to a total volume of 50 µL and left at room temperature for about 5 min for proper adsorption of the sample then placed on the magnetic separator until the liquid is totally clear then the supernatant was discarded. The tube was then removed from the magnet and 100 µL adsorption solution was added, magnetic separation for 2 min and the supernatant was discarded. The last two steps were repeated for two more times. For MS analysis, 100 µL washing solution was added to the bead pellet and re-suspended (desalting step), magnetic separation for 1 min, the supernatant was discarded, 10 µL desorption solution was added to the beads and re-suspended, magnetic separation for 2 min and the liquid was removed for further analysis to a new micro tube. Sample analysis: 1 µL of the eluate and 1 µL of a MALDI-MS matrix (alpha-hydro-cyanocinnamic acid) were mixed. Spotting of 1 µL of the mixture on a MALDI target generated reliable spectra.

3.2. MALDI-TOF MS proteome analysis

The urine peptide profiles were analyzed using an Ultraflextreme MALDI TOF/TOF MS (Bruker Daltonik, Germany). With this spectrum, sample preparation peptide/protein peaks in the *m/z* range of 900–20,000 Da were measured with matrix suppression effect enabled to suppress up was set at 900 Da. Spectral acquisition was done in the positive linear mode. Only peaks with signal/noise (S/N) ratio above 3 were chosen from the spectra generated. For each spot, 3000 shots were done by shooting 500 laser shots at six different spot positions. Shots were gathered into one spectrum.

3.3. Statistical analysis

The ClinPro Tools software V.03 (Bruker Daltonik, Germany) was used for analysis of all sample data derived either from patients or controls. Data analysis began with raw data pretreatment, including baseline subtraction of spectra, normalization of spectra, internal peak alignment using prominent peaks, and a peak picking procedure. The pretreated data were then used for visualization and statistical analysis in ClinPro Tools. Statistically significant different quantities of peptides were determined by means of Wilcoxon/Kruskal–Wallis (W/KW) test. ClinPro Tools support three kinds of algorithms for generating classification models. These algorithms are different in their methodology. They include Genetic Algorithm (GA) and Supervised Neural Network (SNN) and Quick Classifier (QC) algorithms. Class prediction model between each two groups was set up by the algorithm with the best recognition capability, cross-validation, and external validation implemented by the ClinPro Tools software V.03.

While other data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Qualitative data were described using number and percentage. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Chi-square test for categorical variables to compare between different groups and Monte Carlo correction for chi-square when more than 20% of the cells have expected count less than 5. *F*-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and Tukey's post-hoc test for pairwise comparisons.

Kruskal–Wallis test for abnormally distributed quantitative variables to compare between more than two studied groups, and Dunn's multiple comparisons post-hoc test for pairwise comparisons. Significance of the obtained results was judged at the 5% level.

4. Results

4.1. Baseline characteristics of patients

The study included 75 subjects (40 male, 35 female), with mean age (47.04 ± 10.33) in Group I, (46.72 ± 10.6)

in Group II, and (42.9 ± 11.89) in Group III. There was no statistically significant difference between different groups regarding age and sex. Clinical and laboratory data of the two patients and the control group.

The three groups were comparable regarding the baseline characteristics and routine laboratory investigations with no statistically significant difference except for the renal functions which is the differentiating parameter between groups (Table 1).

4.2. Proteomic analysis

A comparative case control analysis between CAN, TN patients and healthy subjects was performed. Using GA analysis, classification models were developed to classify

Table 1. Comparison between the three studied groups according to demographic, clinical and laboratory data.

	Group I (n = 25)		Group II (n = 25)		Group III (n = 25)			
	No.	%	No.	%	No.	%	Test of Sig.	P
Sex								
Male	15	60.0	13	52.0	12	48.0	χ^2 = 0.750	^{Mc}p = 0.687
Female	10	40.0	12	48.0	13	52.0		
Age (years)								
Min.–Max.	25–60		20–59		26–61		F = 1.067	0.349
Mean ± SD	47.04 ± 10.33		46.72 ± 10.6		42.9 ± 11.89			
Weight (kg)								
Min.–Max.	60.0–146.0		60.0–130.0		60.0–117.0		F = 1.073	0.345
Mean ± SD	84.36 ± 15.98		86.78 ± 16.34		81.90 ± 11.96			
Height (cm)								
Min.–Max.	153.0–186.0		156.0–192.0		158.0–1920.0		F = 1.463	0.236
Mean ± SD	168.0 ± 9.31		168.9 ± 8.81		171.2 ± 7.33			
BMI (kg/m$^{2)}$								
Min.–Max.	20.0–45.0		21.0–45.0		20.0–37.0		F = 2.958	0.056
Mean ± SD	29.98 ± 5.77		30.26 ± 5.53		27.76 ± 3.53			
WC (cm)								
Min.–Max.	80.0–140.0		71.0–133.0		80.0–120.0		F = 2.962	0.056
Mean ± SD	99.33 ± 11.40		102.1 ± 11.57		96.38 ± 8.45			
Hemoglobin (g/dl)								
Min.–Max.	8.40–12.0		12.0–17.0		12.0–15.80		F = 144.540*	<0.001*
Mean ± SD	9.60 ± 0.86		14.70 ± 1.40		13.87 ± 1.08			
Sig. bet Grps			p_1 = 0.031*, p_2 < 0.001*, p_3 < 0.001*					
WBCs (cells/mm^3)								
Min.–Max.	4.20–9.20		4.0–10.0		4.20–10.0		F = 0.421	0.658
Mean ± SD	7.10 ± 1.44		7.28 ± 1.63		6.86 ± 1.74			
Platelets (× 10^3/UL)								
Min.–Max.	198.0–418.0		221.0–490.0		204.0–421.0		F = 0.352	0.704
Mean ± SD	323.0 ± 62.51		329.7 ± 63.67		314.6 ± 65.68			
Urea (mg/dl)							F = 582.994*	<0.001*
Min.–Max.	65.0–102.0		19.0–40.0		20.0–38.0			
Mean ± SD	83.08 ± 9.38		30.68 ± 4.66		26.16 ± 4.36			
Sig. bet Grps			p_1 < 0.001*, p_2 < 0.001*, p_3 = 0.045*					
Creatinine (mg/dl)								
Min.–Max.	2.0–4.30		0.60–1.0		0.60–1.0		F = 261.106*	<0.001*
Mean ± SD	2.89 ± 0.61		0.84 ± 0.12		0.84 ± 0.12			
Sig. bet Grps			p_1 < 0.001*, p_2 < 0.001*, p_3 = 1.000					
eGFR-EPI (%)								
Min.–Max.	17.0–44.0		61.0–135.0		61.0–125.8		F = 3.004	0.053
Mean ± SD	187.9 ± 15.42		94.86 ± 19.48		94.61 ± 14.31			
Total cholesterol (mg/dl)								
Min.–Max.	75.0–236.0		106.0–285.0		113.0–272.0		F = 0.323	0.725
Mean ± SD	149.1 ± 34.35		192.9 ± 42.92		187.2 ± 37.68			
Triglycerides (mg/dl mmol/L)								
Min.–Max.	82.0–129.0		43.0–130.0		48.0–110.0		F = 1.604	0.208
Mean ± SD	101.9 ± 9.66		111.0 ± 22.0		103.0 ± 24.0			
HDL-C (mmol/L)								
Min.–Max.	31.0–72.0		26.0–74.0		24.0–63.0		F = 2.910	0.061
Mean ± SD	51.30 ± 11.12		44.63 ± 9.93		46.59 ± 8.98			
LDL-C (mmol/L)								
Min.–Max.	73.0–132.0		51.80–201.0		60.0–174.0		F = 1.123	0.329
Mean ± SD	104.7 ± 13.94		114.5 ± 39.27		108.9 ± 29.68			

Group I (CAN group, chronic allograft nephropathy group), Group II (TN group, transplant with normal renal functions group), and Group III (C group, control group).

FPG: fasting plasma glucose, eGFR-EPI: estimated glumerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration equation, HDL-C: high-density lipoprotein-cholesterol, LDL: low-density lipoprotein-cholesterol.

BMI: body mass index; WC: waist circumference.

χ^2, p: χ^2 and p values for Chi square test for comparing between the three groups.

^{MC}p: p value for Monte Carlo for Chi square test for comparing between the three groups.

Numbers with common letters are not significant (numbers with different letters are significant).

F, p: F and p values for ANOVA test, Sig. bet. grps was done using post-hoc test (LSD).

H, p: H and p values for Kruskal–Wallis test, Sig. bet. grps was done using post-hoc test (Dunn's multiple comparisons test).

p_1: p value for comparing between Group I and Group II p_2: p value for comparing between Group I and Group III.

p_3: p value for comparing between Group II and Group III*: Statistically significant at $p \leq 0.05$.

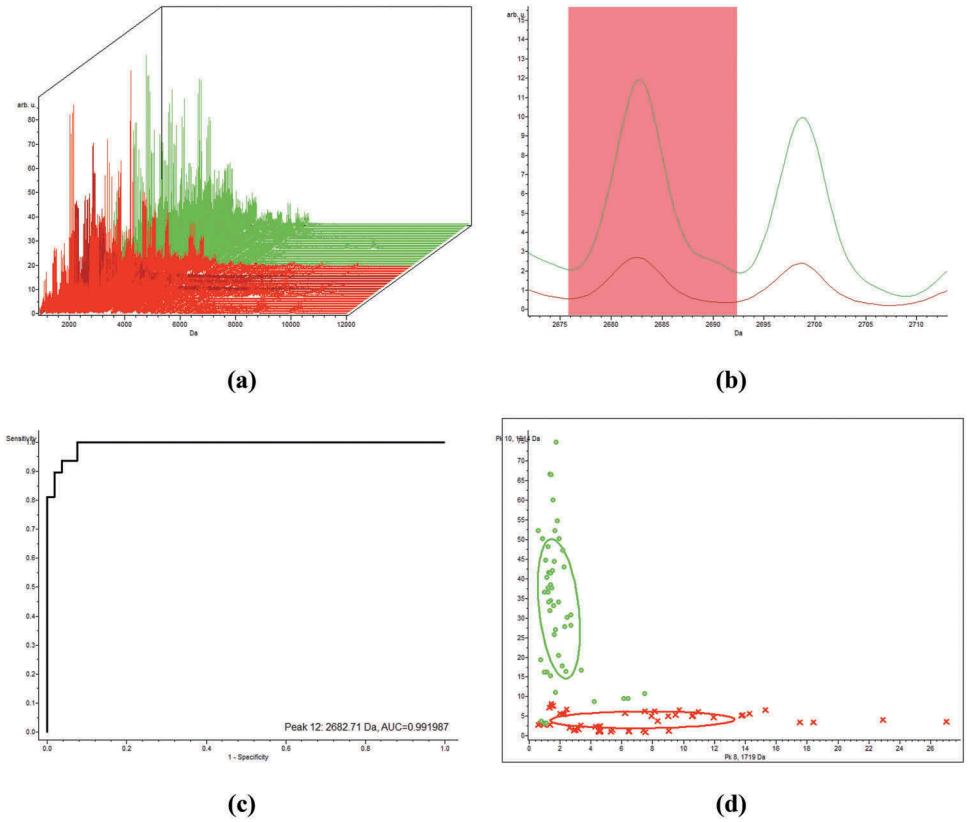

Figure 1. CAN (red color) against control (green color). (a) Spectra view of CAN against control. (b) Spectra view of peak 12 at the mass 2683. (c) ROC curve peak 12 with *m/z* 2683 and AUC = 0.991. (d) 2D distribution of peaks (12, 124 with *m/z* (2683 and 4326 Da).

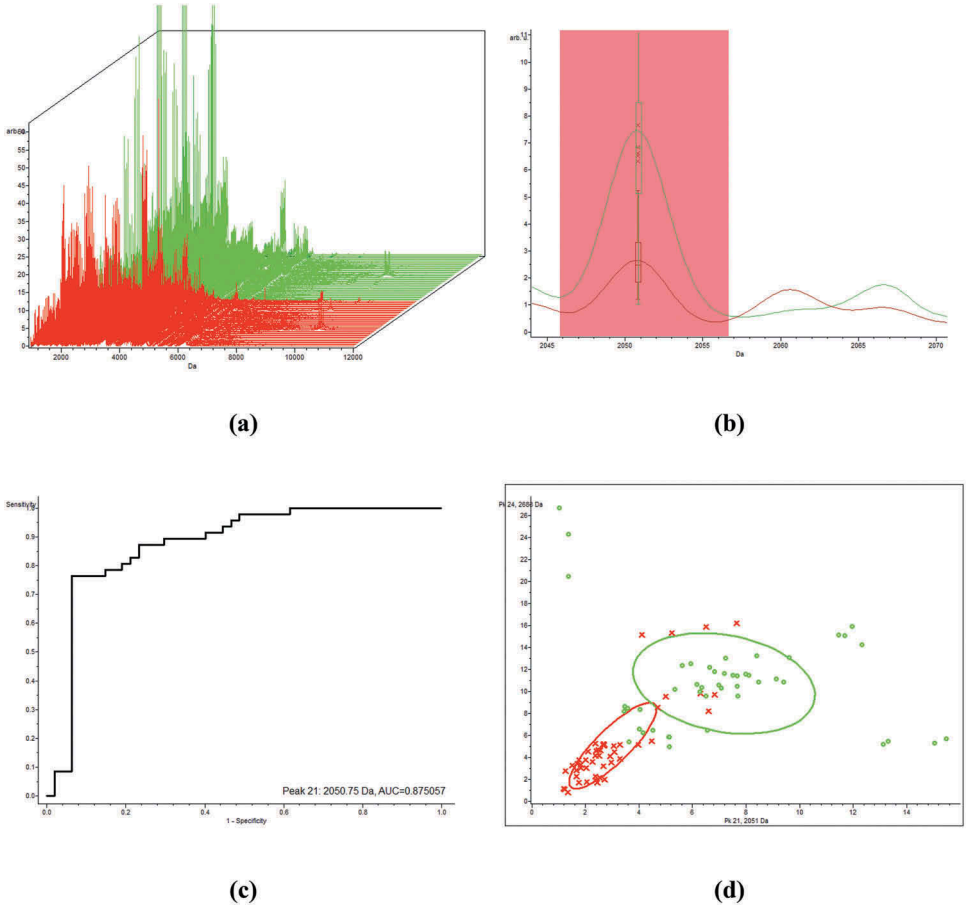

Figure 2. TN (red color) against control (green color). (a) Spectra view of TN against control. (b) Spectra view of peptide peak 21 at the mass 2050. (c) ROC curve of peak 21 with *m/z* 2050 and AUC = 0.875. (d) 2D distribution of peaks (24, 21) with *m/z* (2688 and 2051 Da).

samples between CAN, TN, and normal controls. The blinded verification of the GA classification model proved the correct classification of the CAN patients, TN patients and of the healthy volunteers.

Peptidomic maps associated with the disease were drawn. The present study showed that:

In Group I (CAN vs. controls), proteomic profile of five peaks with masses (2682.71, 1913.8, 4326.47, 1718.58, 6177.84) was generated with GA could discriminate between CAN patients and controls with recognition capability (RC) of 100%, cross-validation (XV) of 100%, sensitivity of 100%, and specificity of 91.7%. Two peaks (24, 8) were up-regulated and three peaks (12, 34, 10) were down-regulated in CAN patients in comparison to controls (Appendix A; Figure 1).

In Group II (TN vs. controls), proteomic profile of five peaks with masses (4659.02, 2237.65, 2050.75, 2682.82, 4371.49) was generated with GA could discriminate between TN cases and controls with a RC of 100%, sensitivity of 92.5%, specificity of 75%, and a XV of 96.8%. There were two up-regulated peaks (52, 22) and three down-regulated peaks (54, 21, 24) in transplanted normal (TN) in comparison to controls (Appendix B; Figure 2).

In Group III (CAN vs. TN patients), proteomic profile of five peaks with masses (1072.22, 3739.03, 3406.88, 2926.33, 10,053.93) was generated with GA could discriminate between CAN cases and TN cases. This model achieved RC of 98.9%, XV of 91.1%, and sensitivity of 64.4% and specificity of 61.7%. Two peaks (3, 23) were up-regulated and three peaks were down-regulated (31, 27, 77) in CAN patients in comparison to TN patients (Appendix C; Figure 3).

Table 2. Distribution of the studied cases according to different histopathological parameters in CAN group (n = 25).

	No.	%
Glomerular		
0	5	20.0
1	11	44.0
2	9	36.0
Interstitial fibrosis		
Mild	11	44.0
Moderate	10	40.0
Severe	4	16.0
Vascular		
Mild	18	72.0
Moderate	7	28.0
Severe	0	0.0

4.3. Histopathological results

CAN is a biopsy-based diagnosis and different pathological findings are summarized in Tables 2 and 3.

5. Discussion

Chronic rejection remains a major problem for transplanted patients, hence, the need for noninvasive diagnostic procedure for early diagnosis and therefore

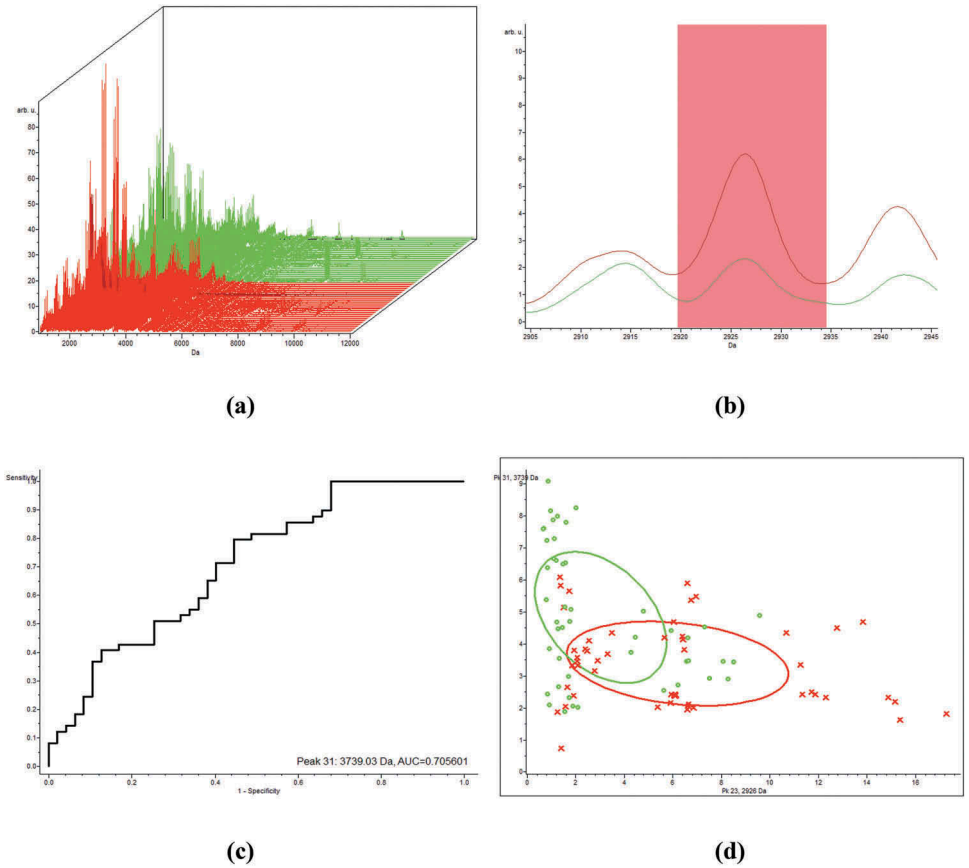

Figure 3. CAN (red color) against TN (green color). (a) Spectra view of CAN against TN. (b) Spectra view of Peak 23. (mass 2926 Da). (c) ROC curve for peak31 with m/z 3739.03 Da and area under the curve (AUC) = 0.705. (d) 2D distribution view of peak 23 and peak 31.

Table 3. Histopathological findings in CAN biopsies.

No.	Glomerular	Interstitial fibrosis	Vascular
1	Transplant glomerulopathy (cg2)	++	Mild fibrous intimal thickening (cv1)
2	Transplant glomerulopathy (cg1)	++	Mild fibrous intimal thickening (cv1)
3	Transplant glomerulopathy (cg2)	++	Moderate fibrous intimal thickening (cv2)
4	Transplant glomerulopathy (cg1)	++	Moderate fibrous intimal thickening (cv2)
5	Transplant glomerulopathy (cg0)	+	Mild fibrous intimal thickening (cv1)
6	Transplant glomerulopathy (cg0)	+	Mild fibrous intimal thickening (cv1)
7	Transplant glomerulopathy (cg1)	++	Moderate fibrous intimal thickening (cv2)
8	Transplant glomerulopathy (cg1)	+	Mild fibrous intimal thickening (cv1)
9	Transplant glomerulopathy (cg1)	+	Mild fibrous intimal thickening (cv1)
10	Transplant glomerulopathy (cg2)	+++	Mild fibrous intimal thickening (cv1)
11	Transplant glomerulopathy (cg0)	+	Mild fibrous intimal thickening (cv1)
12	Transplant glomerulopathy (cg1)	+	Mild fibrous intimal thickening (cv1)
13	Transplant glomerulopathy (cg2)	+++	Moderate fibrous intimal thickening (cv2)
14	Transplant glomerulopathy (cg1)	+	Mild fibrous intimal thickening (cv1)
15	Transplant glomerulopathy (cg2)	++	Mild fibrous intimal thickening (cv1)
16	Transplant glomerulopathy (cg1)	+	Mild fibrous intimal thickening (cv1)
17	Transplant glomerulopathy (cg2)	++	Mild fibrous intimal thickening (cv1)
18	Transplant glomerulopathy (cg1)	+	Moderate fibrous intimal thickening (cv2)
19	Transplant glomerulopathy (cg1)	++	Mild fibrous intimal thickening (cv1)
20	Transplant glomerulopathy (cg2)	+++	Moderate fibrous intimal thickening (cv2)
21	Transplant glomerulopathy (cg0)	+	Mild fibrous intimal thickening (cv1)
22	Transplant glomerulopathy (cg2)	++	Mild fibrous intimal thickening (cv1)
23	Transplant glomerulopathy (cg2)	+++	Moderate fibrous intimal thickening (cv2)
24	Transplant glomerulopathy (cg0)	+	Mild fibrous intimal thickening (cv1)
25	Transplant glomerulopathy (cg1)	++	Mild fibrous intimal thickening (cv1)

+ = Mild interstitial fibrosis.
++ = Moderate interstitial fibrosis.
+++ = Severe interstitial fibrosis.

early intervention that may prevent more deterioration of renal functions.

To the best of our knowledge, this is the first proteomic study using urinary profiling with MALDI-TOF-MS in field of transplantation in Egypt. CAN is still a major complication in transplanted patients and is the leading cause of graft rejection. This is most likely due to the complexity of pathogenic mechanisms of CAN and the difficulties in its early detection. The multifactorial nature of CAN supports the use of combined markers as a diagnostic tool. Therefore, a current research priority is to identify more sensitive and specific biomarkers for the early detection of CAN.

The present study aimed to determine serum peptidome patterns for early diagnosis of CAN. Our aim was to detect new biomarkers potentially involved in CAN pathophysiology and which might be useful for CAN diagnosis and management

In the current study, Peak 2682 was repeated in Group I–III. This mass is believed to be ubiquitin C-terminal hydrolase L1 protein [14], this protein was found to be expressed in human kidney podocytes; it was found that it is important for protein degradation in the human kidney, so it may have a role in pathogenesis of graft dysfunction, it needs further studies to prove this relation [15].

In our study, we use the urine of transplanted patients for studying the proteomic profile, Different from our study, Kurian et al. [16], used the serum of transplanted patients for proteomic analysis, this study identifies several unique signatures of transcript and protein biomarkers with high predictive values for mild and moderate/severe CAN. A consensus analysis reveals 393 (mild) and 63 (moderate/severe) final candidates as CAN markers with predictive accuracy of 80% (mild) and 92% (moderate/severe). Proteomic profiles show over 500 candidates each, for both stages of CAN including 302 proteins unique to mild and 509 unique to moderate/severe CAN.

In our study, we use MALDI-TOF MS for proteomic profiling, using other proteomic technique for detecting subclinical rejection (SCR), Mao et al. [17], analyzed total of 73 urine samples by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) combined with bioinformatics tools. The diagnostic pattern comprised of four biomarkers could differentiate SCR group from stable group with sensitivity of 81.5% and specificity of 81.8%. The remaining 14 samples from stable group and 10 samples from SCR were analyzed on the second day as an independent test set. The independent tests yielded a specificity of 71.4% and sensitivity of 90%.

6. Conclusion

Potential biomarkers for early chronic rejection can be detected using urinary proteomic profile.

Disclosure statement

The authors declare that they have no conflicts of interest.

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Appendix A. Differences of the serum proteome profiles of CAN group in comparison to C group

S	Index	Mass	Dave	PTTA	PWKW	PAD	Ave1	Ave2	StdDev1	StdDev2	CV1	CV2
X	14	3268.66	8.33	<0.000001	<0.000001	0.00000571	4.48	12.81	2.3	2.54	51.36	19.85
X	11	2050.84	6.14	<0.000001	<0.000001	0.0000018	2.62	8.76	1.05	2.83	40.19	32.25
X	12	2682.71	9.16	<0.000001	<0.000001	<0.000001	3.54	12.7	1.71	3.65	48.3	28.72
X	19	3460.72	15.95	<0.000001	<0.000001	0.00000455	7.34	23.29	4.48	5.84	61.11	25.08
X	18	3444.75	9.91	<0.000001	<0.000001	0.00000108	6.17	16.08	3.26	2.75	52.88	17.08
X	10	1913.8	31.38	<0.000001	<0.000001	<0.000001	4.58	35.96	2.37	19.71	51.67	54.83
X	32	5850.4	1.26	0.0000119	<0.000001	<0.000001	1.72	0.46	1.7	0.22	98.86	48.91
X	22	4294.81	5.16	<0.000001	<0.000001	0.405	5.42	10.58	2.28	2.09	42.1	19.72
X	15	3284.71	7.77	<0.000001	<0.000001	0.0117	5.44	13.22	2.9	3.7	53.31	28
X	9	1831.73	11.81	<0.000001	<0.000001	<0.000001	13.35	1.54	13.79	1.06	103.29	68.67
X	43	8073.29	0.44	0.000206	<0.000001	<0.000001	0.61	0.17	0.74	0.04	120.85	24.18
X	46	8675.14	0.28	<0.000001	<0.000001	<0.000001	0.41	0.13	0.29	0.03	71.15	23.14
X	17	3407.48	7.28	<0.000001	<0.000001	0.0607	5.87	13.15	3.32	3.45	56.48	26.22
X	52	10,540.56	0.19	0.00000227	<0.000001	<0.000001	0.27	0.07	0.24	0.03	88.87	35.5
X	36	7514.95	0.41	0.0000771	<0.000001	<0.000001	0.6	0.2	0.62	0.05	102.97	24.64
X	16	3389.64	9.55	<0.000001	<0.000001	0.00702	6.44	15.99	4.56	5.08	70.87	31.74
X	47	8788.29	2.11	0.0000135	<0.000001	<0.000001	2.33	0.22	2.87	0.33	123.38	150.42
X	51	10,068.48	0.95	<0.000001	<0.000001	<0.000001	1.06	0.11	1.05	0.1	99.41	89.98
X	37	7577.37	0.48	0.000313	<0.000001	<0.000001	0.67	0.19	0.83	0.04	123.92	21.94
X	45	8660.09	0.36	0.00413	<0.000001	<0.000001	0.48	0.13	0.81	0.03	167.1	26.6
X	20	3724.46	4.17	<0.000001	<0.000001	0.000992	4.48	8.65	2.07	3.03	46.2	35.07
X	38	7655.58	0.76	0.00148	<0.000001	<0.000001	0.96	0.2	1.53	0.07	159.35	34.15
X	34	6177.84	3.09	<0.000001	<0.000001	<0.000001	0.75	3.84	0.32	2.87	42.8	74.68
X	35	6193.07	4.74	<0.000001	<0.000001	<0.000001	2	6.73	2.08	3.54	104.37	52.6
X	39	7756.33	0.68	0.00121	<0.000001	<0.000001	0.87	0.19	1.33	0.04	153.33	22.62
X	24	4326.47	5.39	<0.000001	<0.000001	<0.000001	7.81	2.42	4.96	1.12	63.54	46.29
X	13	2755.91	17	0.00000128	<0.000001	<0.000001	22.13	5.13	19.66	7.4	88.84	144.19
X	44	8314.4	0.33	0.00561	<0.000001	<0.000001	0.46	0.13	0.77	0.04	166.25	28.43
X	49	9843.08	0.24	0.00017	<0.000001	<0.000001	0.37	0.13	0.38	0.11	103.27	82.25
X	7	1525.56	4.11	<0.000001	<0.000001	<0.000001	4.09	8.2	1.9	3.96	46.39	48.34
X	8	1718.58	6.01	<0.000001	<0.000001	<0.000001	8.17	2.15	6.62	1.52	81.05	70.46
X	40	7907.64	0.39	0.00223	<0.000001	<0.000001	0.56	0.17	0.82	0.04	147	25.88
X	31	5233.37	1.47	<0.000001	<0.000001	0.000258	1.79	3.26	1.11	1.18	61.88	36.16
X	50	9942.6	0.24	0.000087	<0.000001	<0.000001	0.35	0.11	0.37	0.06	105.73	55.43
X	41	7972.51	0.39	0.0000868	<0.000001	<0.000001	0.56	0.17	0.61	0.04	108.24	25.69
X	42	7988.1	0.4	0.00125	0.00000107	<0.000001	0.57	0.17	0.79	0.05	137.45	30.08
X	30	5217.4	0.92	0.0000206	0.00000129	<0.000001	1.86	2.78	0.93	1.05	49.97	37.92
X	26	4821.28	2.77	0.000016	0.0000051	<0.000001	5.22	2.45	3.74	1.14	71.69	46.6
X	27	4836.58	2.36	0.00000395	0.00000531	0.00000152	4.9	2.53	2.87	1.2	58.71	47.52
X	48	9750.26	0.1	0.397	0.0000188	<0.000001	0.24	0.34	0.22	0.8	91.08	237.25
X	33	6130.76	0.37	0.00000623	0.0000267	0.00000115	0.68	1.05	0.25	0.46	36.29	43.61
X	6	1442.01	3.81	0.00017	0.0000508	<0.000001	9.81	5.99	5.58	3.4	56.95	56.69
X	2	1072.37	2.41	0.00017	0.000149	<0.000001	5.62	3.21	3.55	2.12	63.14	65.93
X	23	4310.57	2.02	0.000272	0.000267	0.126	6.49	8.51	2.98	2.07	45.84	24.34
X	4	1369.74	4.6	0.137	0.00213	<0.000001	8.25	3.65	20.57	1.91	249.21	52.28
X	21	4102.54	1.74	0.000383	0.00245	0.00784	5.15	6.88	2.09	2.51	40.71	36.48
X	29	4867.8	0.83	0.000592	0.00308	0.0473	2.38	3.21	1.21	1.05	50.92	32.68
X	25	4807.1	1.78	0.00155	0.00703	<0.000001	4.29	2.52	3.51	0.93	81.65	36.82
X	3	1082.8	1.89	0.0262	0.0145	<0.000001	5.74	3.85	4.71	3.34	81.96	86.77
X	1	1066.8	2.42	0.0899	0.0461	<0.000001	9.19	6.78	7.33	6.37	79.74	93.99
X	28	4852.36	0.26	0.413	0.27	0.401	3.31	3.06	1.77	1.26	53.59	41.29
X	5	1427.18	0.65	0.387	0.569	0.0012	7.36	8.01	3.42	3.82	46.41	47.63

Appendix B. Differences of the serum proteome profiles of TN group in comparison to C group

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	StdDev1	StdDev2	CV1	CV2
X	8	1219.43	5.87	<0.000001	<0.000001	<0.000001	7.57	1.69	4.74	0.99	62.65	58.35
X	23	2665.12	5.13	<0.000001	<0.000001	0.0000799	4.4	9.53	3.38	3.91	76.83	41.06
X	24	2682.82	6.29	<0.000001	<0.000001	0.0000953	5.66	11.95	4.35	5.08	76.85	42.55
X	21	2050.75	4.61	<0.000001	<0.000001	0.00000119	3.36	7.97	1.81	3.77	53.8	47.3
X	32	3389.59	6.39	<0.000001	<0.000001	0.132	8.09	14.48	3.78	5.13	46.75	35.42
X	35	3444.71	5.18	<0.000001	<0.000001	0.000591	8.85	14.03	4.02	4.06	45.44	28.93
X	33	3407.27	4.74	<0.000001	<0.000001	0.0101	7.75	12.49	3.59	3.75	46.35	30.02
X	69	5836	0.47	0.0000027	<0.000001	<0.000001	0.97	0.5	0.51	0.23	53.06	45.04
X	25	2698.72	4.26	0.000147	<0.000001	<0.000001	5.41	9.66	5.21	4.05	96.38	41.88
X	7	1127.19	3.14	<0.000001	<0.000001	0.00000146	5.88	2.75	3.11	1.3	52.88	47.23
X	76	6697.5	0.12	<0.000001	<0.000001	0.00113	0.4	0.28	0.11	0.07	27.48	23.48
X	20	1913.76	20.22	<0.000001	0.00000137	<0.000001	7.84	28.06	3.37	20.38	42.96	72.64
X	36	3460.69	7.65	0.00000197	0.00000202	0.00291	12.65	20.31	6.23	7.03	49.23	34.6
X	29	3252.56	2.89	0.00000101	0.00000425	0.00355	5.18	8.07	2.23	2.61	43.09	32.41
X	70	5852.83	0.41	0.0000186	0.00000564	<0.000001	0.95	0.54	0.47	0.28	50.11	52.76
X	37	3475.42	2.04	0.00000966	0.0000101	0.00592	4.04	6.08	1.93	1.9	47.67	31.26
X	52	4371.49	1.62	0.00439	0.0000237	<0.000001	3.44	1.82	2.86	1.85	83.02	101.61
X	34	3427.41	2.56	0.0000294	0.0000423	<0.000001	5.65	8.21	2.74	2.36	48.45	28.72
X	65	5233.39	0.89	0.015	0.0000433	0.00034	2.56	3.45	1.86	1.16	72.58	33.79
X	54	4659.02	1.51	0.0000163	0.0000605	0.000231	2.17	3.68	1.18	1.67	54.2	45.49
X	49	4294.79	2.55	0.000536	0.000067	0.374	6.91	9.46	3.37	2.79	48.83	29.45
X	5	1082.77	2.25	0.00024	0.0000832	<0.000001	5.05	2.8	3.04	1.9	60.13	67.81
X	77	6712.72	0.1	0.0000726	0.000148	0.0000605	0.39	0.29	0.13	0.08	33.38	27.33
X	55	4751.72	0.68	0.0934	0.000158	<0.000001	1.4	2.08	1.53	1.82	109.29	87.41
X	74	6193.08	2.02	0.0898	0.000386	<0.000001	4.75	6.77	5.57	4.17	117.09	61.55
X	28	3015.19	2.5	0.000856	0.000424	0.0141	5.83	8.32	3.27	3.03	56.11	36.37
X	88	9188.38	0.2	0.0347	0.000515	<0.000001	0.32	0.12	0.56	0.03	174.96	29.24
X	68	5796.52	0.17	0.00106	0.000604	0.00058	0.73	0.57	0.25	0.18	33.49	31.68
X	61	4896.52	1.06	0.000259	0.000662	<0.000001	2.37	1.31	1.56	0.6	65.77	46.13
X	66	5248.13	0.25	0.00588	0.000662	0.0152	1.08	1.33	0.32	0.45	29.87	33.83
X	51	4326.3	4.11	0.000856	0.000775	<0.000001	6.89	2.78	6.9	1.61	100.09	58.06
X	97	10,053.89	0.67	0.047	0.00103	<0.000001	0.79	0.12	1.99	0.08	252.28	65.04
X	2	1050.78	2.11	0.0021	0.00123	<0.000001	4.66	2.55	3.71	1.6	79.74	62.78
X	30	3268.53	3.09	0.000407	0.00123	0.0000478	7.77	10.86	4.43	2.59	57.05	23.85
X	73	6178.05	3.16	0.00632	0.00139	<0.000001	1.25	4.41	0.96	6.76	76.78	153.35
X	63	5048.19	0.45	0.00221	0.00166	0.00321	1.36	1.81	0.42	0.77	31.09	42.51
X	48	4279.46	0.47	0.0105	0.00172	0.0857	2.17	2.65	0.81	0.77	37.24	29.25
X	3	1066.8	5.11	0.00136	0.00188	<0.000001	10.68	5.57	8.38	4.42	78.41	79.3
X	64	5217.46	0.63	0.00398	0.00188	0.578	2.17	2.81	0.93	0.93	42.96	33.23
X	12	1442.01	4.5	0.000276	0.0019	<0.000001	10.57	6.07	6.66	2.62	63.05	43.25
X	81	8523.71	0.03	0.0447	0.0019	<0.000001	0.18	0.15	0.06	0.07	34.47	47.61
X	96	10,013.72	0.03	0.262	0.00202	<0.000001	0.15	0.11	0.13	0.11	88.11	97.58
X	11	1427.18	2.99	0.000959	0.00277	0.000433	9.69	6.71	4.6	2.76	47.42	41.19
X	99	10,525.22	0.04	0.0045	0.0043	<0.000001	0.13	0.09	0.08	0.03	61.21	31.44
X	46	4240.7	1.02	0.00262	0.00432	<0.000001	3.25	2.23	1.79	0.93	54.98	41.76
X	78	7276.17	0.19	0.229	0.00457	0	0.39	0.21	0.9	0.05	228.94	22.76
X	90	9525.18	0.09	0.284	0.00788	0	0.2	0.11	0.5	0.03	252.72	30.42
X	80	8377.58	0.05	0.00484	0.0106	<0.000001	0.2	0.15	0.1	0.04	48.41	30.33
X	9	1277.81	2.37	0.00538	0.0156	<0.000001	5.56	3.19	4.6	2.17	82.73	67.85
X	82	8660.51	0.09	0.0216	0.016	<0.000001	0.22	0.14	0.22	0.03	100.02	24.87
X	10	1369.65	0.93	0.0113	0.0184	0.009	3.73	2.8	1.7	1.4	45.72	50.17
X	98	10,182.92	0.05	0.0286	0.0185	<0.000001	0.14	0.09	0.13	0.02	91.86	24.83
X	31	3284.62	2.39	0.0447	0.0194	0.494	9.31	11.7	5.63	4.34	60.48	37.12
X	27	2766.75	3.55	0.172	0.0217	<0.000001	4.43	7.98	2.58	14.64	58.35	183.37
X	16	1718.57	4.76	0.000959	0.0228	<0.000001	6.73	1.98	8.23	0.71	122.24	36.11
X	87	9076.26	0.02	0.72	0.0249	<0.000001	0.19	0.18	0.16	0.2	82.04	110.78
X	26	2756.1	6.15	0.00262	0.0359	<0.000001	9.92	3.77	11.73	2.33	118.25	61.87
X	22	2237.65	1.01	0.137	0.0359	0.000958	6.46	5.45	2.55	2.99	39.47	54.88
X	18	1831.72	7.86	0.00106	0.036	<0.000001	10.35	2.49	13.73	2.3	132.68	92.5
X	67	5590.51	0.1	0.591	0.0361	<0.000001	0.88	0.78	0.72	0.79	81.33	101.37
X	83	8773.04	0.48	0.0286	0.037	<0.000001	0.7	0.22	1.26	0.28	179.39	123.97
X	95	9940.66	0.06	0.244	0.0453	<0.000001	0.2	0.14	0.29	0.12	147.35	91.98
X	72	6131.12	0.17	0.327	0.0501	<0.000001	0.94	1.1	0.71	0.73	75.95	66.05
X	19	1882.63	14.85	0.117	0.0543	<0.000001	2.72	17.57	0.81	54.56	29.61	310.47
X	45	4174.62	0.57	0.211	0.0681	0.541	4.49	5.06	2.16	1.51	48.17	29.73
X	6	1088.72	1.14	0.013	0.0721	<0.000001	4.03	2.89	2.4	1.35	59.49	46.65
X	75	6337.43	0	0.999	0.0777	<0.000001	0.51	0.51	0.67	0.62	133.11	121.78
X	13	1509.28	0.3	0.229	0.0836	0.563	3.08	3.38	0.76	1.22	24.72	35.97
X	4	1072.3	1.85	0.015	0.0868	<0.000001	5.11	3.26	4.15	1.92	81.12	59.06
X	89	9509.58	0.06	0.249	0.0949	0	0.17	0.12	0.29	0.03	165.94	28.71
X	91	9709.14	0.04	0.257	0.141	<0.000001	0.13	0.16	0.03	0.19	25.15	118.42
X	94	9912.7	0.04	0.324	0.15	<0.000001	0.12	0.16	0.06	0.24	47.5	144.96
X	14	1525.59	0.93	0.189	0.188	0.157	6.57	7.5	2.73	3.07	41.54	41

(*Continued*)

(Continued).

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	StdDev1	StdDev2	CV1	CV2
X	60	4867.91	0.39	0.253	0.263	0.76	2.99	3.38	1.55	1.26	51.7	37.35
X	86	8934.87	0.02	0.306	0.263	<0.000001	0.16	0.14	0.06	0.06	40.78	44.84
X	53	4542.32	0.06	0.784	0.332	0.000221	1.9	1.84	0.71	0.99	37.55	53.77
X	40	3739.35	0.51	0.435	0.45	0.285	5.8	6.3	2.9	2.44	50.07	38.77
X	50	4310.59	0.45	0.578	0.464	0.0495	7.58	8.03	4	2.5	52.83	31.19
X	56	4807.69	0.14	0.662	0.469	0.0121	2.92	3.06	1.58	1.1	54.08	36.06
X	71	6078.54	0.03	0.534	0.478	0.00000383	0.59	0.56	0.23	0.23	38.04	40.93
X	42	4028.25	0.03	0.923	0.524	0.564	2.85	2.88	1.11	0.76	38.73	26.27
X	38	3707.15	0.23	0.59	0.539	0.000591	4.24	4	1.78	1.81	42	45.09
X	85	8873.05	0	0.997	0.56	<0.000001	0.19	0.19	0.1	0.11	52.93	59.19
X	47	4256.54	0.19	0.516	0.576	0.428	3.04	3.23	1.22	1.18	39.97	36.37
X	58	4836.48	0.48	0.18	0.591	0.00166	3.16	2.68	1.85	0.93	58.57	34.67
X	17	1769.51	0.61	0.294	0.65	0.0000011	3.8	4.41	2.01	2.78	52.82	63.12
X	41	4010.75	0.52	0.158	0.65	0.00000173	3.74	3.21	1.94	0.88	51.99	27.39
X	79	8183.23	0	0.994	0.65	0.000116	0.19	0.19	0.05	0.06	26.82	34.53
X	15	1682.44	1.21	0.175	0.683	<0.000001	3.75	4.96	2.48	4.52	66.14	91.12
X	39	3723.93	0.52	0.527	0.733	0.000231	7.49	6.97	3.65	3.09	48.74	44.27
X	62	5032.16	0.2	0.435	0.733	<0.000001	2.08	1.88	1.34	0.69	64.25	36.91
X	92	9751.1	0.44	0.0934	0.734	<0.000001	0.13	0.57	0.04	1.53	31.25	267.72
X	57	4821.32	0.37	0.299	0.734	<0.000001	3.12	2.74	1.85	1.09	59.32	39.74
X	44	4118.43	0.34	0.482	0.75	<0.000001	3.34	3	2.67	0.92	79.93	30.51
X	1	1002.15	5.71	0.154	0.85	<0.000001	1.79	7.5	0.93	22.85	51.99	304.56
X	84	8846.35	0.03	0.229	0.902	<0.000001	0.18	0.21	0.07	0.13	37.28	61.28
X	59	4852.37	0.14	0.705	0.929	0.0647	3.24	3.11	1.78	1.1	55.01	35.55
X	43	4102.62	0	0.999	0.968	0.283	6.44	6.44	3.11	1.92	48.3	29.78
X	93	9848.97	0.05	0.181	0.976	<0.000001	0.12	0.17	0.04	0.2	30.67	116.79

Appendix C. Differences of the serum proteome profiles of CAN group in comparison to TN group

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	StdDev1	StdDev2	CV1	CV2
X	56	6177.42	1	0.00000184	0.00000521	<0.000001	0.61	1.61	0.25	1.01	40.59	62.83
X	17	1913.55	6.31	0.00164	0.0000338	<0.000001	4.57	10.87	2.03	9.71	44.45	89.34
X	19	2237.66	2.5	0.00147	0.0000422	<0.000001	3.96	6.47	2.49	3.1	62.9	47.94
X	23	2926.33	3.53	0.00164	0.000132	<0.000001	6.89	3.37	5.08	3.01	73.68	89.22
X	57	6192.7	2.85	0.00669	0.00107	<0.000001	1.76	4.61	1.85	4.97	104.9	107.82
X	64	7756.85	0.46	0.0454	0.00215	<0.000001	0.76	0.31	1.25	0.32	163.1	104.38
X	67	8073.46	0.3	0.0189	0.00215	<0.000001	0.55	0.24	0.71	0.09	130.09	38.26
X	78	10,067.17	0.65	0.00779	0.00273	<0.000001	0.95	0.3	1.23	0.47	129.17	155.8
X	62	7630.59	0.23	0.0738	0.00273	<0.000001	0.6	0.37	0.6	0.43	99.79	116.05
X	31	3739.03	1.6	0.00172	0.00309	0.000122	3.76	5.36	1.46	2.27	38.83	42.4
X	47	5031.95	0.49	0.0738	0.00309	0.0000152	2.52	2.03	0.97	1.25	38.33	61.58
X	63	7655.32	0.45	0.093	0.00309	<0.000001	0.92	0.46	1.45	0.64	158.43	138.73
X	50	5512.62	0.49	0.0152	0.00312	<0.000001	1.52	1.03	0.94	0.6	61.75	57.87
X	55	6129.35	0.34	0.00779	0.00312	<0.000001	0.61	0.96	0.26	0.63	41.72	65.66
X	74	9845	0.1	0.0142	0.00312	<0.000001	0.23	0.13	0.22	0.04	93.61	30
X	54	5851.95	0.36	0.0132	0.00635	0.225	1.47	1.11	0.44	0.66	30.05	59.5
X	60	7515.28	0.28	0.0179	0.00739	<0.000001	0.6	0.32	0.62	0.22	103.51	67.14
X	42	4836.62	1.5	0.00694	0.00755	0.00543	4.61	3.11	2.34	1.78	50.75	57.11
X	41	4821.29	1.43	0.00957	0.00814	0.0000482	4.55	3.13	2.37	1.82	51.99	58.11
X	46	4919.44	0.93	0.0982	0.011	<0.000001	2.81	1.88	2.96	1.63	105.21	86.99
X	37	4326.37	2.47	0.0842	0.0116	<0.000001	9.38	6.91	5.37	6.35	57.24	91.85
X	73	8788.05	1.23	0.0119	0.0129	<0.000001	1.82	0.6	2.43	0.99	133.3	166.46
X	68	8313.6	0.24	0.0615	0.0153	<0.000001	0.42	0.18	0.73	0.06	174.55	31.19
X	29	3460.53	2.58	0.0393	0.0177	0.0000349	8.18	10.77	4.6	5.25	56.19	48.75
X	66	7906.47	0.28	0.0454	0.0191	<0.000001	0.55	0.27	0.78	0.14	141.2	53.76
X	75	9941.4	0.15	0.0172	0.0201	<0.000001	0.3	0.15	0.35	0.09	114.56	57.84
X	24	3268.35	1.89	0.0172	0.029	<0.000001	4.94	6.83	2.24	3.74	45.34	54.71
X	25	3284.4	2.61	0.0122	0.0294	<0.000001	6.01	8.62	2.74	4.87	45.56	56.52
X	38	4372.36	1.29	0.147	0.0294	<0.000001	5.6	4.31	3.85	3.72	68.68	86.33
X	1	921.99	2.73	0.00694	0.0304	<0.000001	2.58	5.31	1	5.08	38.73	95.61
X	21	2698.53	2.23	0.0237	0.0356	<0.000001	3.35	5.58	1.71	5.21	51.22	93.31
X	71	8727.99	0.08	0.0842	0.0359	<0.000001	0.31	0.23	0.2	0.17	64.89	74.01
X	70	8659.64	0.23	0.0842	0.0403	<0.000001	0.46	0.23	0.76	0.16	165.45	70.29
X	76	10,009.06	0.05	0.0678	0.0407	<0.000001	0.21	0.16	0.13	0.11	60.09	70.57
X	51	5797.02	0.16	0.0454	0.0474	0.000377	0.96	0.79	0.35	0.3	36.46	38.12
X	26	3389.36	0.85	0.361	0.0499	0.0000168	6.81	7.66	4.58	2.88	67.26	37.57
X	20	2682.41	1.89	0.0203	0.0501	<0.000001	3.47	5.36	1.55	4.25	44.68	79.22
X	61	7577.68	0.4	0.11	0.0501	<0.000001	0.77	0.36	1.47	0.33	191.73	91.55
X	59	6420.89	0.42	0.0198	0.0615	<0.000001	0.89	0.47	0.94	0.37	106.29	79.96
X	49	5233.05	0.28	0.219	0.0646	0.0141	1.83	2.11	1.1	0.76	60.43	36.11
X	69	8524.7	0.02	0.482	0.0666	<0.000001	0.28	0.26	0.11	0.16	39.8	62.26
X	81	10,956.74	0.05	0.0521	0.07	<0.000001	0.17	0.11	0.15	0.06	88.34	56.14
X	4	1082.69	1.6	0.0454	0.0749	<0.000001	5.64	4.04	4.02	1.96	71.41	48.43
X	40	4806.95	0.75	0.0751	0.0759	0.0000465	3.72	2.97	1.85	1.57	49.88	52.93
X	45	4896.94	0.38	0.22	0.0797	0.0256	2.56	2.17	1.15	1.45	45	66.57
X	6	1219.34	2.91	0.0613	0.0912	<0.000001	5.91	8.82	4.64	7.46	78.61	84.55
X	7	1241.65	1.98	0.0198	0.0956	<0.000001	3.04	5.03	2.28	4.17	75.02	82.93
X	10	1409.79	4.07	0.0521	0.104	<0.000001	7.17	3.1	11.74	1.38	163.75	44.47
X	77	10,053.93	0.32	0.41	0.105	<0.000001	0.66	0.98	0.77	2.04	116.08	208.66
X	72	8773.09	0.9	0.254	0.119	<0.000001	1.15	2.04	1.46	4.32	127.34	211.48
X	30	3724.17	0.98	0.133	0.135	0.000506	5.72	6.7	2.67	2.84	46.68	42.41
X	80	10,525.58	0.01	0.854	0.139	<0.000001	0.17	0.18	0.1	0.19	55.67	103.62
X	27	3406.88	0.81	0.262	0.152	0.00443	6.31	7.12	3.4	2.66	53.92	37.35
X	2	1066.76	2.47	0.147	0.16	<0.000001	10.5	8.04	8.49	5.85	80.8	72.78
X	34	4174.12	0.68	0.145	0.16	<0.000001	3.24	3.93	1.75	2.16	53.83	55.06
X	43	4852.36	0.69	0.0919	0.177	0.0113	3.66	2.97	1.92	1.48	52.38	49.86
X	58	6405.16	0.11	0.606	0.195	<0.000001	0.65	0.77	0.5	1.08	76.57	141.51
X	65	7887.54	0.17	0.0935	0.206	<0.000001	0.46	0.29	0.53	0.26	116.5	88.15
X	8	1277.74	0.56	0.515	0.216	0.00000584	5.29	4.73	3.36	3.45	63.54	72.83
X	52	5814.78	0.22	0.117	0.216	0.00000131	1.18	0.96	0.67	0.51	56.47	52.67
X	79	10,396.8	0.02	0.522	0.218	<0.000001	0.15	0.17	0.08	0.16	52.61	95.29
X	13	1525.44	0.55	0.407	0.233	0.00000278	5.87	6.42	2.74	2.57	46.75	40.06
X	5	1127.13	0.33	0.609	0.255	0.0048	6.04	5.71	2.28	2.9	37.81	50.89
X	28	3444.67	0.89	0.262	0.278	0.00000382	6.56	7.45	3.3	3.45	50.38	46.26
X	53	5835.76	0.11	0.461	0.291	0.301	1.27	1.16	0.46	0.71	36.42	60.79
X	11	1426.69	6.36	0.0542	0.308	<0.000001	15.64	9.28	18.31	4.05	117.05	43.63
X	12	1441.95	0.7	0.572	0.342	0.0899	10.12	9.42	3.78	5.77	37.4	61.24
X	14	1682.28	1.01	0.0733	0.426	<0.000001	2.68	3.69	1.4	2.87	52.18	77.59
X	18	1944.96	1.41	0.0961	0.562	<0.000001	2.76	4.18	2.14	4.51	77.42	107.88
X	15	1718.51	0.57	0.719	0.575	<0.000001	5.33	5.9	4.95	6.64	92.77	112.65
X	3	1072.22	0.07	0.948	0.578	<0.000001	5.08	5.01	3.37	3.71	66.34	74.18
X	48	5217.22	0.01	0.983	0.721	0.000625	1.92	1.93	1.01	0.75	52.29	38.87
X	36	4310.47	0.24	0.772	0.764	0.000162	7.23	6.98	3.37	2.71	46.59	38.77

(*Continued*)

(Continued).

S	Index	Mass	DAve	PTTA	PWKW	PAD	Ave1	Ave2	StdDev1	StdDev2	CV1	CV2
X	16	1831.67	3.93	0.147	0.79	<0.000001	7.55	11.47	8.78	13.52	116.36	117.85
X	33	4157.86	1.64	0.262	0.79	<0.000001	4.59	2.94	8.33	2.76	181.69	93.91
X	22	2755.87	0.07	0.983	0.797	<0.000001	14.55	14.48	16.48	16.22	113.24	112.03
X	35	4294.73	0.09	0.906	0.799	0.137	5.88	5.97	2.53	2.34	43.06	39.16
X	9	1369.6	6.51	0.0869	0.87	<0.000001	9.87	3.35	22.22	1.55	225.25	46.16
X	39	4658.69	0.1	0.778	0.93	0.0991	2.78	2.69	1.25	1.27	44.73	47.12
X	44	4867.52	0.04	0.906	0.96	0.00518	2.63	2.67	1.3	1.18	49.59	44.01
X	32	4102.27	0.09	0.908	1	0.324	6.01	5.92	2.61	2.84	43.42	47.95