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APPENDICES

APPENDIX A

Buffers and Reagents

Serum Albumin depletion

(1) Monobasic dihydrogen phosphate (0.5 M KH_2PO_4)

6.81 g KH_2PO_4

Deionized water fill up to 100 mL

(2) Dibasic monohydrogen phosphate (0.5 M K_2HPO_4)

8.72 g K_2HPO_4

Deionized water fill up to 100 mL

(3) Binding buffer (50 mM potassium phosphate buffer, pH 7.0)

19.25 mL 0.5 M KH_2PO_4

30.75 mL 0.5 M K_2HPO_4

Deionized water fill up to 500 mL

(4) Elution buffer (50 mM potassium phosphate buffer, pH 7.0 with 1.5 M KCl)

22.37 g KCl

50 mM potassium phosphate buffer, pH 7.0 fill up to 200 mL

Serum immunoglobulin depletion

(1) Mono-sodium dihydrogen phosphate (0.2 M NaH2PO4)

6.0 g NaH_2PO_4

Deionized water fill up to 100 mL

(2) Di-sodium monohydrogen phosphate (0.2 M Na_2HPO_4)

7.1 g Na_2HPO_4

Deionized water fill up to 100 mL

(3) Binding buffer (20 mM sodium phosphate buffer, pH 7.0)

19.5 mL 0.2 M NaH_2PO_4

30.5 mL 0.2 M Na_2HPO_4

Deionized water fill up to 500 mL

(4) Elution buffer (0.1M glycin-HCl, pH 2.7)

2.2	g	Glycine
160	mL	Deionized water

Mix and adjust pH to 2.7 with HCl

Deionized water fill up to 200 mL

Protein determination (Lowry et al., 1951)

(1) Solution A (2% Na_2CO_3 in 0.1 M NaOH)

4.0	g	NaOH
20.0	g	Na_2CO_3

Distilled water fill up to 1 L

(2) Solution B (1% $CuSo_4$)

1.0	g	$CuSO_4$

Distilled water fill up to 100 mL

(3) Solution C (2% sodium potassium tartrate)

2.0	g	$NaKC_4H_4O_6.4(H_2O)$

Distilled water fill up to 100 mL

(4) Solution D

49	mL	Solution A
0.5	mL	Solution B
0.5	mL	Solution C

(5) Standard protein (1 mg/mL)

Procedure:

100	µl	Sample*
1	mL	Solution D

Mix well and leave at room temperature for 30 minutes

100	µl	1 N Folin phenol

Mix well and leave at room temperature for 30 minutes

Record absorbance at 650 nm

* Distilled water for blank tube

Standard protein (50-600 µg) for standard tube

Diluted serum fractions (2-8 µl) for test tube

Sample preparation

(1) Rehydration stock solution without IPG buffer (7 M urea, 2 M thiourea, 4% CHAPS)

10.5	g	Urea
3.8	g	Thiourea
1.0	g	CHAPS
a few grains		Bromophenol blue

Deionized water fill up to 25 mL (13.5 mL required)

Store in 750 µl aliquots at -20 °C

(2) DTT solution (1 M)

15.42	mg	DTT
Deionized water		100 µl

Store in 100 µl aliquots at -20 °C

(3) IPG buffer pH 4-7 Linear

Procedure:

A	µl	Sample (500 µg of protein)

Rehydration stock solution fill up to 330 µl

13.6	µl	1 M DTT

Mix well

1.8	µl	IPG buffer pH 4-7 Linear*

Mix well and leave on ice for 10 minutes before apply to strip holder

* Addition of the IPG buffer to the rehydration solution

IEF system: IPGphor

pH range of IPG strip: pH 4-7 L

Suggestion carrier ampholytes for rehydration solution: pH 4-7 L IPG buffer

Recommended concentration: 2% IPG buffer

Equilibration solution

(1) SDS equilibration buffer (6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% glycerol, 2% SDS)

72.1	g	Urea
10.0	mL	Tris-HCl, pH 8.8
69.0	mL	Glycerol
4.0	g	SDS
a few grains		Bromophenol blue

Distilled water fill up to 200 mL

Store in 15 mL aliquots at -20 °C

(2) Freshly prepare working solution 1

150	mg	DTT
15	mL	SDS equilibration buffer

(3) Freshly prepare working solution 2

375	mg	Iodoacetamide
15	mL	SDS equilibration buffer

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970)

(1) Monomer stock solution (30% T, 2.6% C Bis)

300	g	Acrylamide
8.0	g	Bis

Deionized water fill up to 1 L

(2) 4x resolving gel buffer solution

181.7 g Tris base

750 mL Deionized water

Mix and adjust pH to 8.8 by HCl

Deionized water fill up to 1 L

(3) 10% SDS

5.0 g SDS

Deionized water fill up to 50 mL

(4) Initiator (10% ammonium persulfate, APS) (freshly prepare)

0.1 g APS

1.0 mL Deionized water

(5) Resolving gel overlay (water saturated butanol)

50 mL n-Butanol

10 mL Deionized water

Vigorous shake and use the top phase to overlay gel

(6) 10x SDS electrophoresis buffer (250 mM Tris base, 1.92 M glycine, 1% SDS)

30.3 g Tris base

144.1 g Glycine

10.0 g SDS

Distilled water fill up to 1 L

(7) Upper chamber buffer (4x SDS electrophoresis buffer)

400 mL 10x SDS electrophoresis buffer

600 mL Distilled water

(8) Lower chamber buffer (1x SDS electrophoresis buffer)

100 mL 10x SDS electrophoresis buffer

900 mL Distilled water

(9) Agarose sealing solution

100	mL	1x SDS electrophoresis buffer
0.5	g	Agarose
a few grains		Bromophenol blue

Recipes for 2 homogenous 12% gel SDS-PAGE (20.5x25.5x0.1 cm^3/1 gel)

Step no.	Reagent/Procedure	Volume (mL)
1	Monomer stock solution	80
2	4x Resolving gel buffer pH 8.8	50
3	10% SDS	2
4	Deionized water	s u
5	Degassing with gentle stir for 30 minutes	
6	10% ammonium persulfate	1
7	TEMED	0.066
Total		200

Colloidal coomassie staining (Neuhoff et al., 1988)

(1) 5% Coomassie Blue G-250 stock (5% coomassie blue G-250)

0.5	g	Coomassie blue G-250

Distilled water fill up to 10 mL

(2) Colloidal coomassie blue G-250 dye stock solution (10% ammonium sulfate, 1% (w/w) phosphoric acid, 0.1% coomassie blue G-250)

50	g	Ammonium sulfate
6	mL	Phosphoric acid
10	mL	5% Coomassie blue G-250 stock

Distilled water fill up to 500 mL

(3) Colloidal coomassie blue G-250 working solution (8% ammonium sulfate, 0.8% phosphoric acid, 0.08% coomassie blue G-250, 20% methanol)

400 mL Colloidal coomassie blue G-250 dye stock solution

100 mL Methanol

Silver staining (Heukeshoven and Dernick, 1988)

(1) Fixative (40% ethanol, 10% acetic acid)

400 mL Ethanol

100 mL Acetic acid

Distilled water fill up to 1 L

(2) Sensitizing solution (30% ethanol, 6.8% sodium acetate, 0.2% sodium thiosulfate, 0.125% glutaraldehyde)

68.0 g Sodium acetate

2.0 g Sodium thiosulfate.$5H_2O$

300 mL Ethanol

Distilled water fill up to 1 L

2.5 mL 50% Glutaraldehyde

(3) Silver nitrate solution (0.25% silver nitrate, 0.015% formaldehyde)

2.5 g Silver nitrate

Distilled water fill up to 1 L

400 µl 37% Formaldehyde

(4) Developing solution (2.5% sodium carbonate, 0.0074% formaldehyde)

25 g Sodium carbonate

Distilled water fill up to 1 L

200 µl 37% Formaldehyde

(5) Stop solution (1.5% Na_2EDTA)

15 g Na_2EDTA

Distilled water fill up to 1 L

In gel digestion

(1) 100 mM ammonium bicarbonate (NH_4HCO_3)

0.4 g NH_4HCO_3

Steriled milli Q water fill up to 50 mL

(2) 50 mM NH_4HCO_3 / 50% Methanol

Combine 100 mM NH_4HCO_3 to absolue Methanol with ratio 1:1

(3) 20 mM NH_4HCO_3

3 mL 100 mM NH_4HCO_3

12 mL Steriled milli Q water

(4) 10 mM NH_4HCO_3

1.5 mL 100 mM NH_4HCO_3

13.5 mL Steriled milli Q water

(5) 10 mM DTT / 10 mM NH_4HCO_3

7 mg DTT

4.5 mL 10 mM NH_4HCO_3

(6) 100 mM IAA / 10 mM NH_4HCO_3

82.8 mg IAA

4.5 mL 10 mM NH_4HCO_3

(7) 50% acetronitrile / 10 mM NH_4HCO_3

Combine 20 mM NH_4HCO_3 to 100% acetronitril with ratio 1:1

(8) 10 ng Trypsin in 50% acetronitrile / 10 mM NH_4HCO_3

20	ug	Trypsin
2	mL	50% acetronitrile / 10 mM NH_4HCO_3

(9) 50% acetronitrile / 0.1% formic acid (FA)

15	ul	FA
7.5	mL	Acetronitrile
7.485	mL	Steriled milli Q water

(10) 0.1% FA

50	ul	FA
49.95	mL	Steriled milli Q water

(11) 30% acetronitrile

1.5	mL	100% Acetronitrile
3.5	mL	Steriled milli Q water

APPENDIX B

2D Gel Images

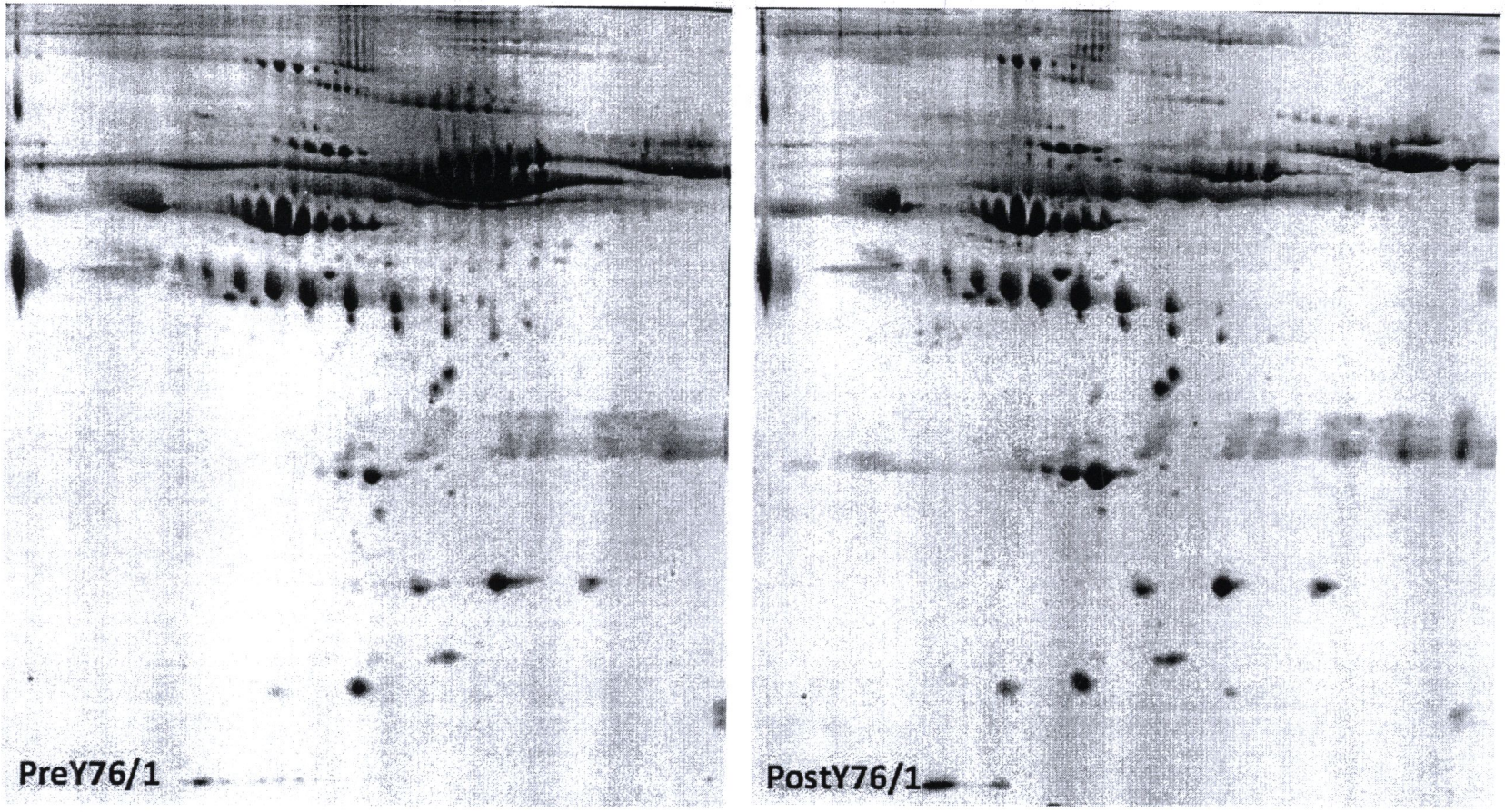

Figure 4-2a Coomassie stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #1 (left) and 2D gel of Y76 post-operative serum #1 (right).

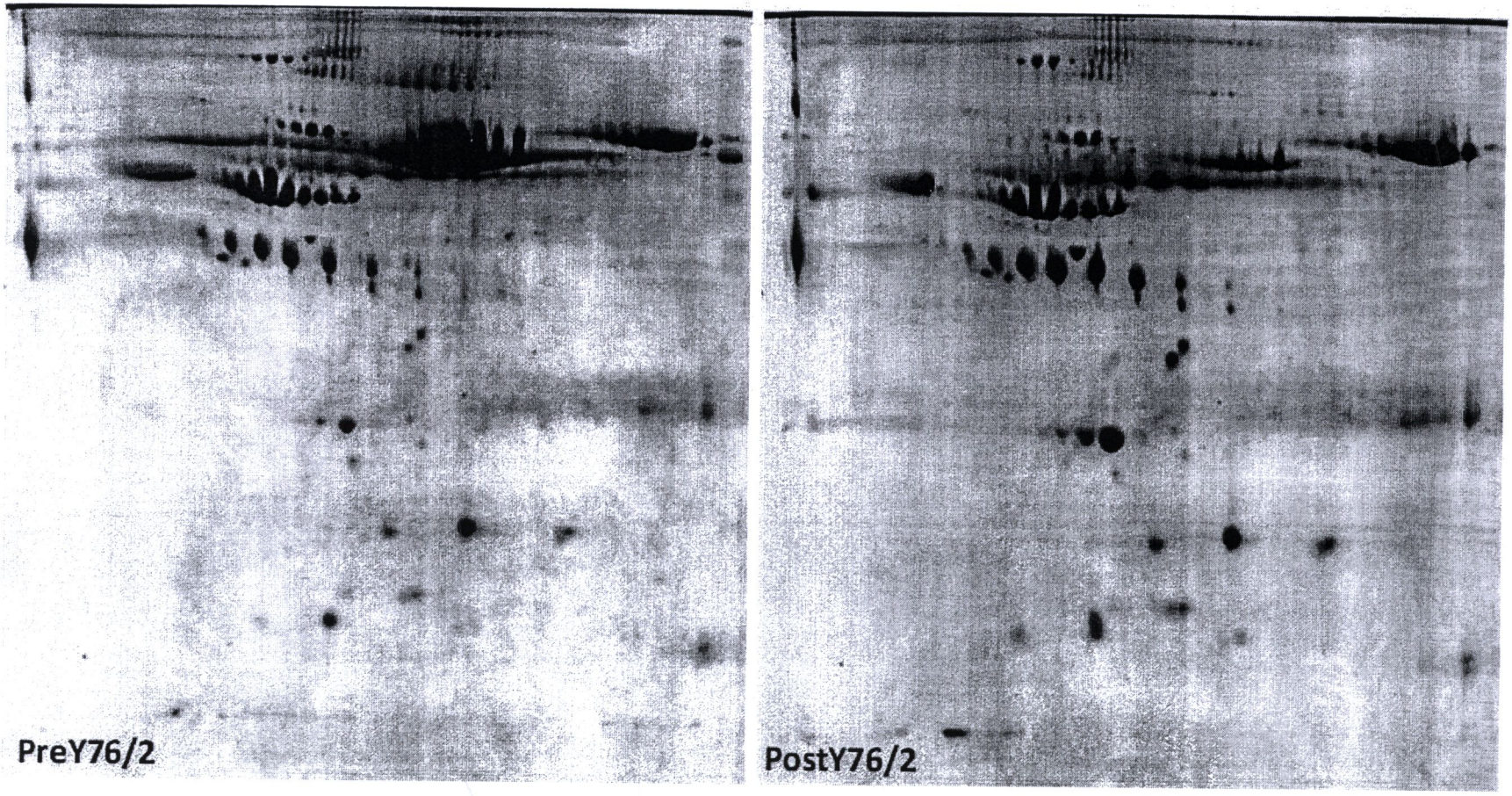

Figure 4-2b Coomassie stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #2 (left) and 2D gel of Y76 post-operative serum #2 (right).

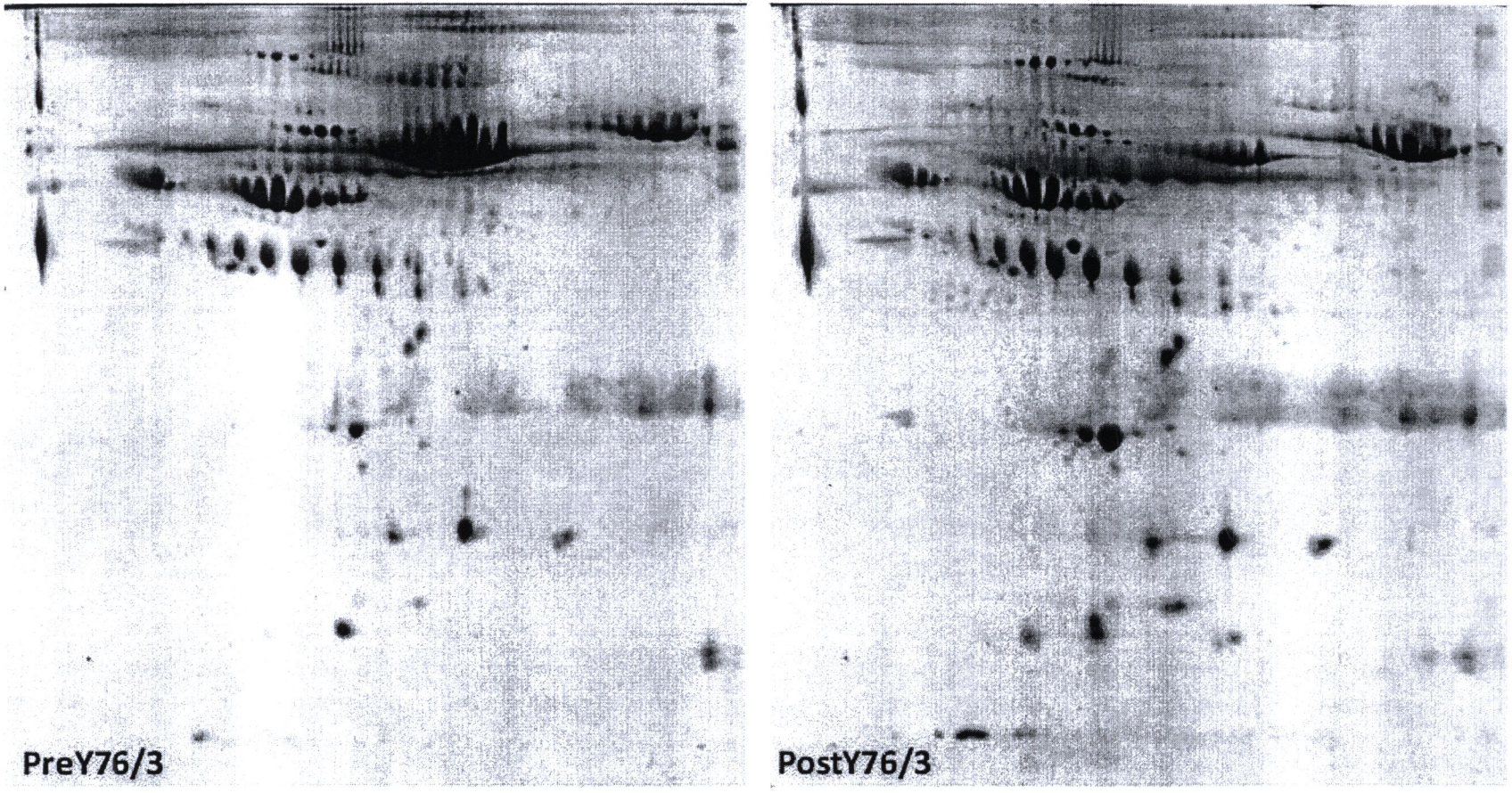

Figure 4-2c Coomassie stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #3 (left) and 2D gel of Y76 post-operative serum #3 (right).

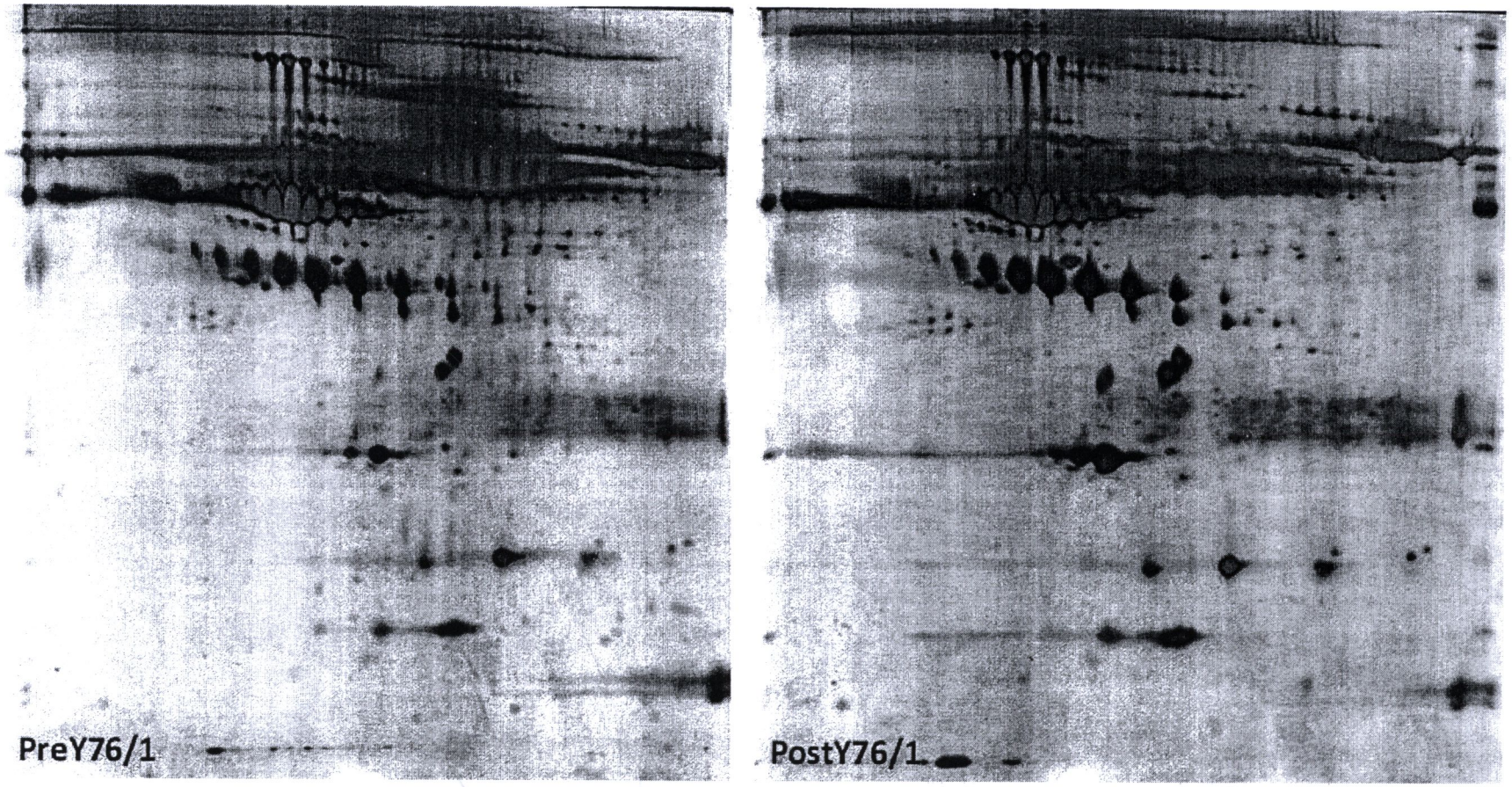

Figure 4-2d Silver stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #1 (left) and 2D gel of Y76 post-operative serum #1 (right).

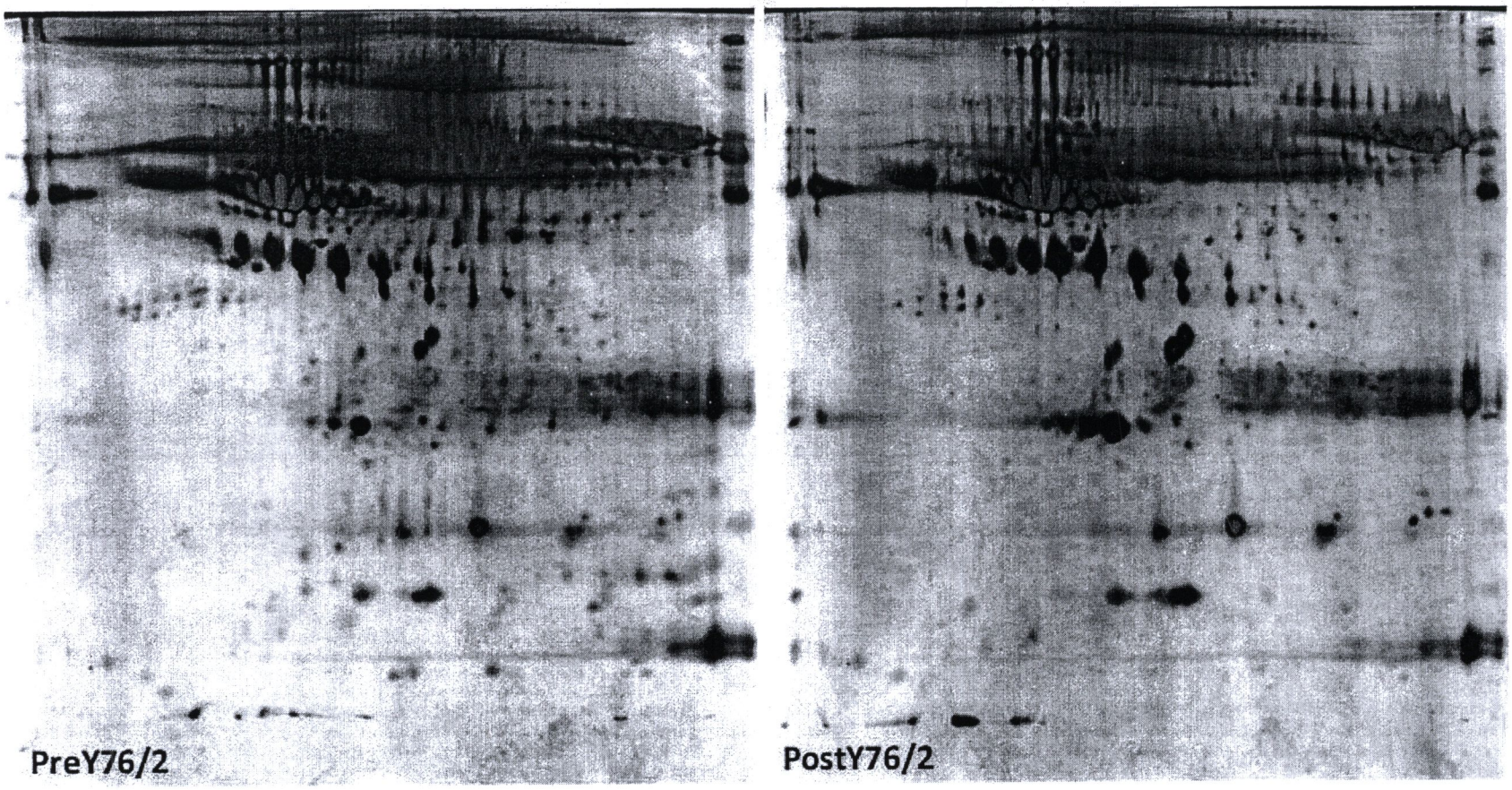

Figure 4-2e Silver stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #2 (left) and 2D gel of Y76 post-operative serum #2 (right).

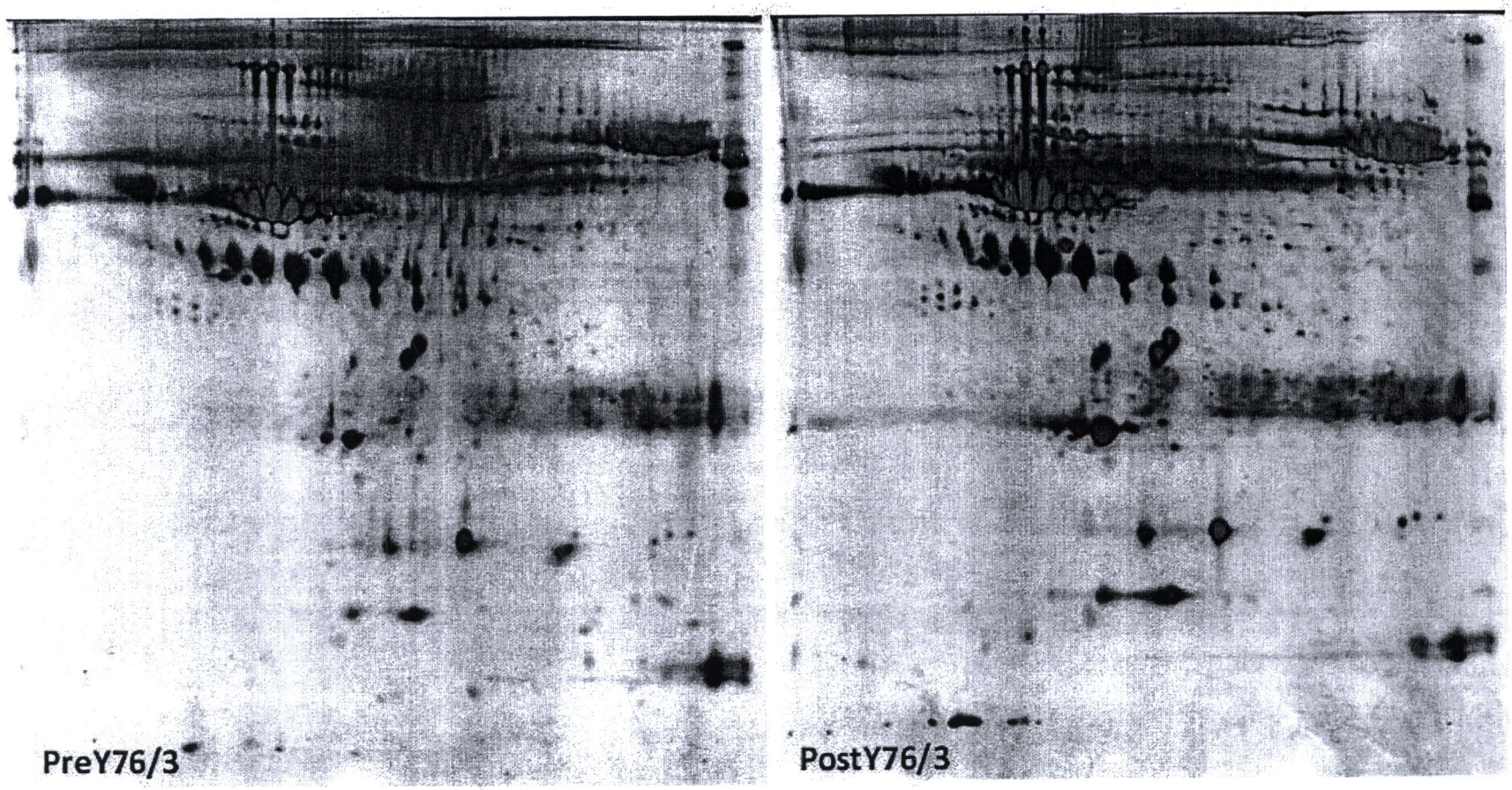

Figure 4-2f Silver stained gels serum 2D pattern of CCA case Y76 in both pre- and post-operation: 2D gel of Y76 pre-operative serum #3 (left) and 2D gel of Y76 post-operative serum #3 (right).

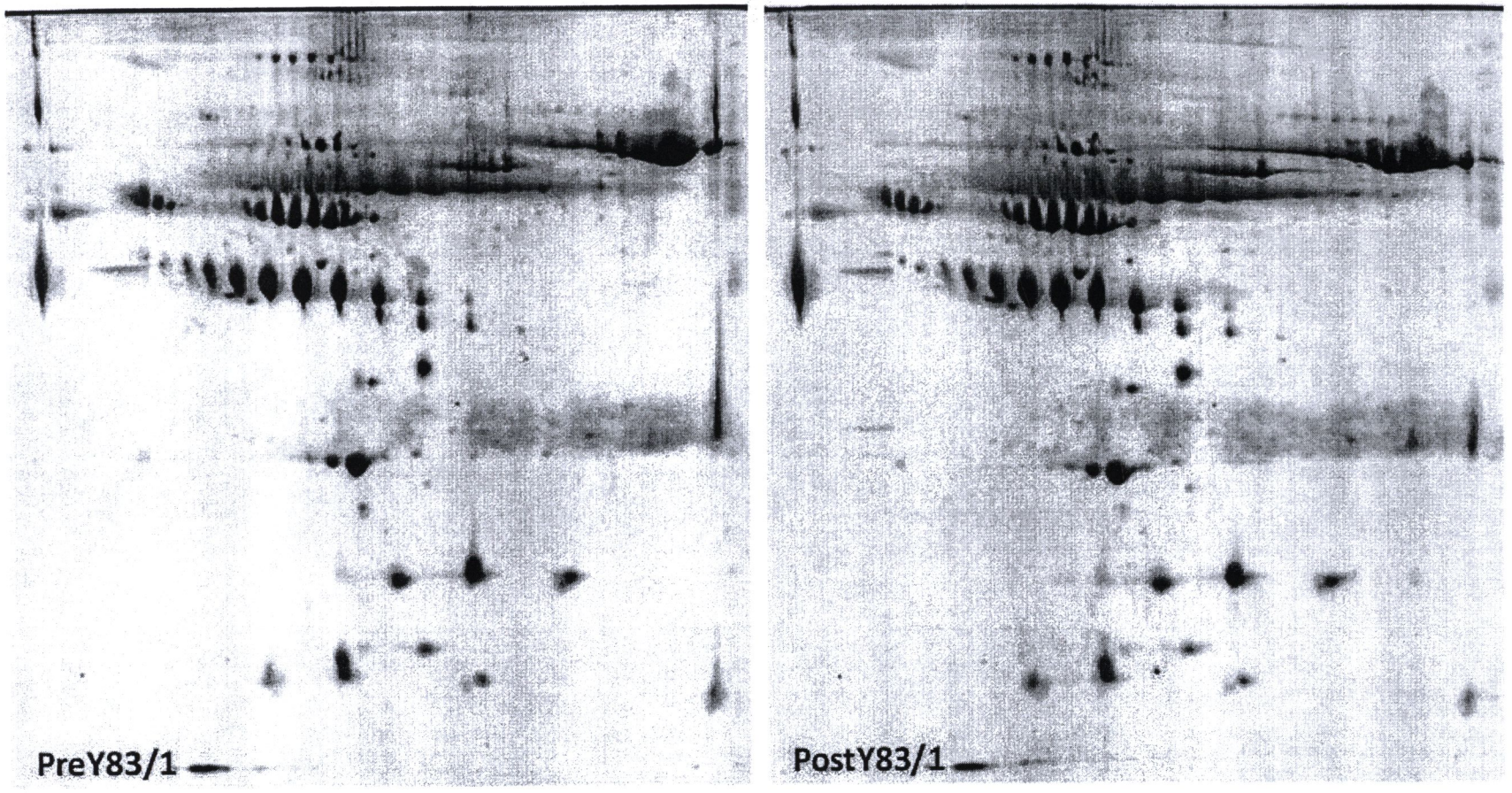

Figure 4-3a Coomassie stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #1 (left) and 2D gel of Y83 post-operative serum #1 (right).

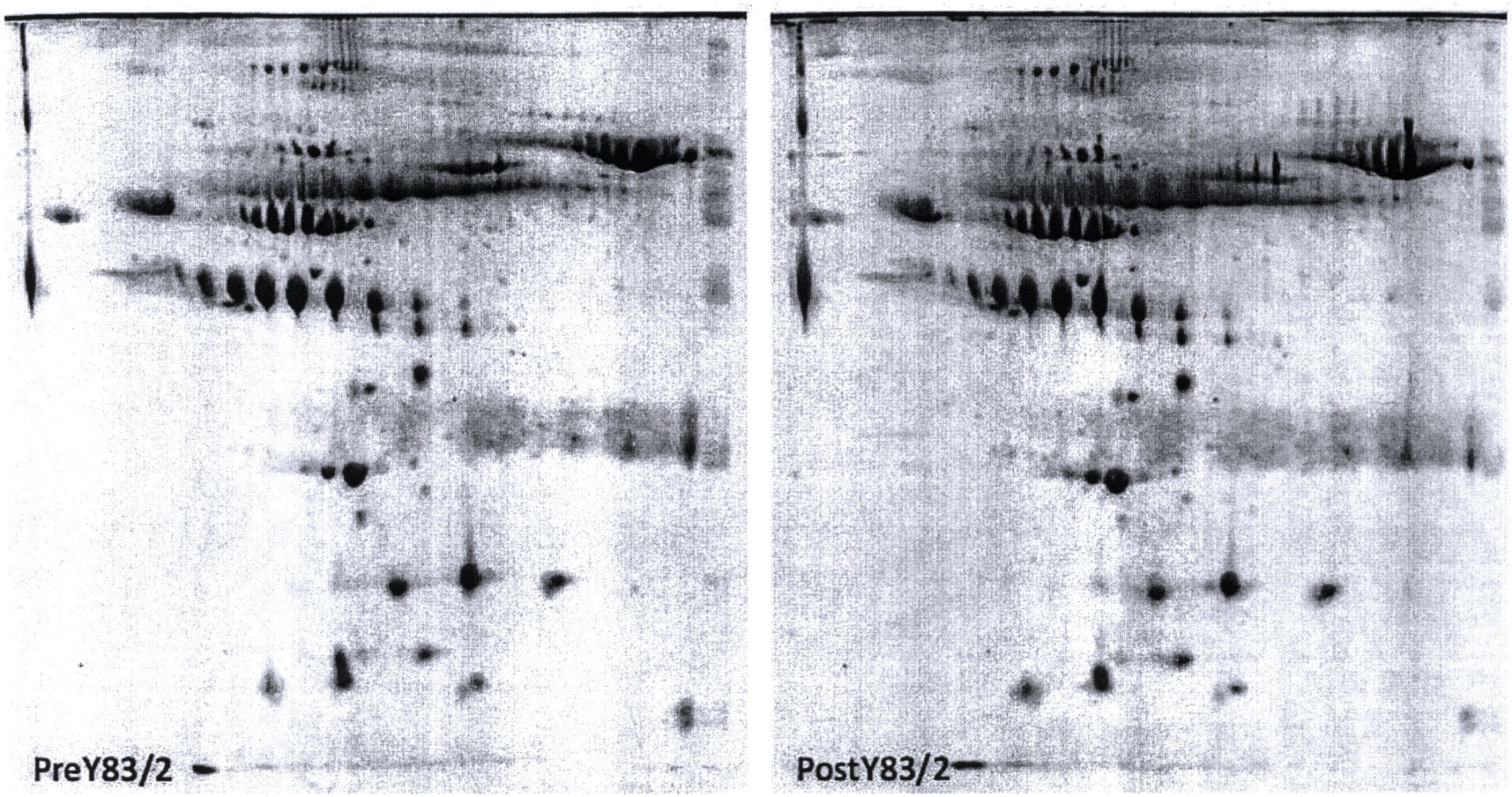

Figure 4-3b Coomassie stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #2 (left) and 2D gel of Y83 post-operative serum #2 (right).

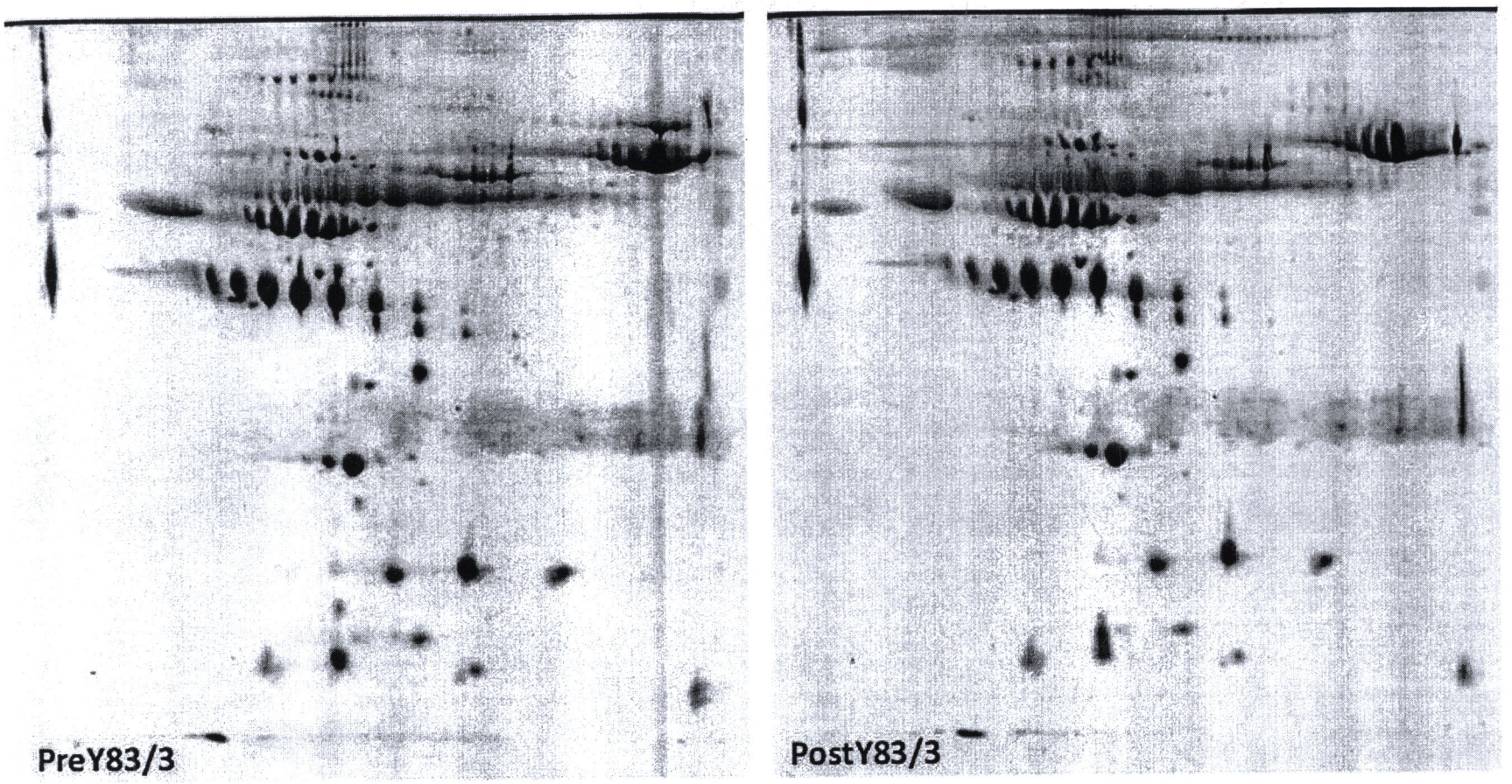

Figure 4-3c Coomassie stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #3 (left) and 2D gel of Y83 post-operative serum #3 (right).

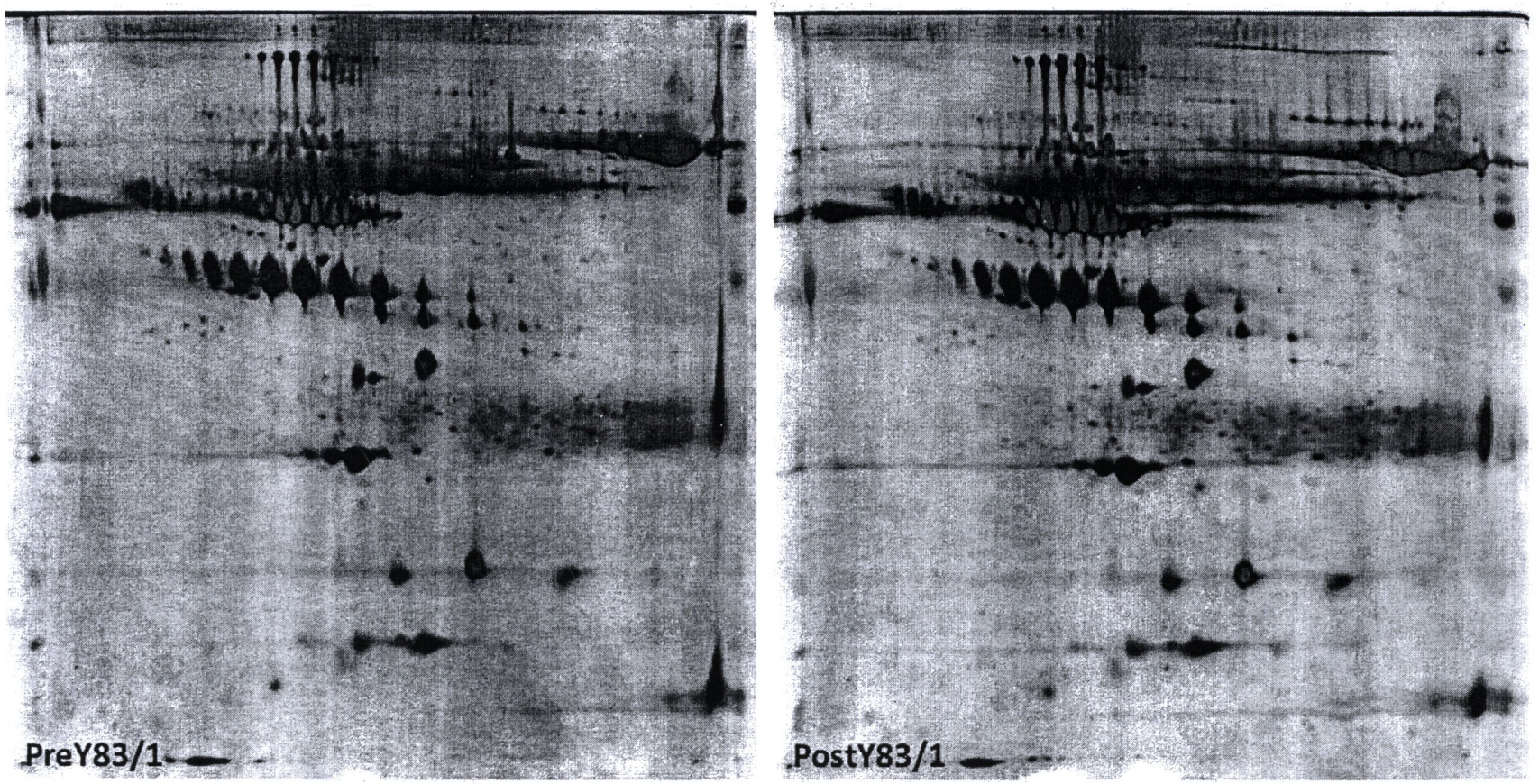

Figure 4-3d Silver stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #1 (left) and 2D gel of Y83 post-operative serum #1 (right).

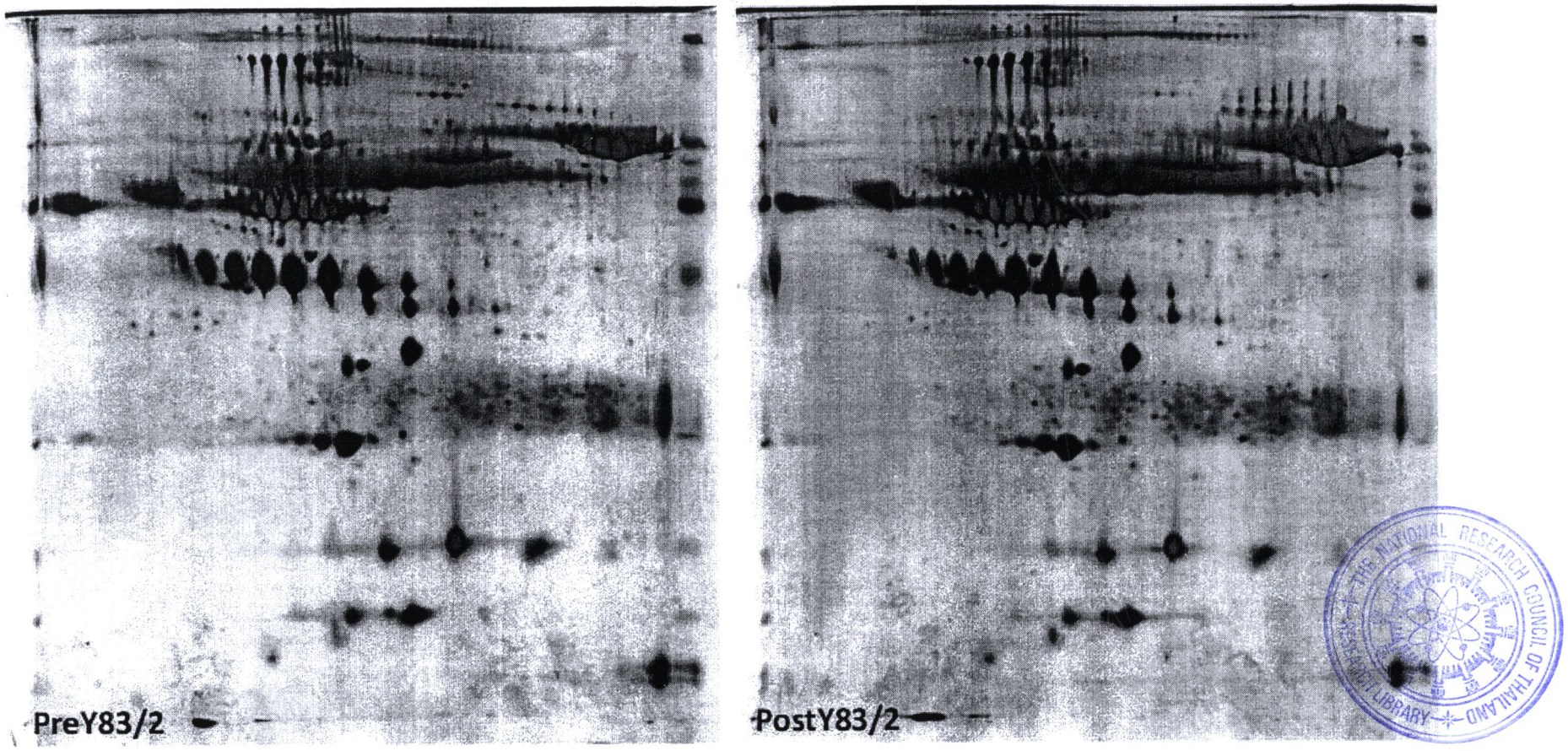

Figure 4-3e Silver stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #2 (left) and 2D gel of Y83 post-operative serum #2 (right).

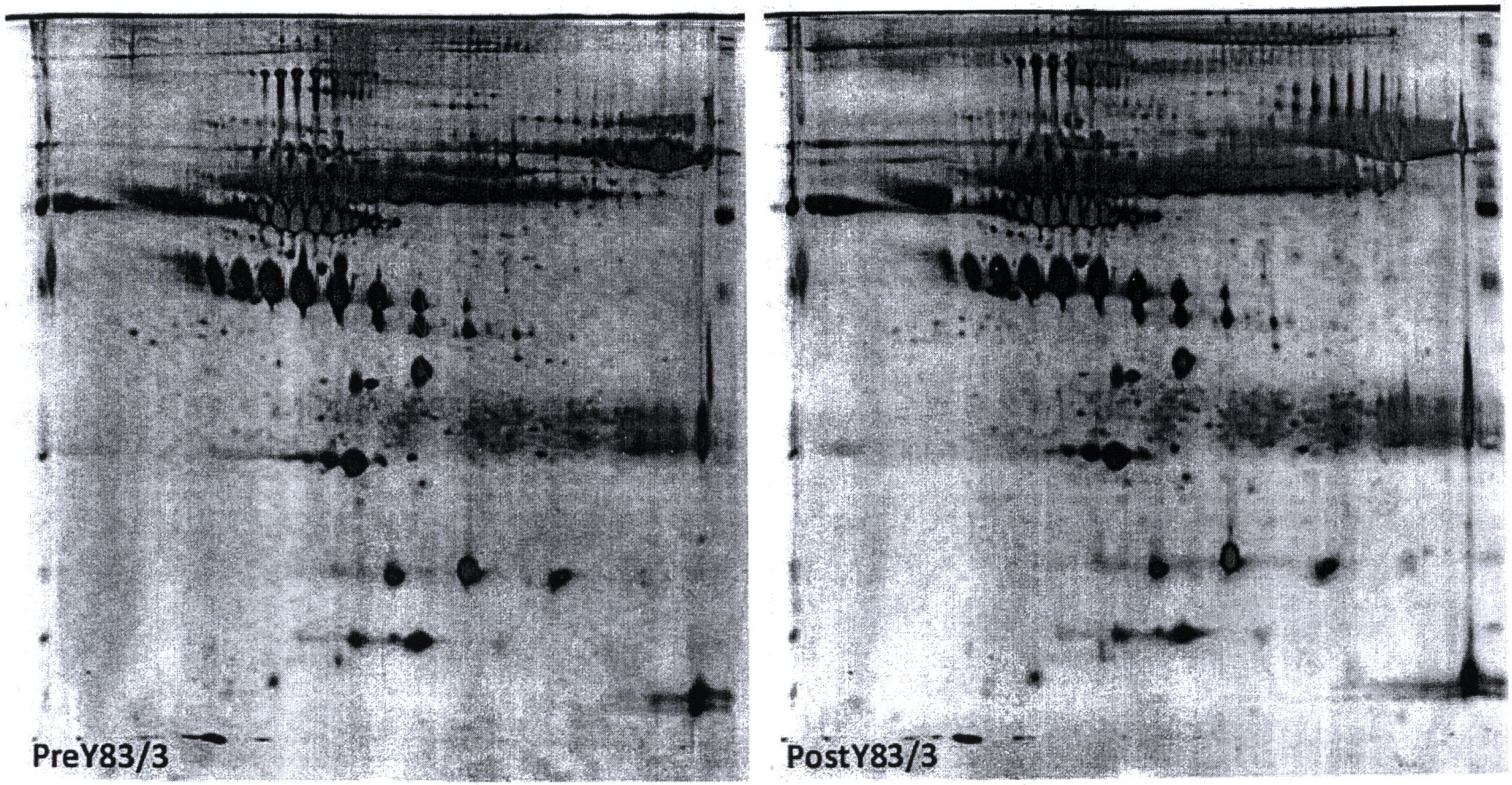

Figure 4-3f Silver stained gels serum 2D pattern of CCA case Y83 in both pre- and post-operation: 2D gel of Y83 pre-operative serum #3 (left) and 2D gel of Y83 post-operative serum #3 (right).

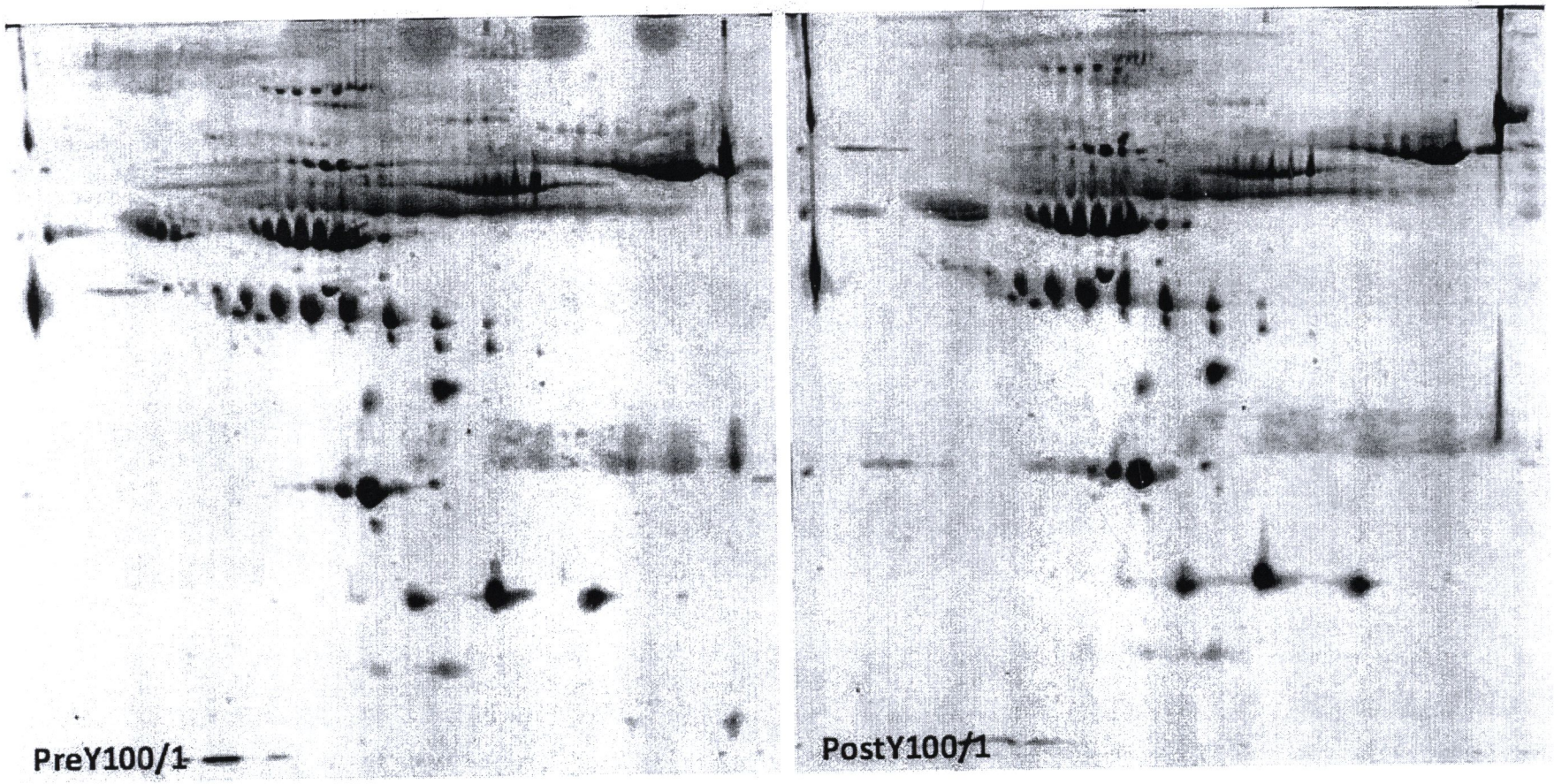

Figure 4-4a Coomassie stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #1 (left) and 2D gel of Y100 post-operative serum #1 (right).

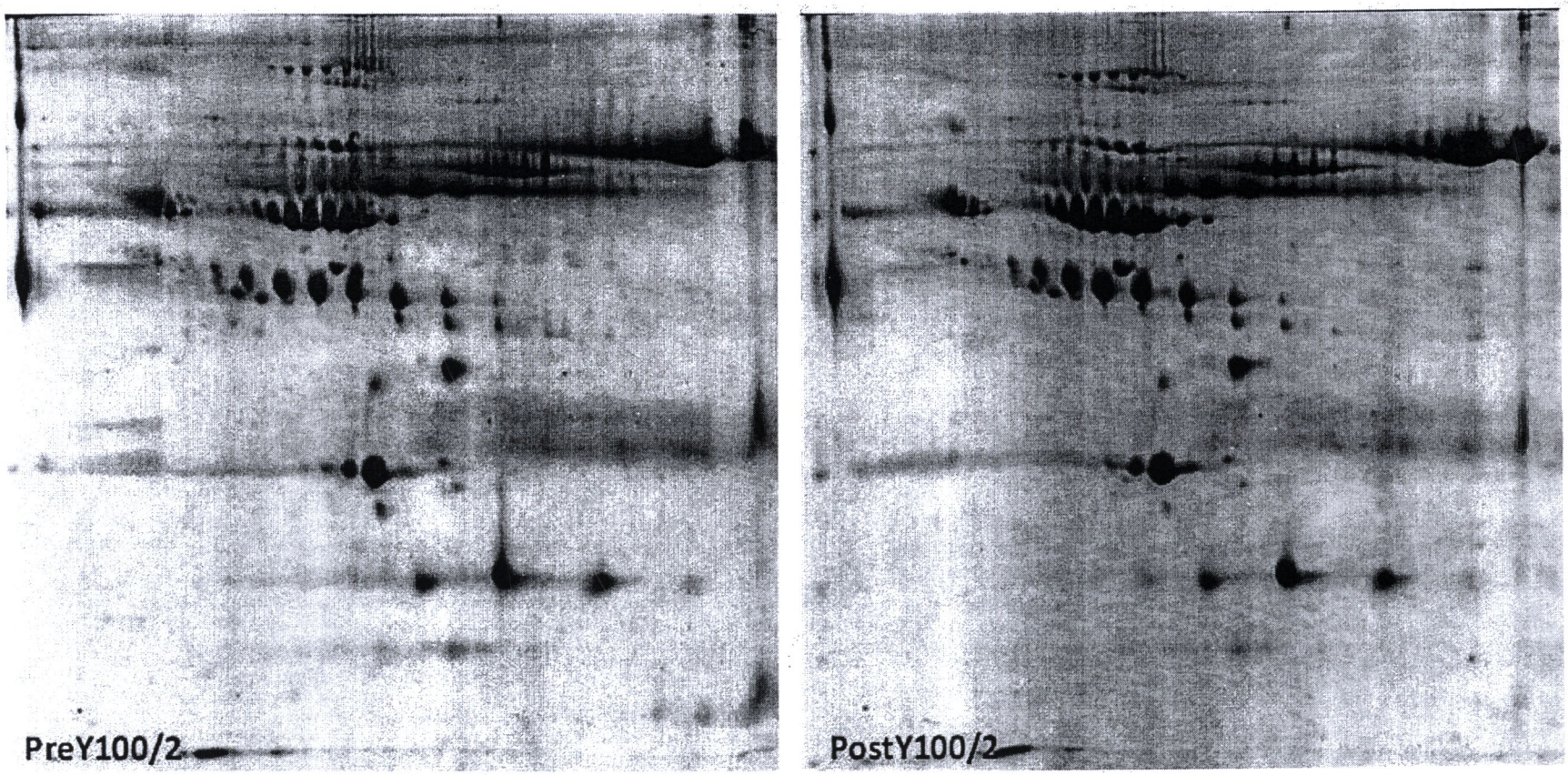

Figure 4-4b Coomassie stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #2 (left) and 2D gel of Y100 post-operative serum #2 (right).

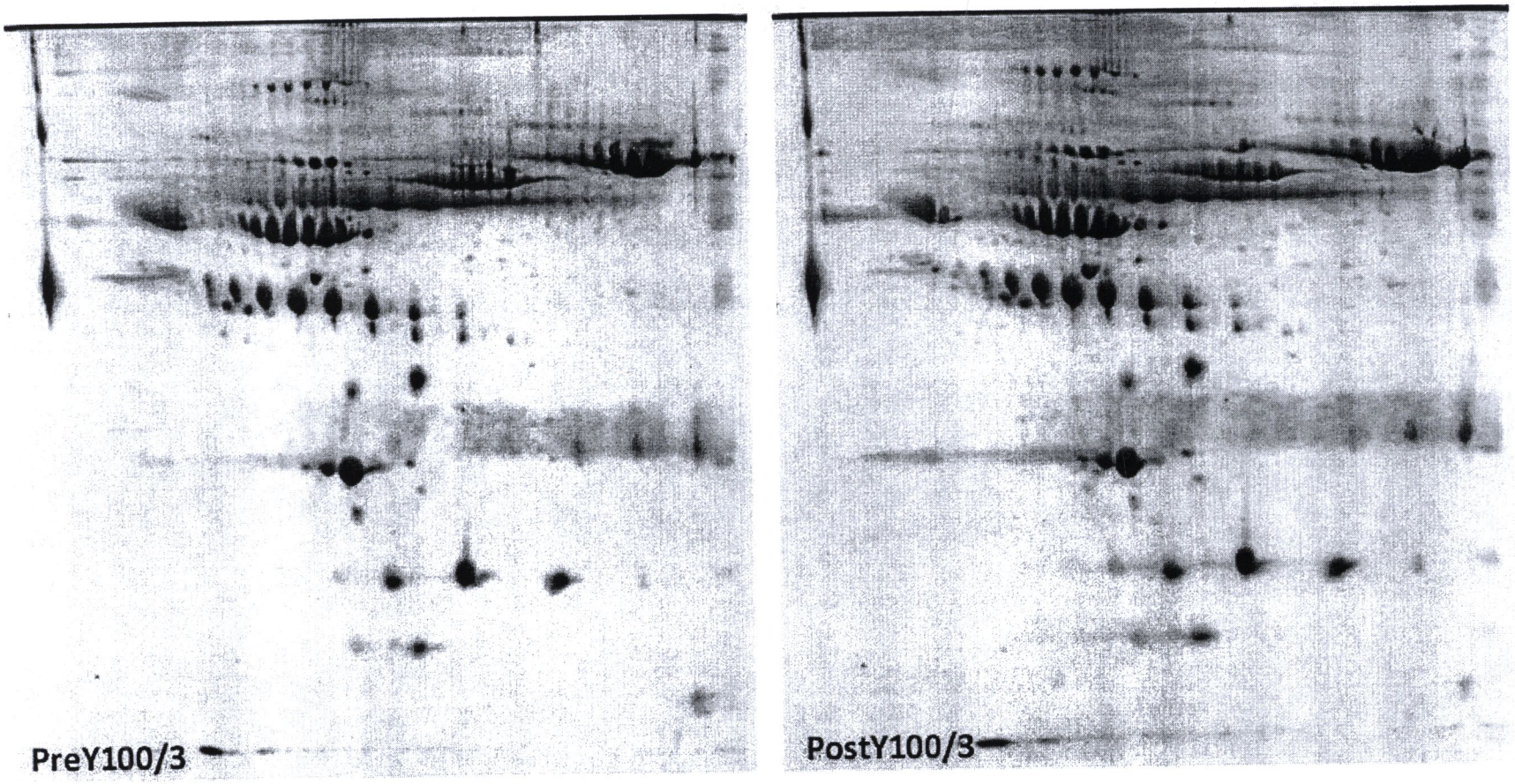

Figure 4-4c Coomassie stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #3 (left) and 2D gel of Y100 post-operative serum #3 (right).

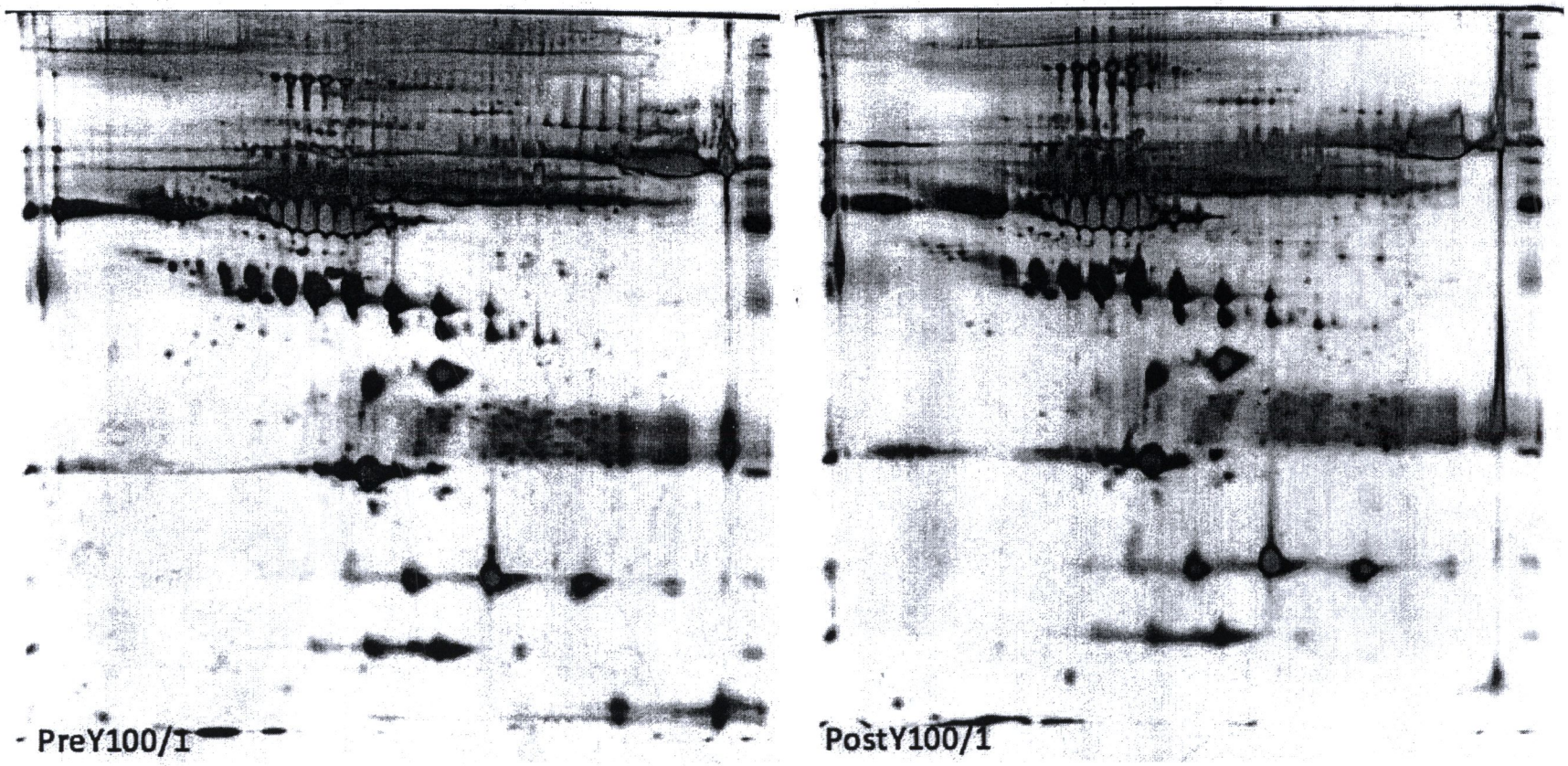

Figure 4-4d Silver stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #1 (left) and 2D gel of Y100 post-operative serum #1 (right).

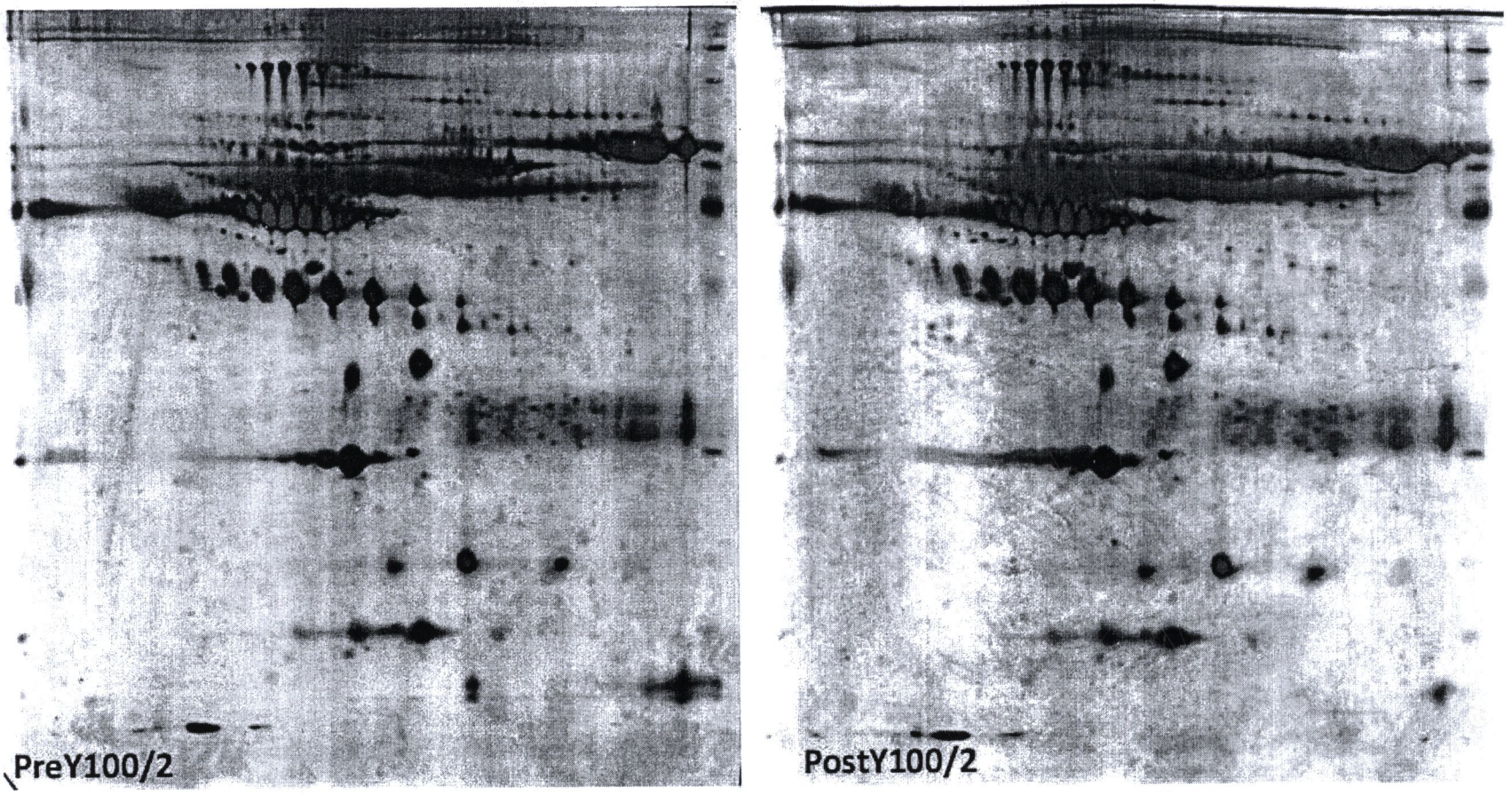

Figure 4-4e Silver stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #2 (left) and 2D gel of Y100 post-operative serum #2 (right).

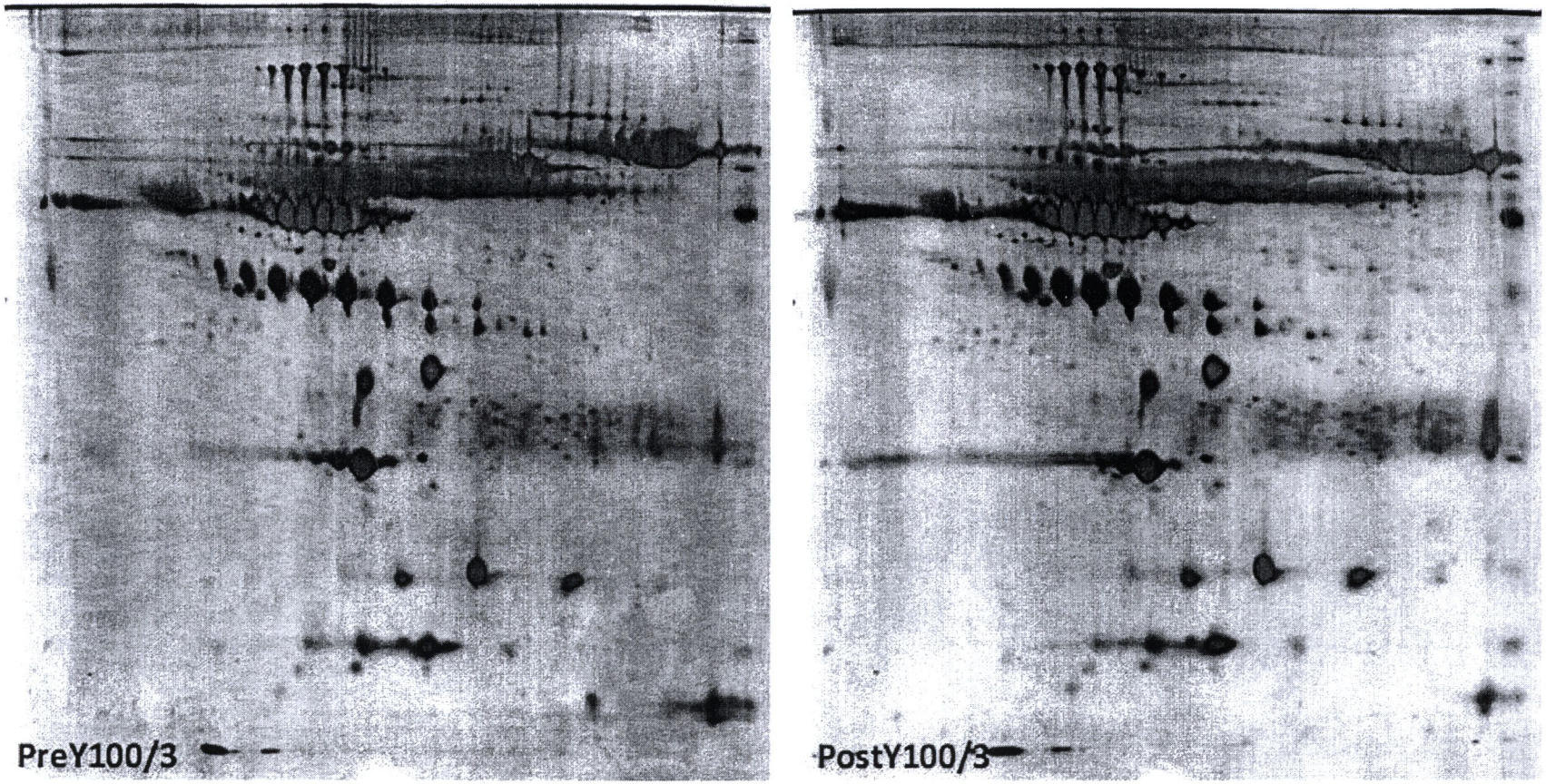

Figure 4-4f Silver stained gels serum 2D pattern of CCA case Y100 in both pre- and post-operation: 2D gel of Y100 pre-operative serum #3 (left) and 2D gel of Y100 post-operative serum #3 (right).

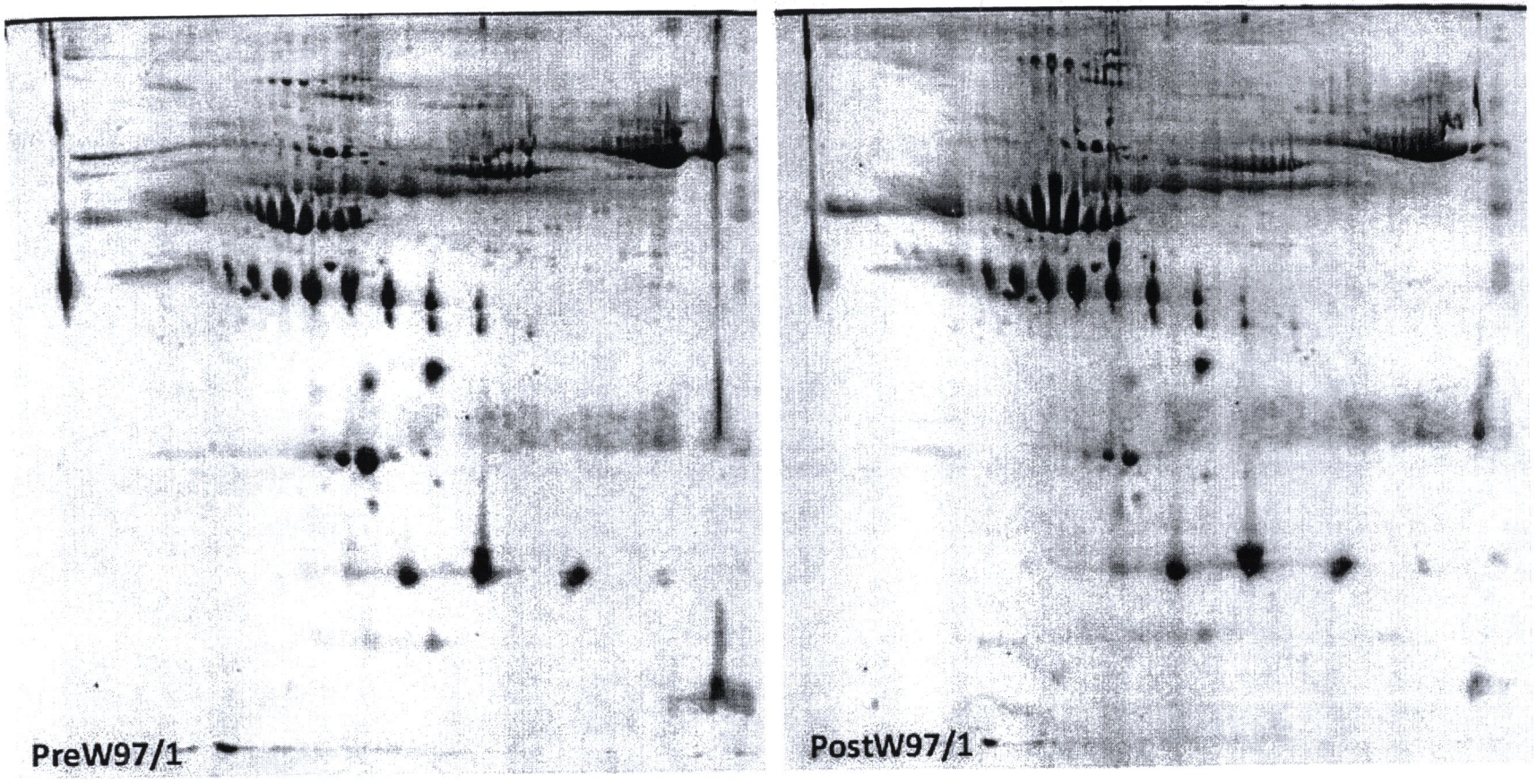

Figure 4-5a Coomassie stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #1 (left) and 2D gel of W97 post-operative serum #1 (right).

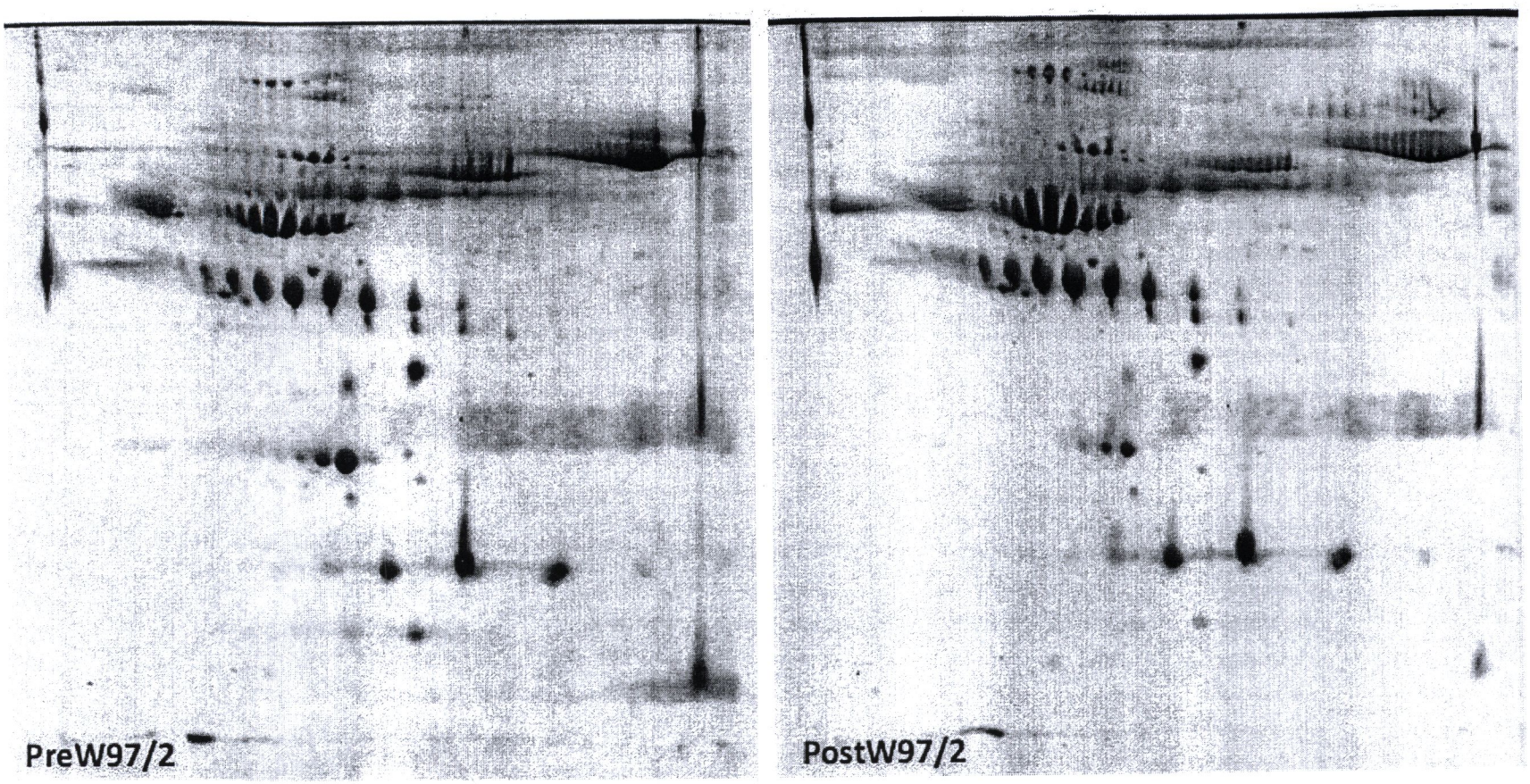

Figure 4-5b Coomassie stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #2 (left) and 2D gel of W97 post-operative serum #2 (right).

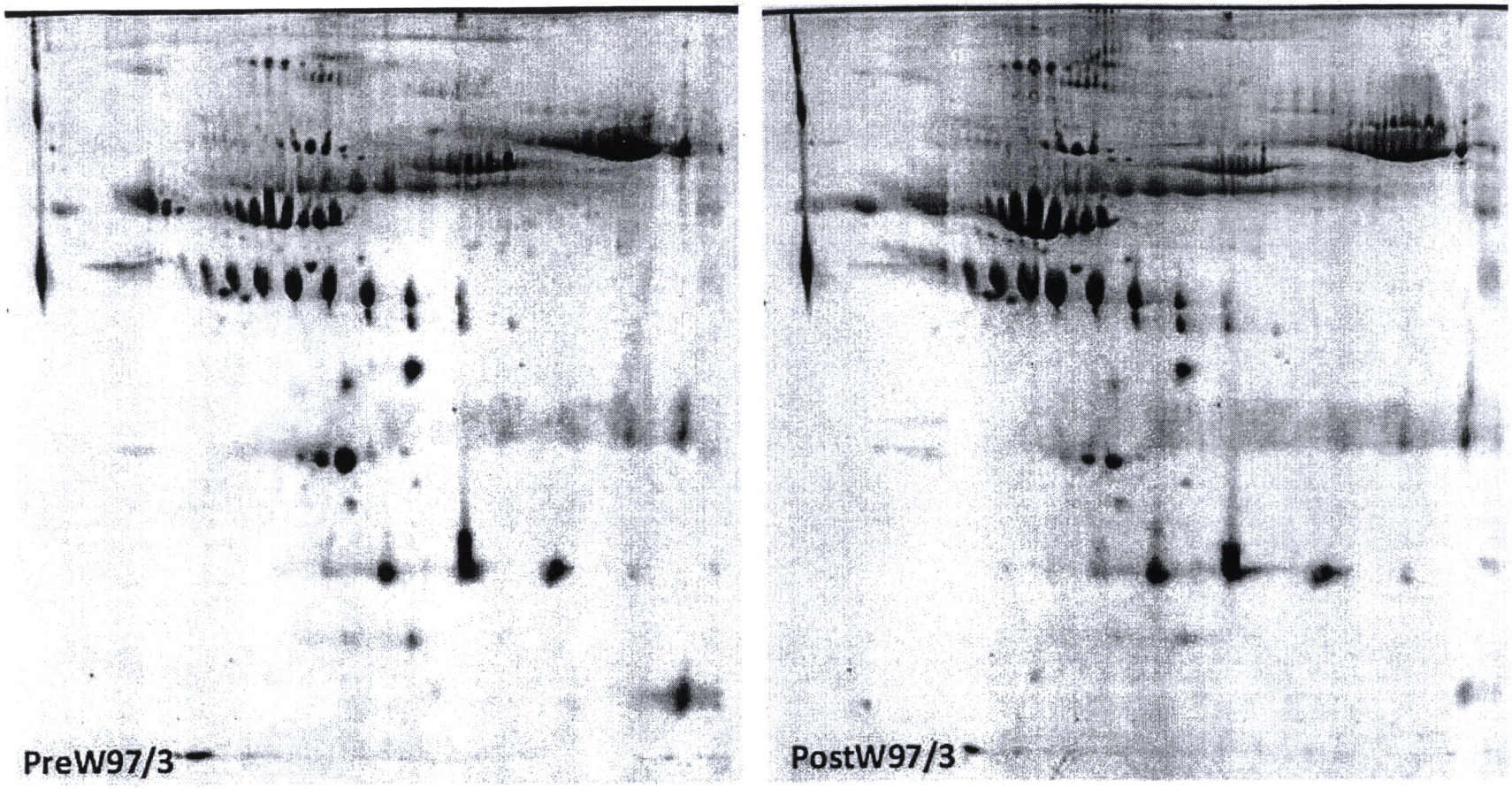

Figure 4-5c Coomassie stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #3 (left) and 2D gel of W97 post-operative serum #3 (right).

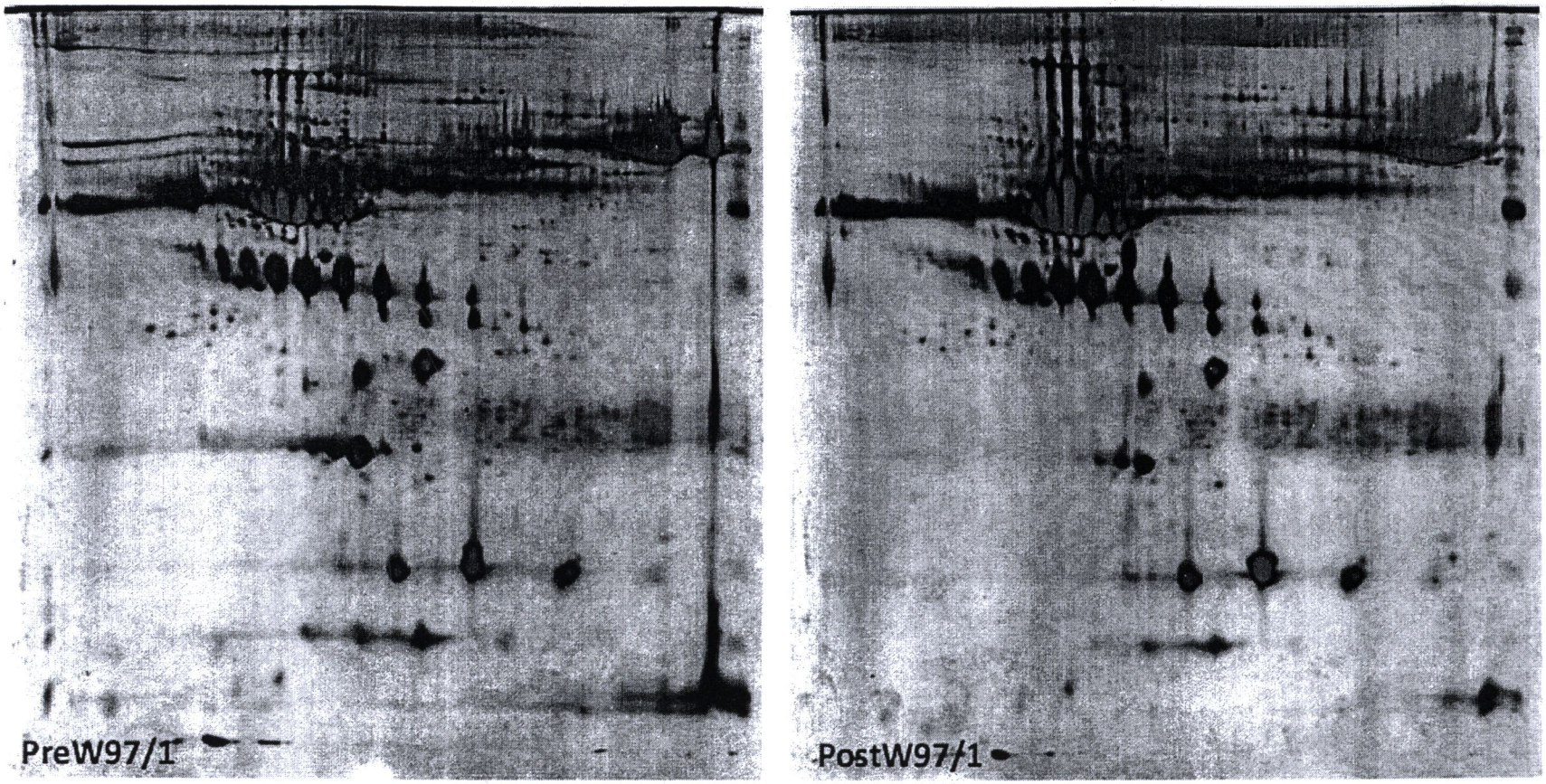

Figure 4-5d Silver stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #1 (left) and 2D gel of W97 post-operative serum #1 (right).

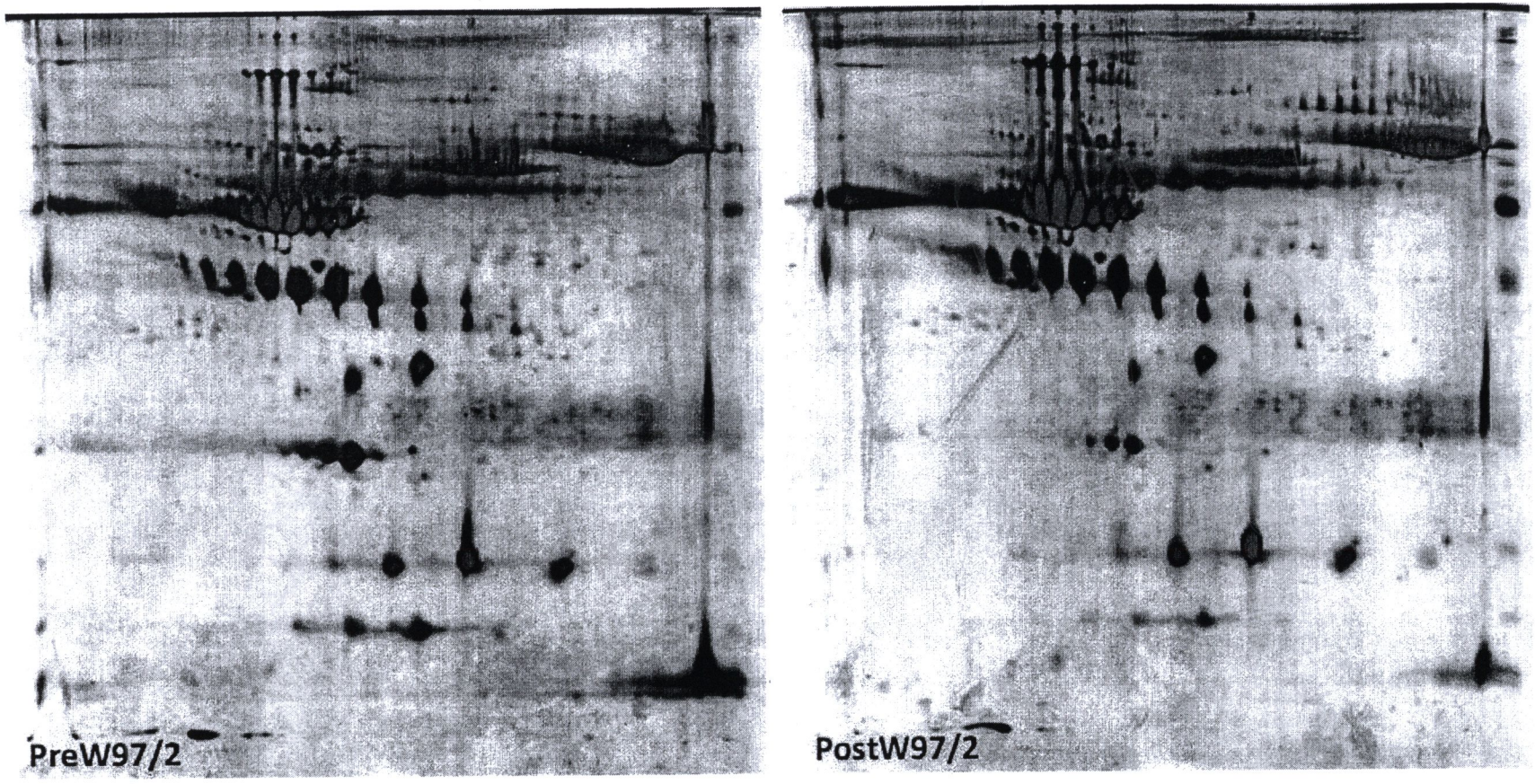

Figure 4-5e Silver stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #2 (left) and 2D gel of W97 post-operative serum #2 (right).

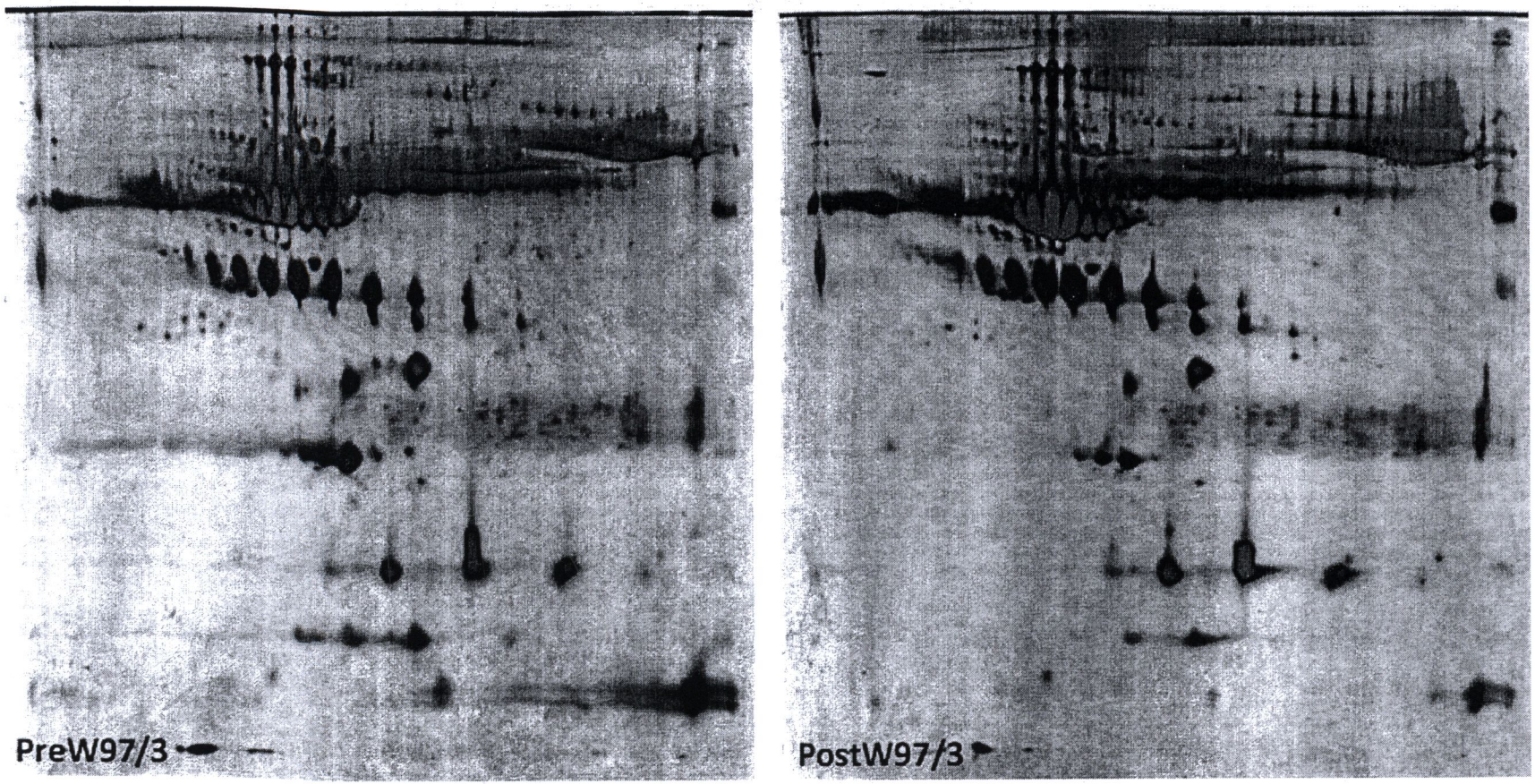

Figure 4-5f Silver stained gels serum 2D pattern of CCA case W97 in both pre- and post-operation: 2D gel of W97 pre-operative serum #3 (left) and 2D gel of W97 post-operative serum #3 (right).

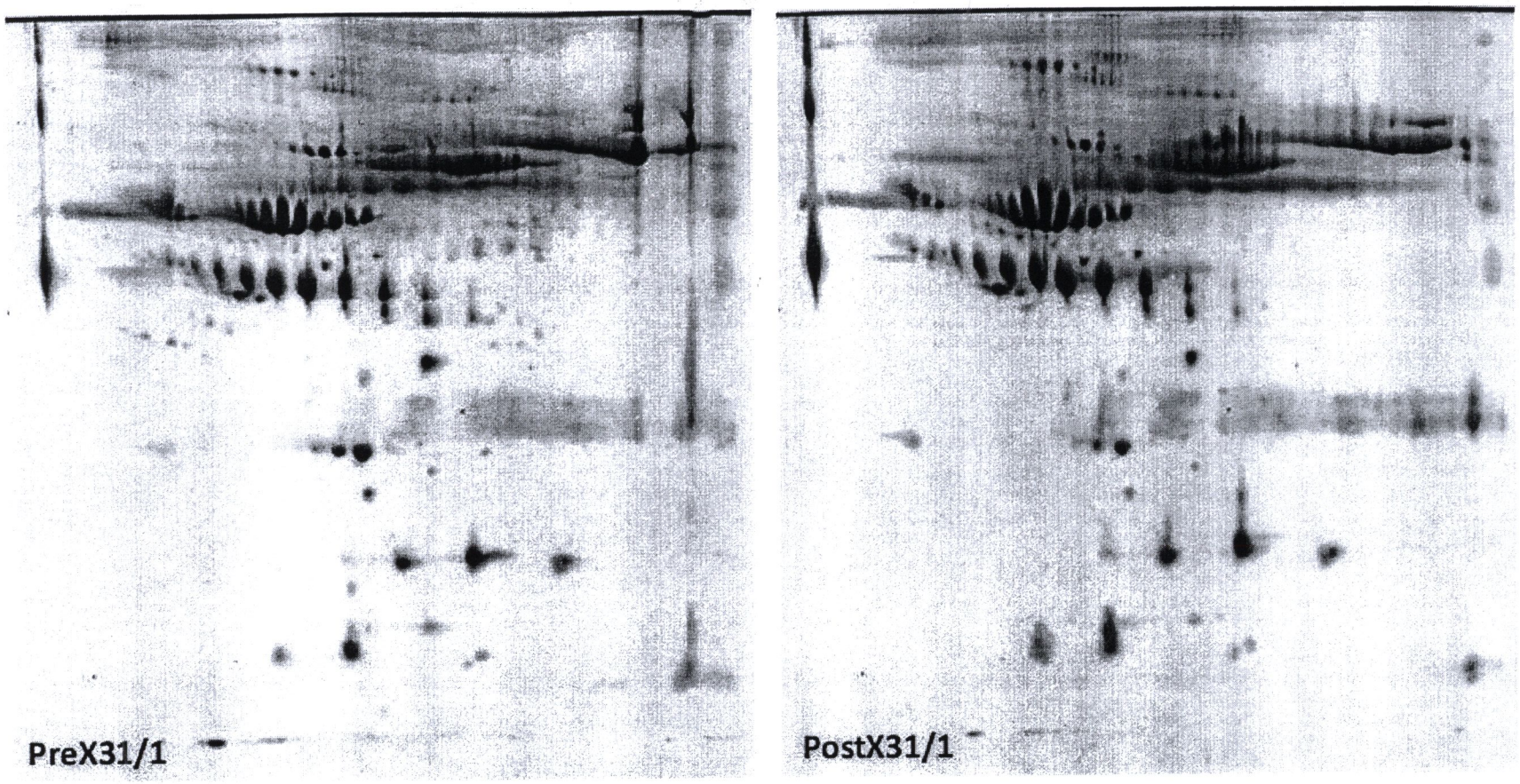

Figure 4-6a Coomassie stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #1 (left) and 2D gel of X31 post-operative serum #1 (right).

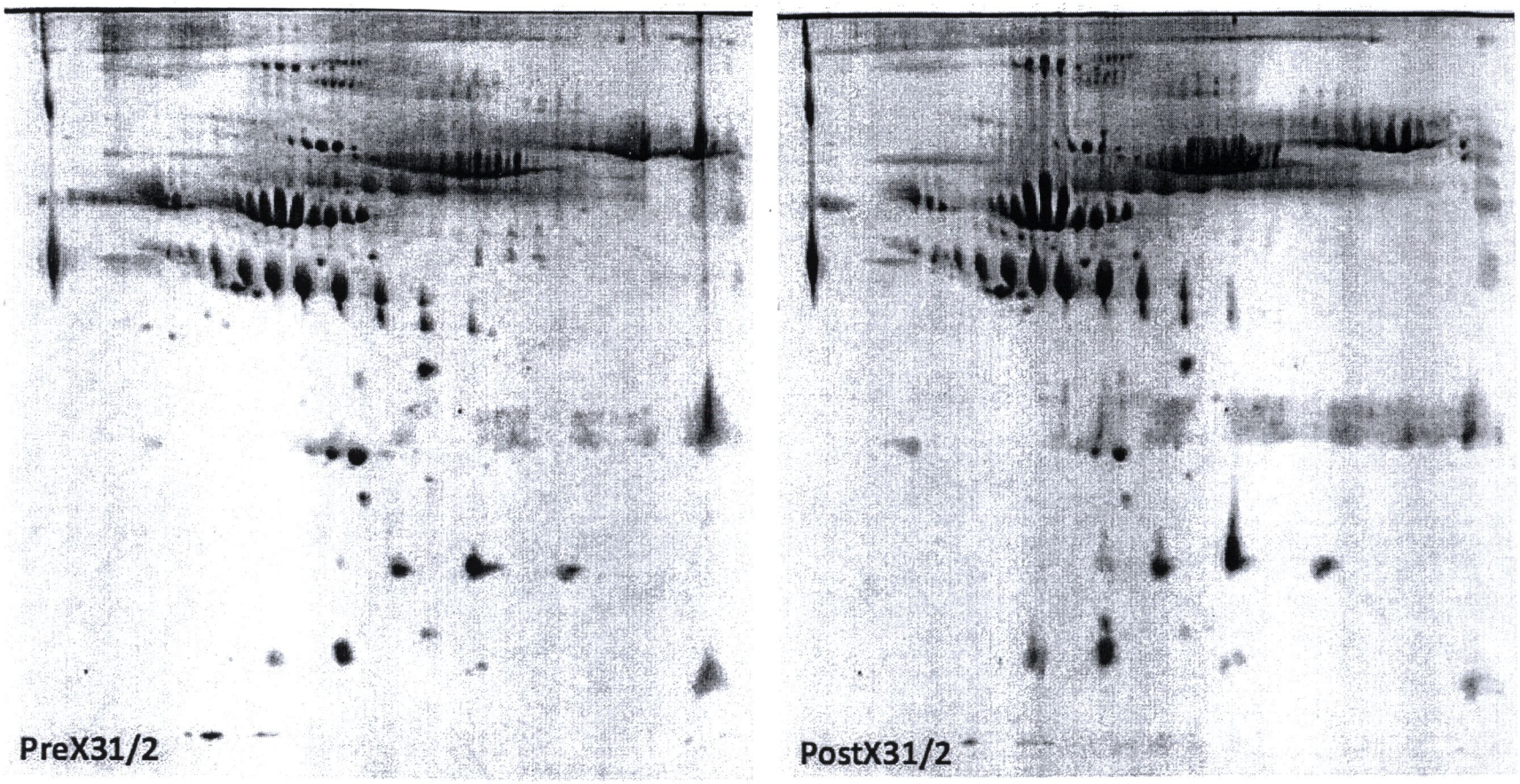

Figure 4-6b Coomassie stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #2 (left) and 2D gel of X31 post-operative serum #2 (right).

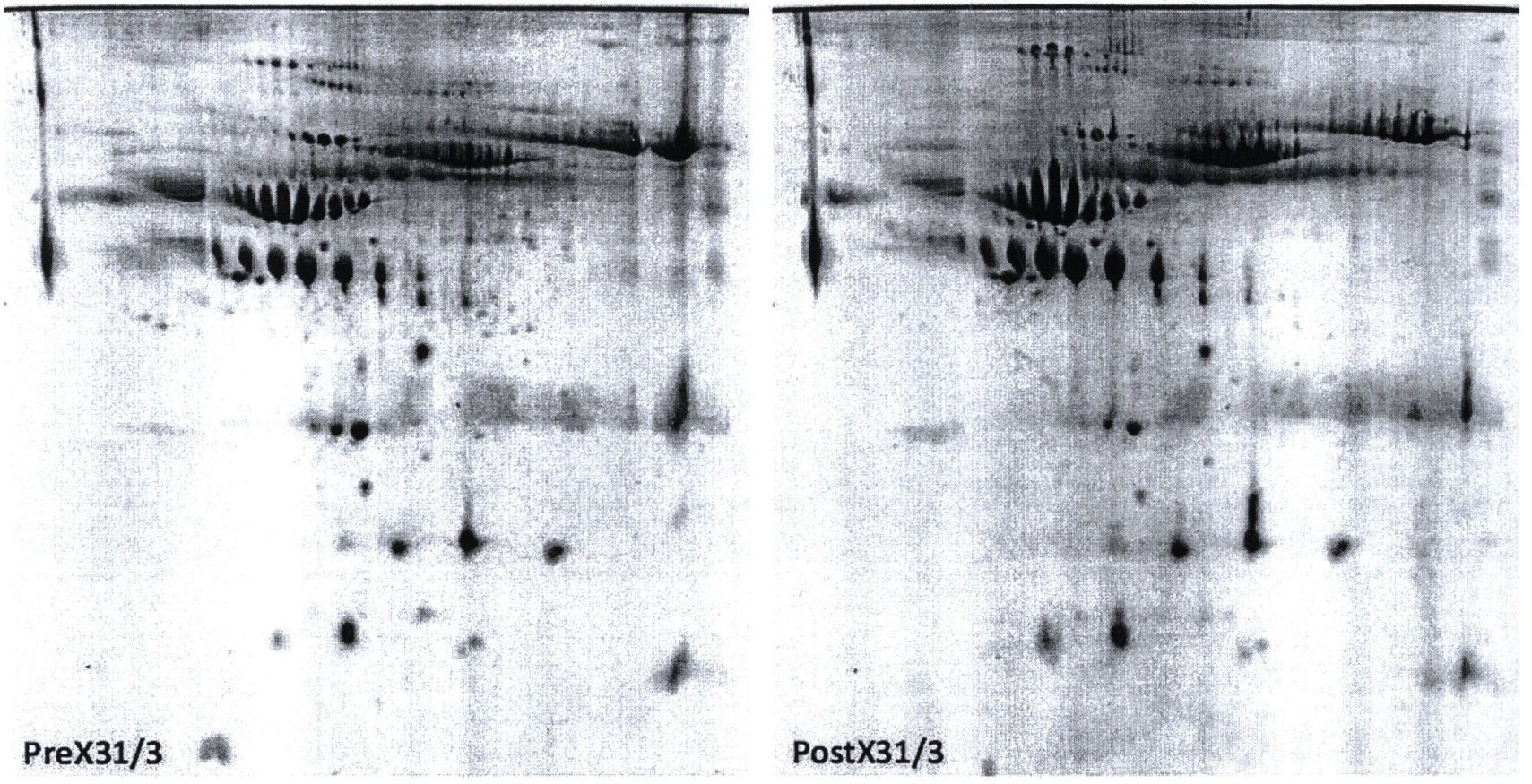

Figure 4-6c Coomassie stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #3 (left) and 2D gel of X31 post-operative serum #3 (right).

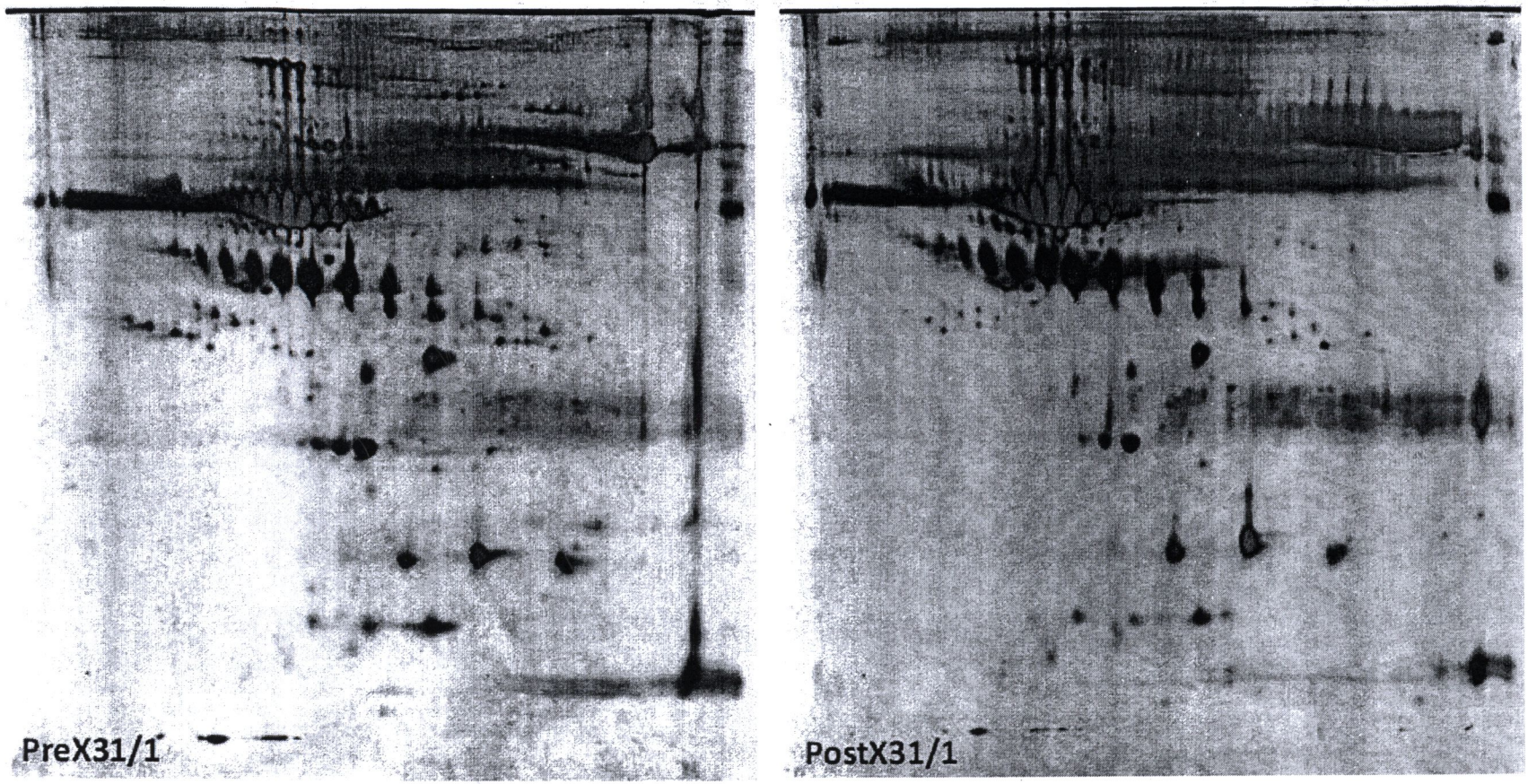

Figure 4-6d Silver stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #1 (left) and 2D gel of X31 post-operative serum #1 (right).

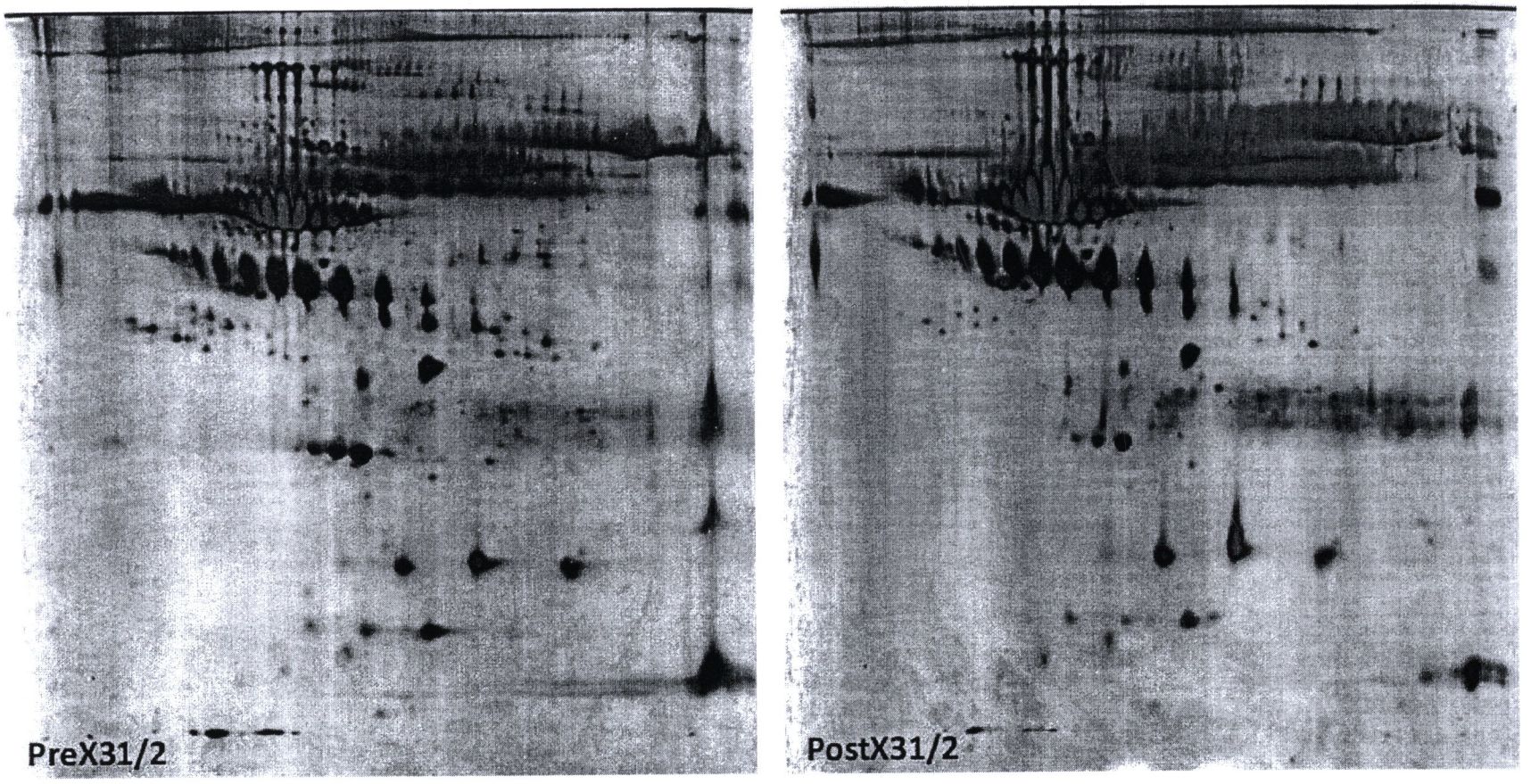

Figure 4-6e Silver stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #2 (left) and 2D gel of X31 post-operative serum #2 (right).

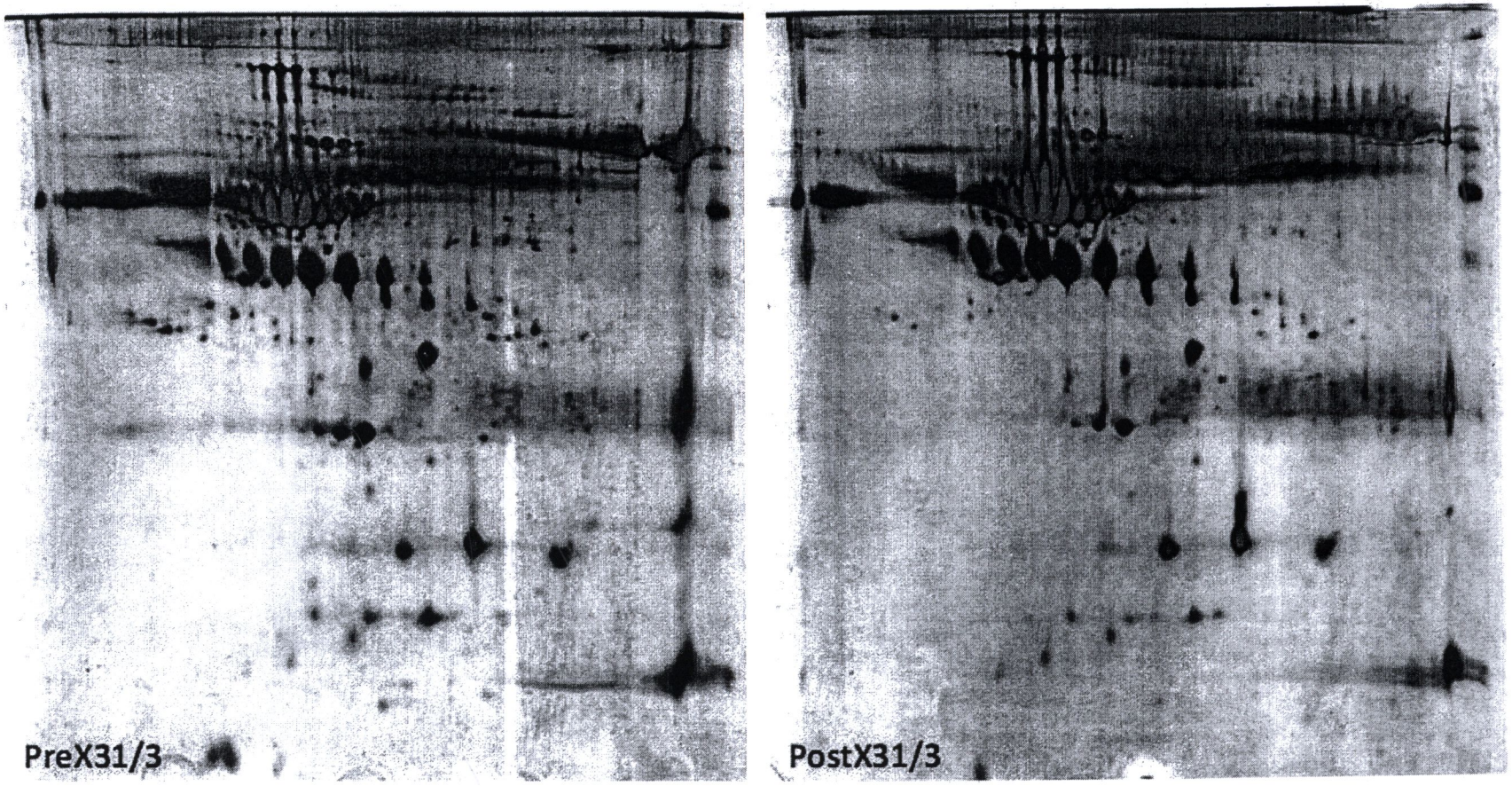

Figure 4-6f Silver stained gels serum 2D pattern of CCA case X31 in both pre- and post-operation: 2D gel of X31 pre-operative serum #3 (left) and 2D gel of X31 post-operative serum #3 (right).

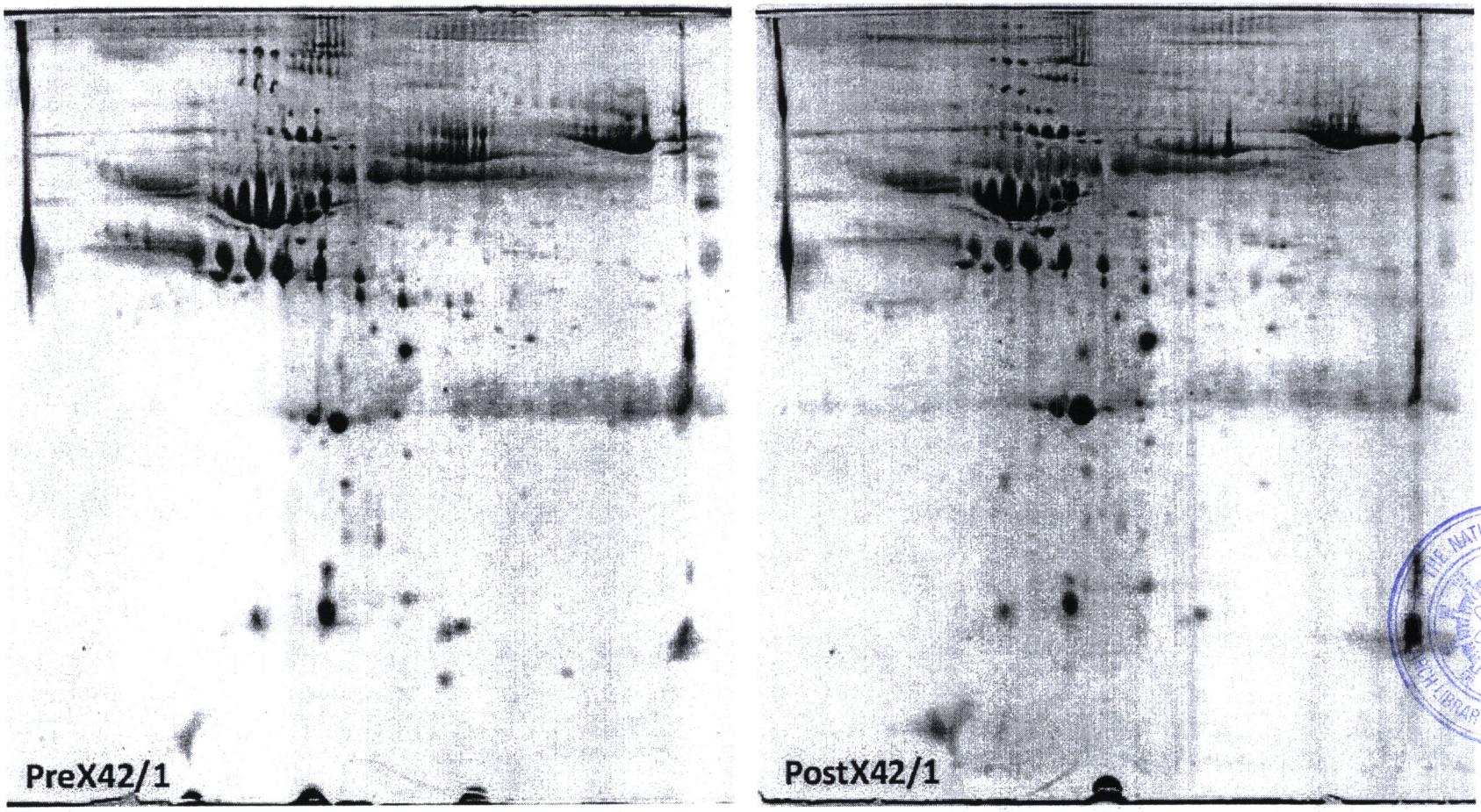

Figure 4-7a Coomassie stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #1 (left) and 2D gel of X42 post-operative serum #1 (right).

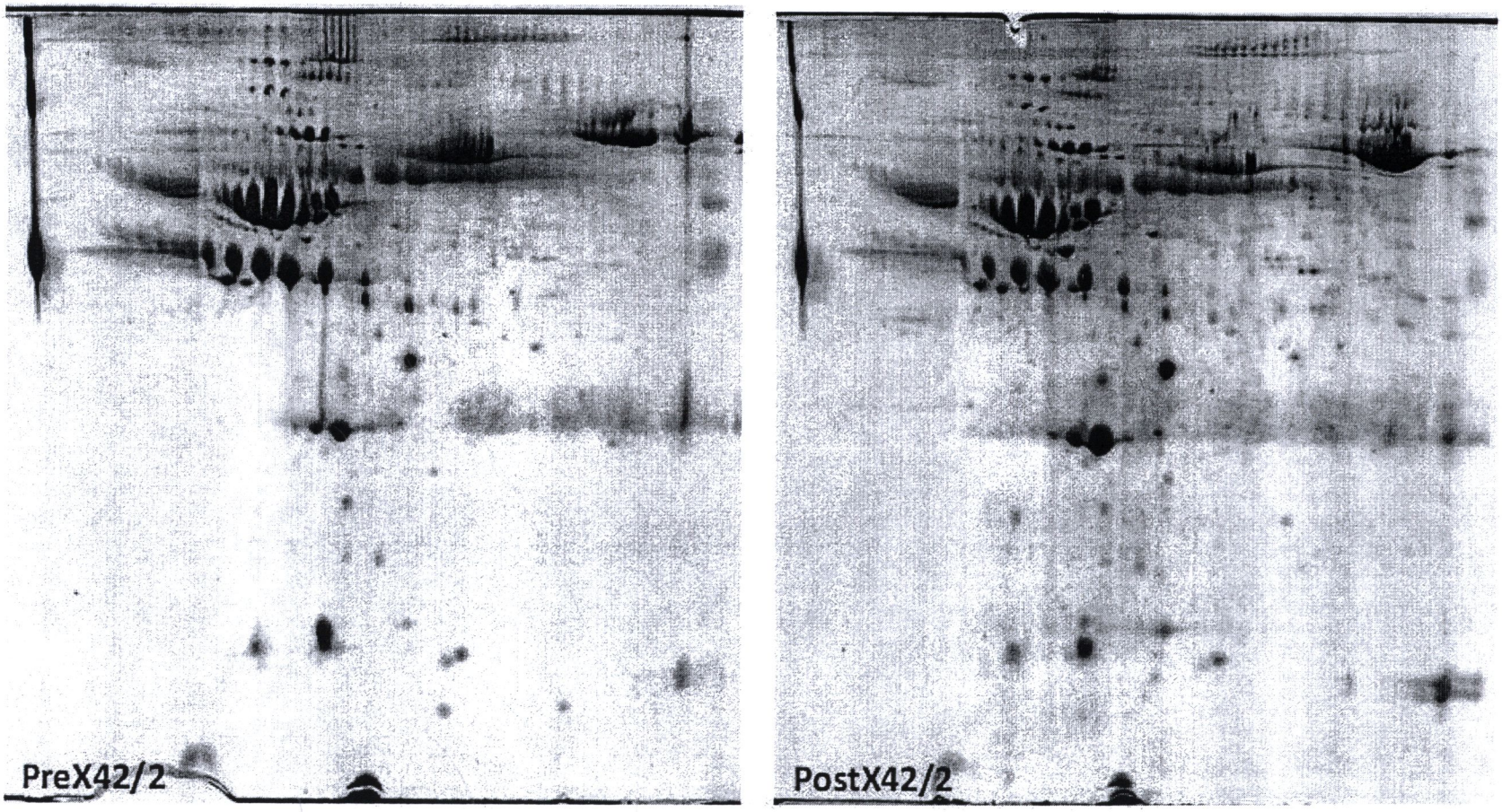

Figure 4-7b Coomassie stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #2 (left) and 2D gel of X42 post-operative serum #2 (right).

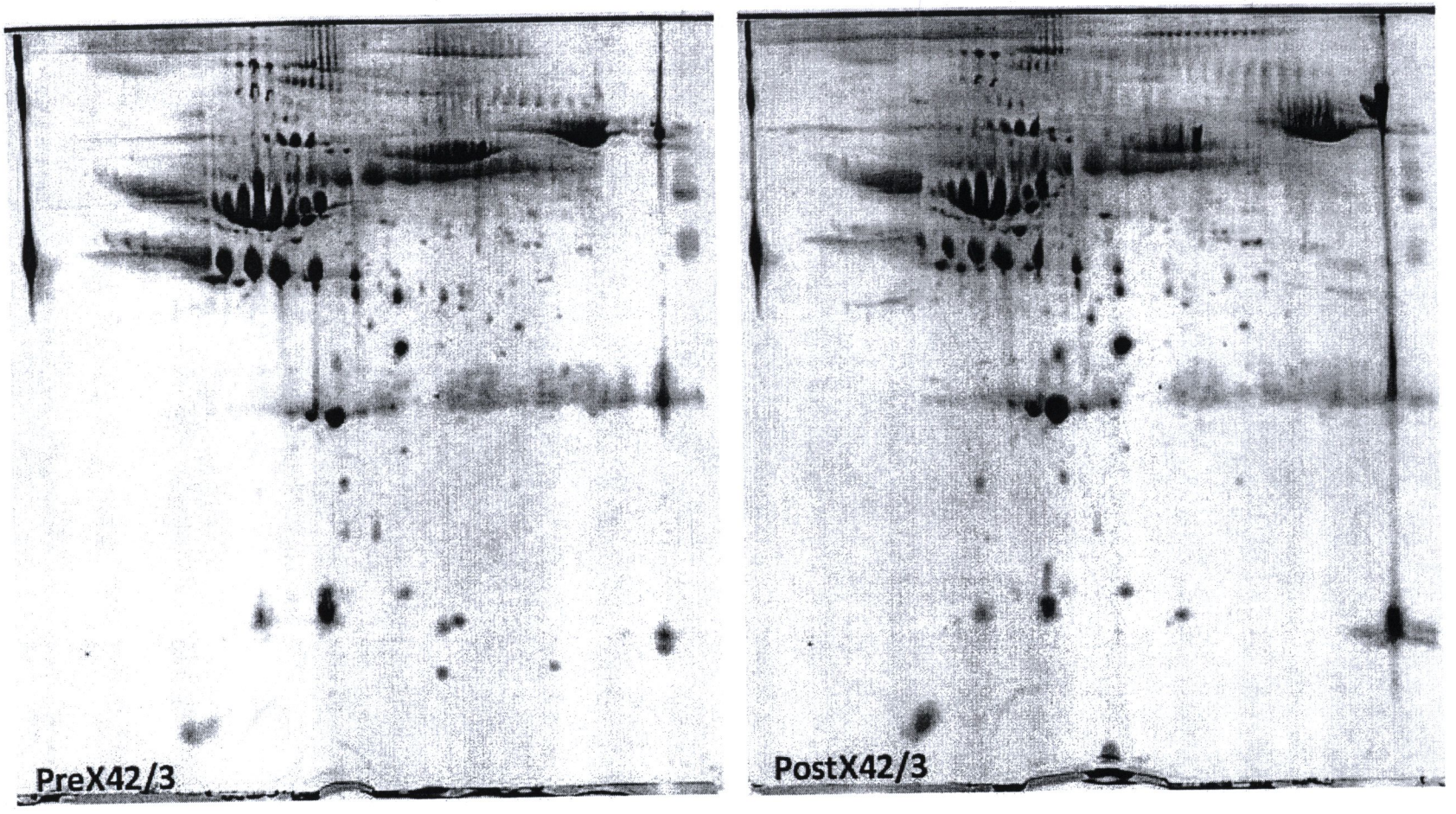

Figure 4-7c Coomassie stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #3 (left) and 2D gel of X42 post-operative serum #3 (right).

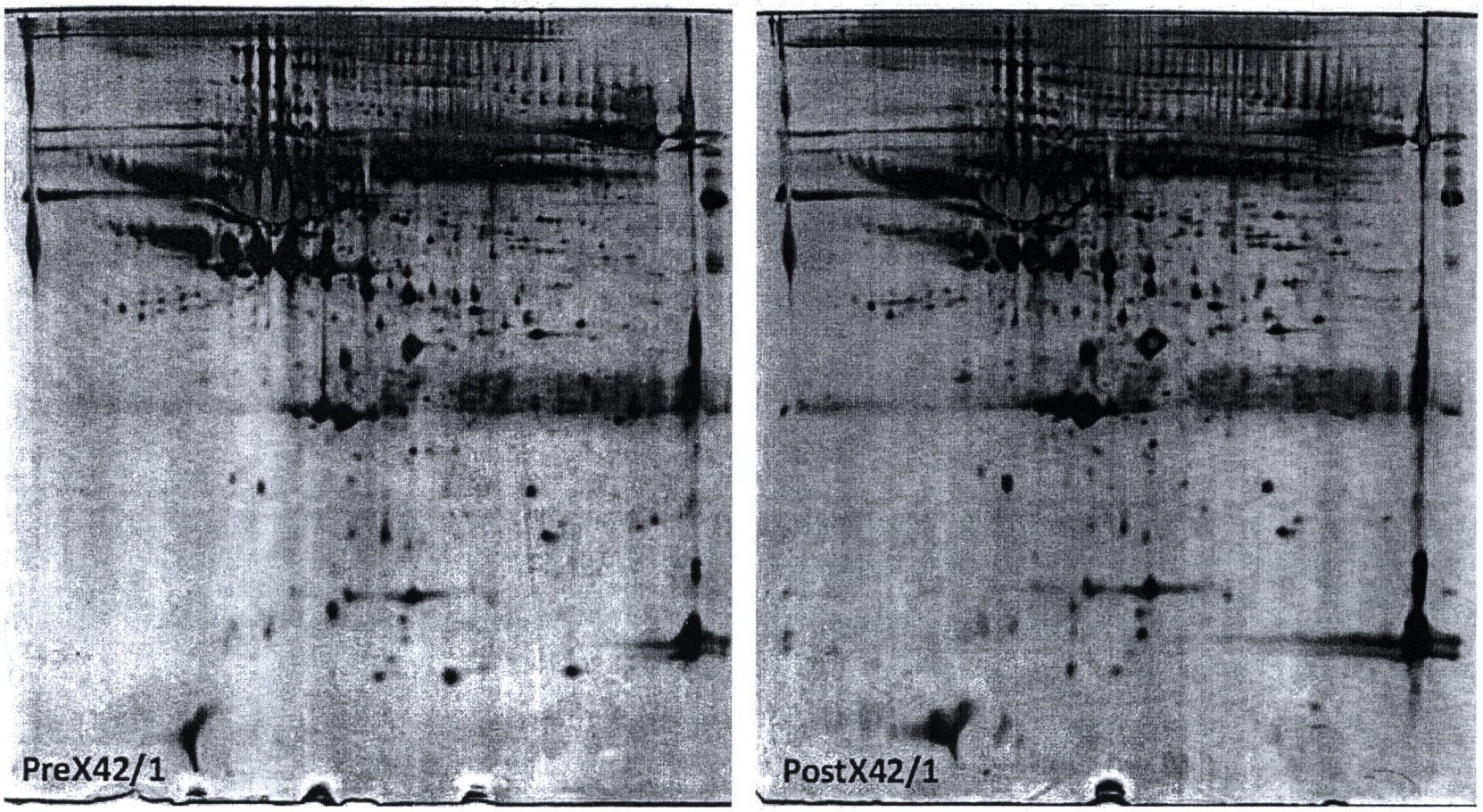

Figure 4-7d Silver stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #1 (left) and 2D gel of X42 post-operative serum #1 (right).

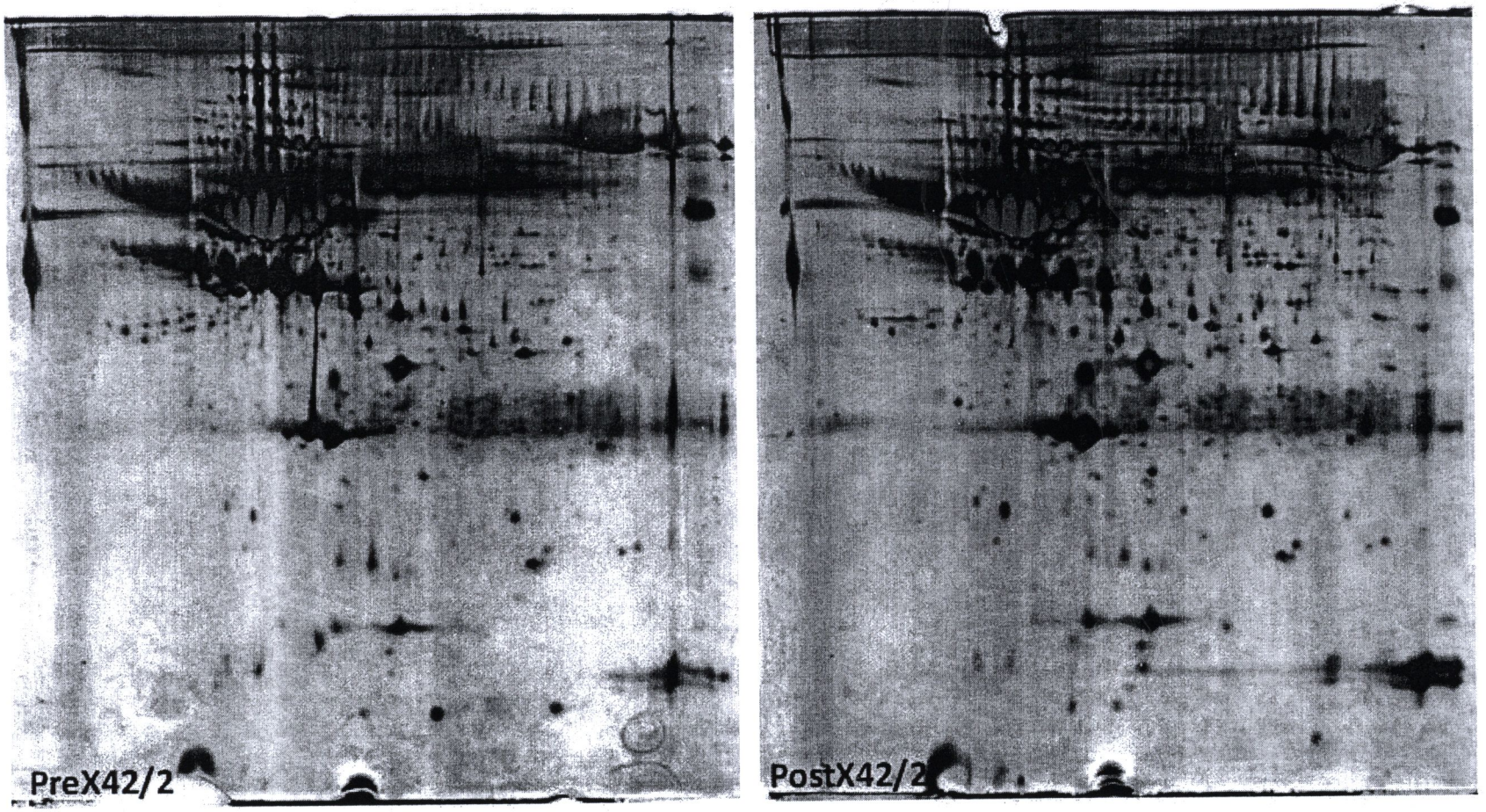

Figure 4-7e Silver stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #2 (left) and 2D gel of X42 post-operative serum #2 (right).

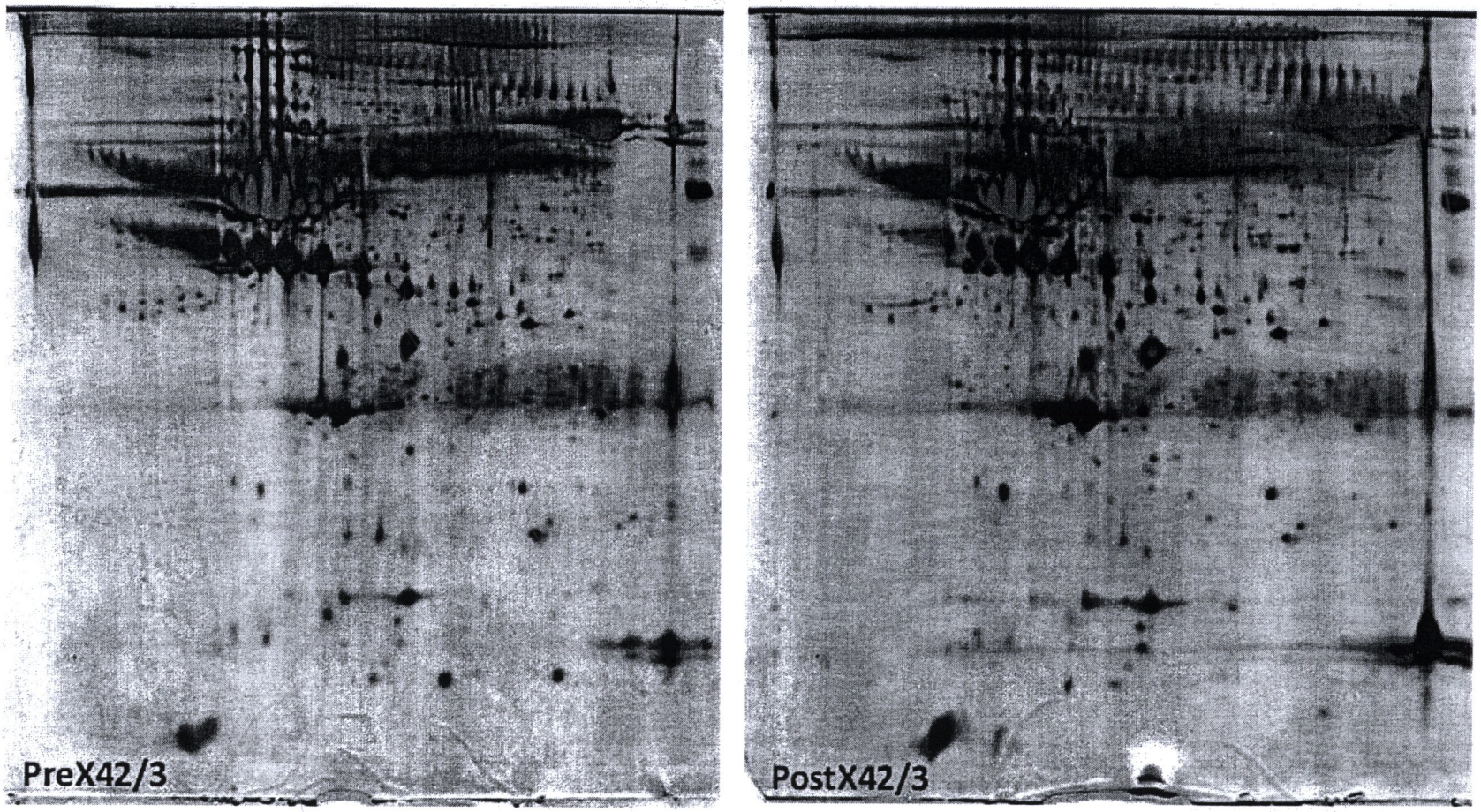

Figure 4-7f Silver stained gels serum 2D pattern of CCA case X42 in both pre- and post-operation: 2D gel of X42 pre-operative serum #3 (left) and 2D gel of X42 post-operative serum #3 (right).

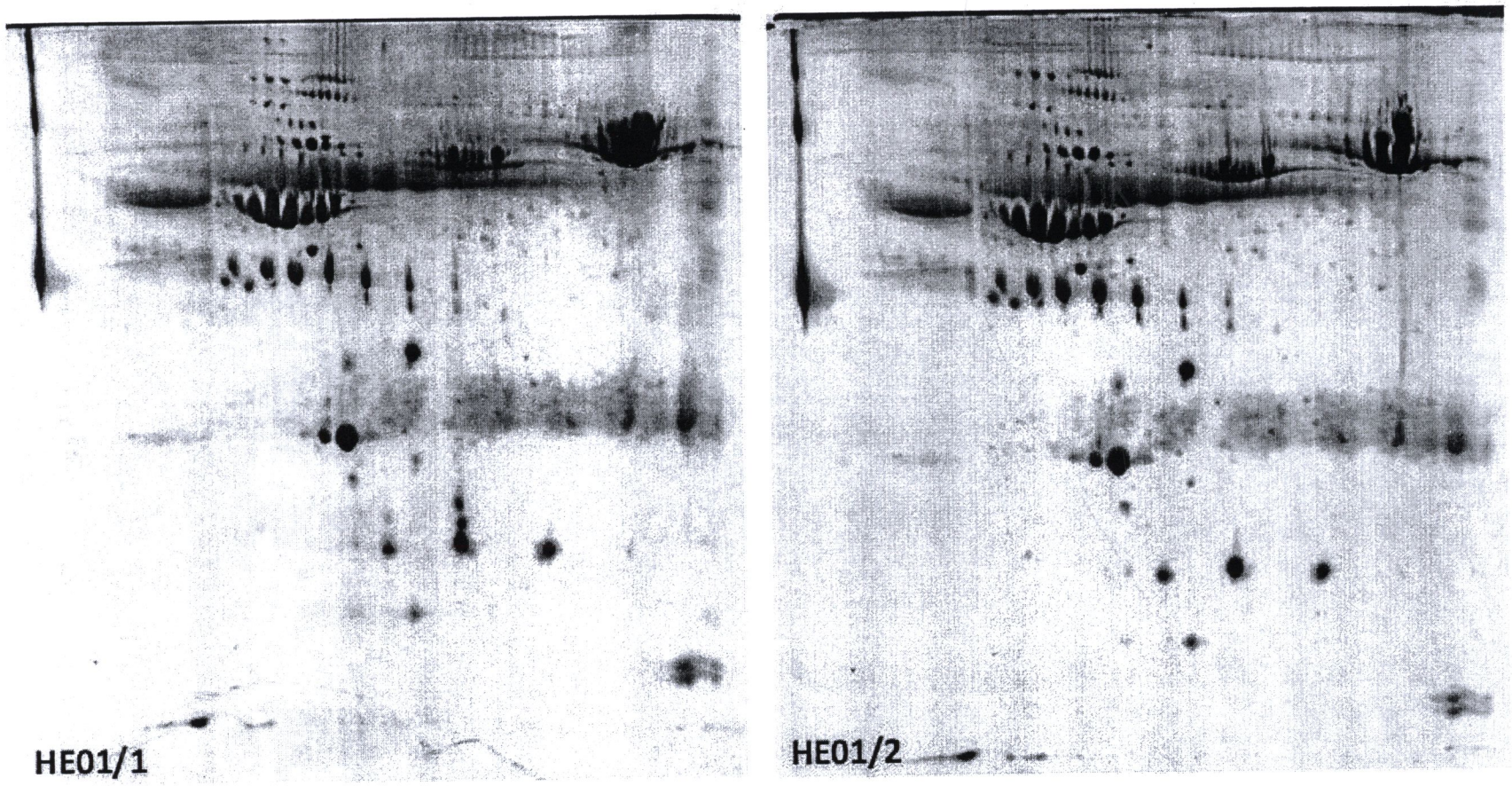

Figure 4-8a Coomassie stained gels serum 2D pattern of healthy subject HE01: 2D gel of HE01 #1 (left) and 2D gel of HE01 #2 (right).

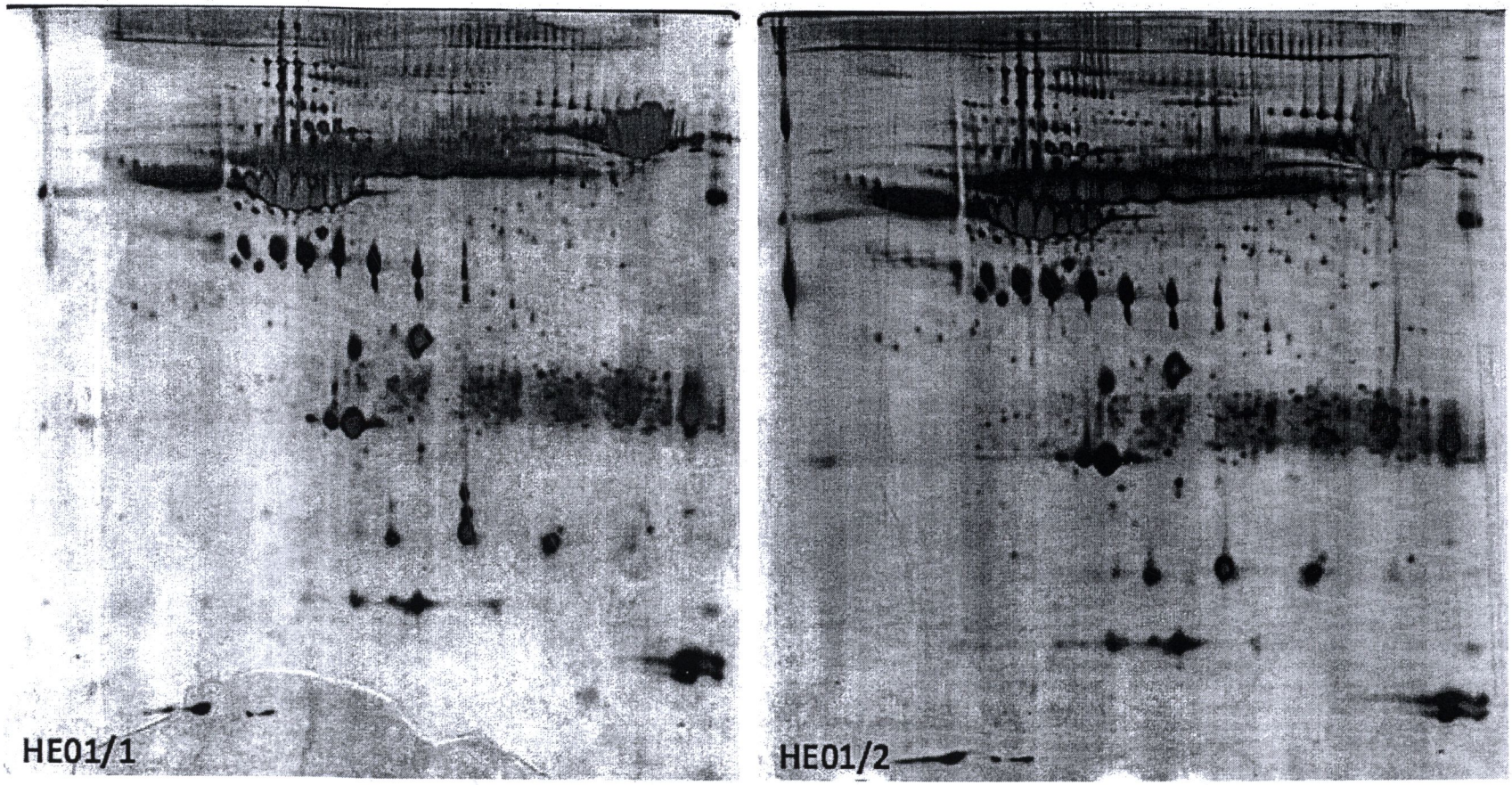

Figure 4-8b Silver stained gels serum 2D pattern of healthy subject HE01: 2D gel of HE01 #1 (left) and 2D gel of HE01 #2 (right).

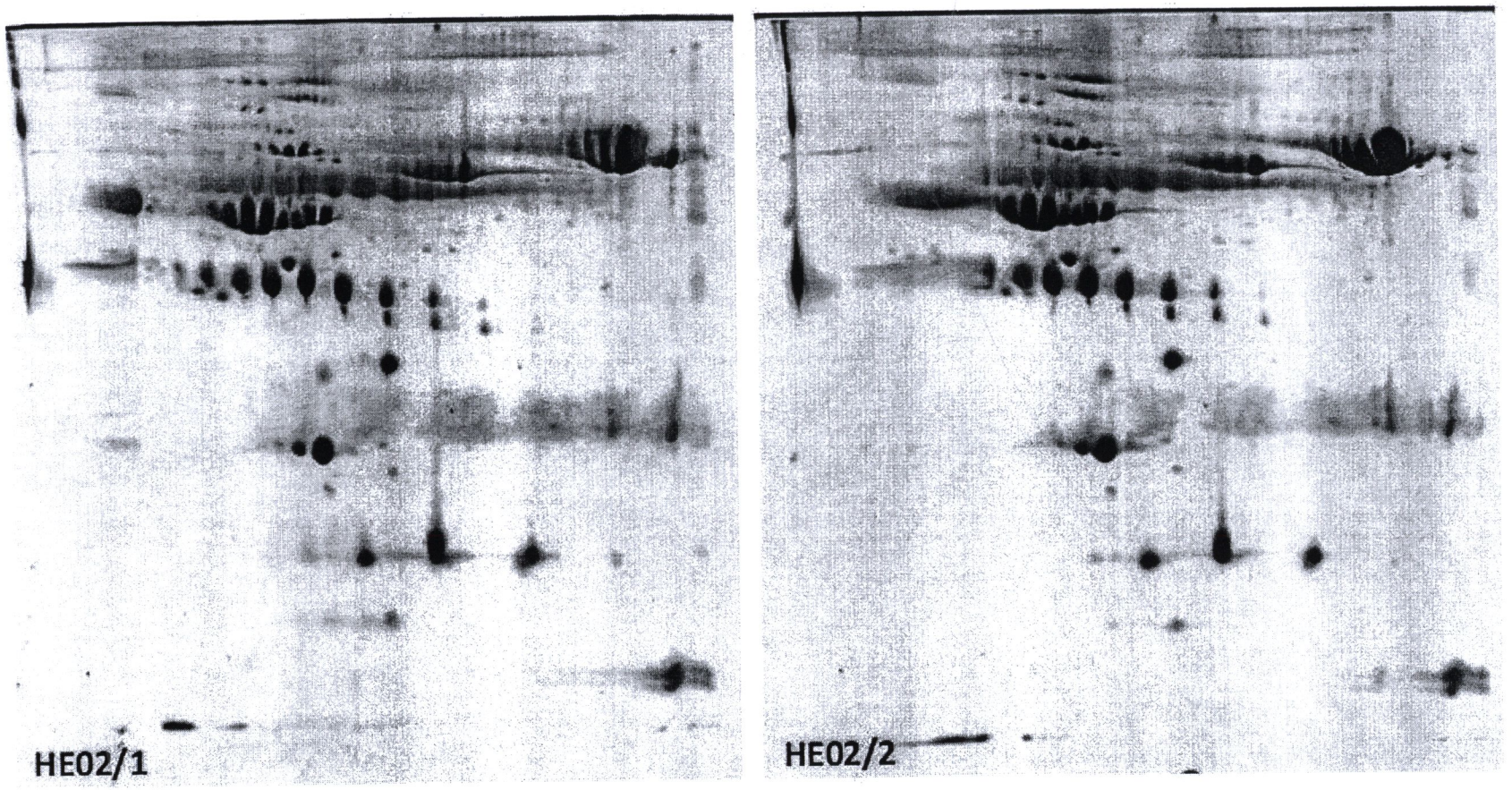

Figure 4-9a Coomassie stained gels serum 2D pattern of healthy subject HE02: 2D gel of HE02 #1 (left) and 2D gel of HE02 #2 (right).

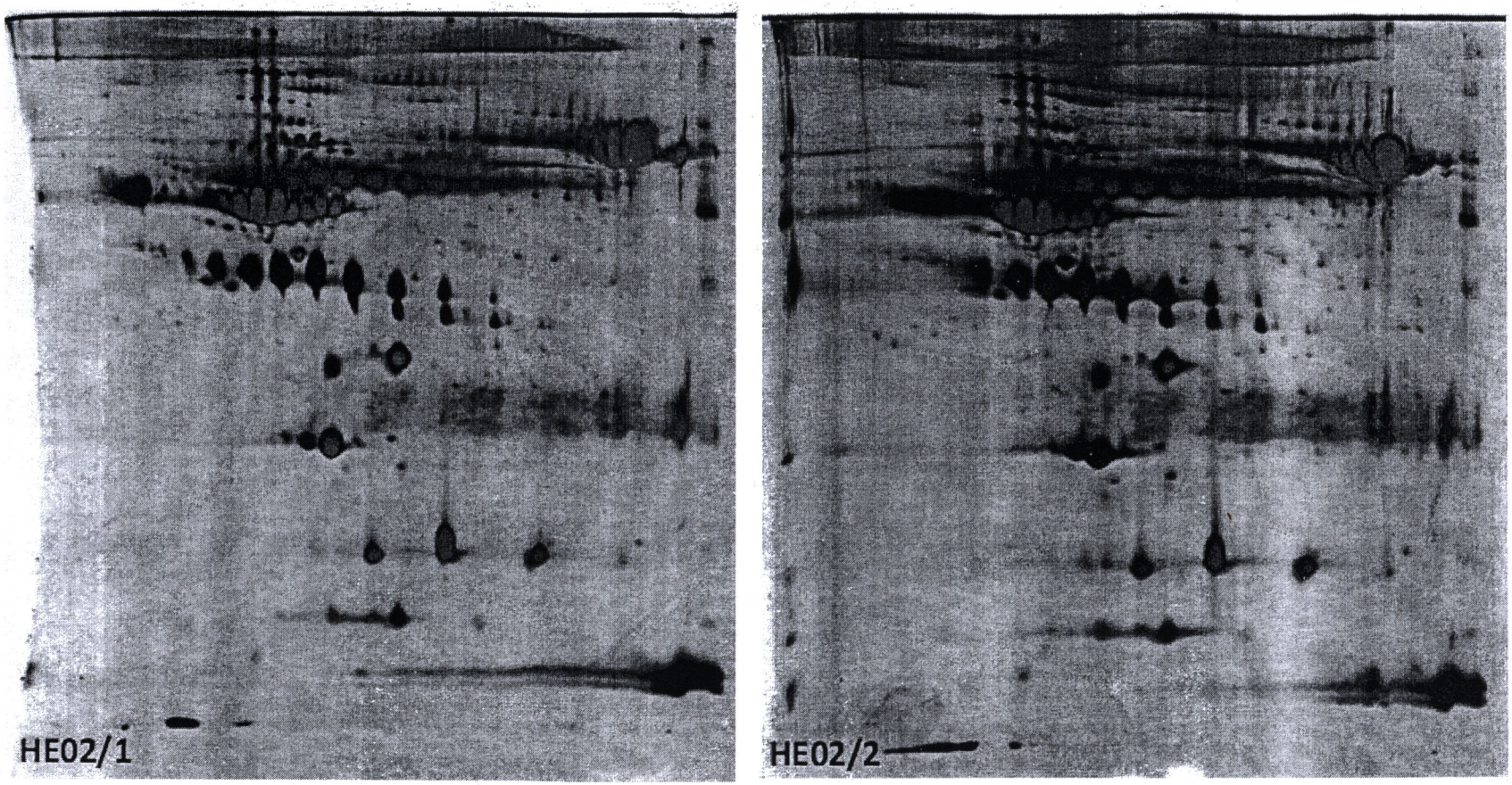

Figure 4-9b Silver stained gels serum 2D pattern of healthy subject HE02: 2D gel of HE02 #1 (left) and 2D gel of HE02 #2 (right).

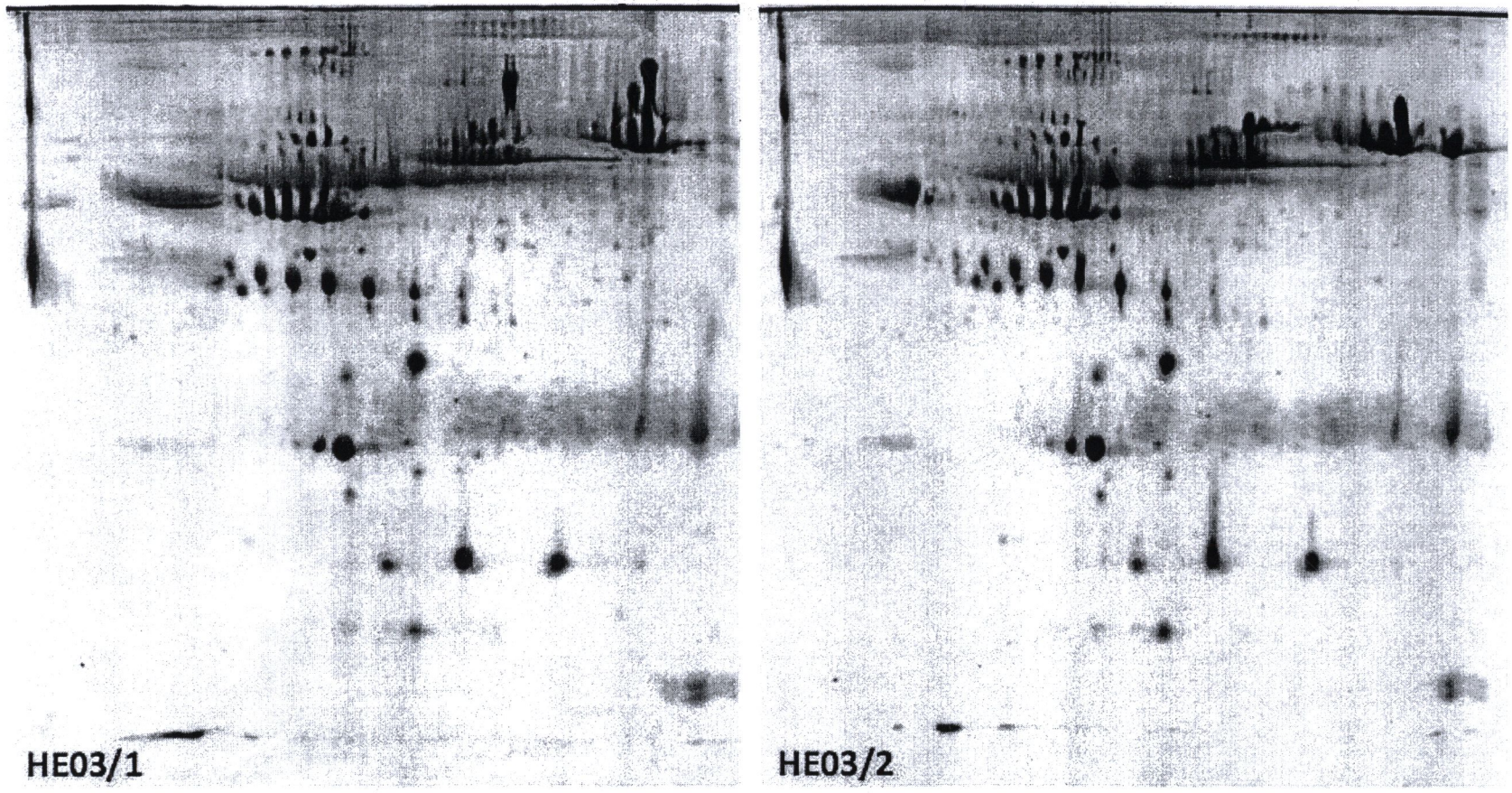

Figure 4-10a Coomassie stained gels serum 2D pattern of healthy subject HE03: 2D gel of HE03 #1 (left) and 2D gel of HE03 #2 (right).

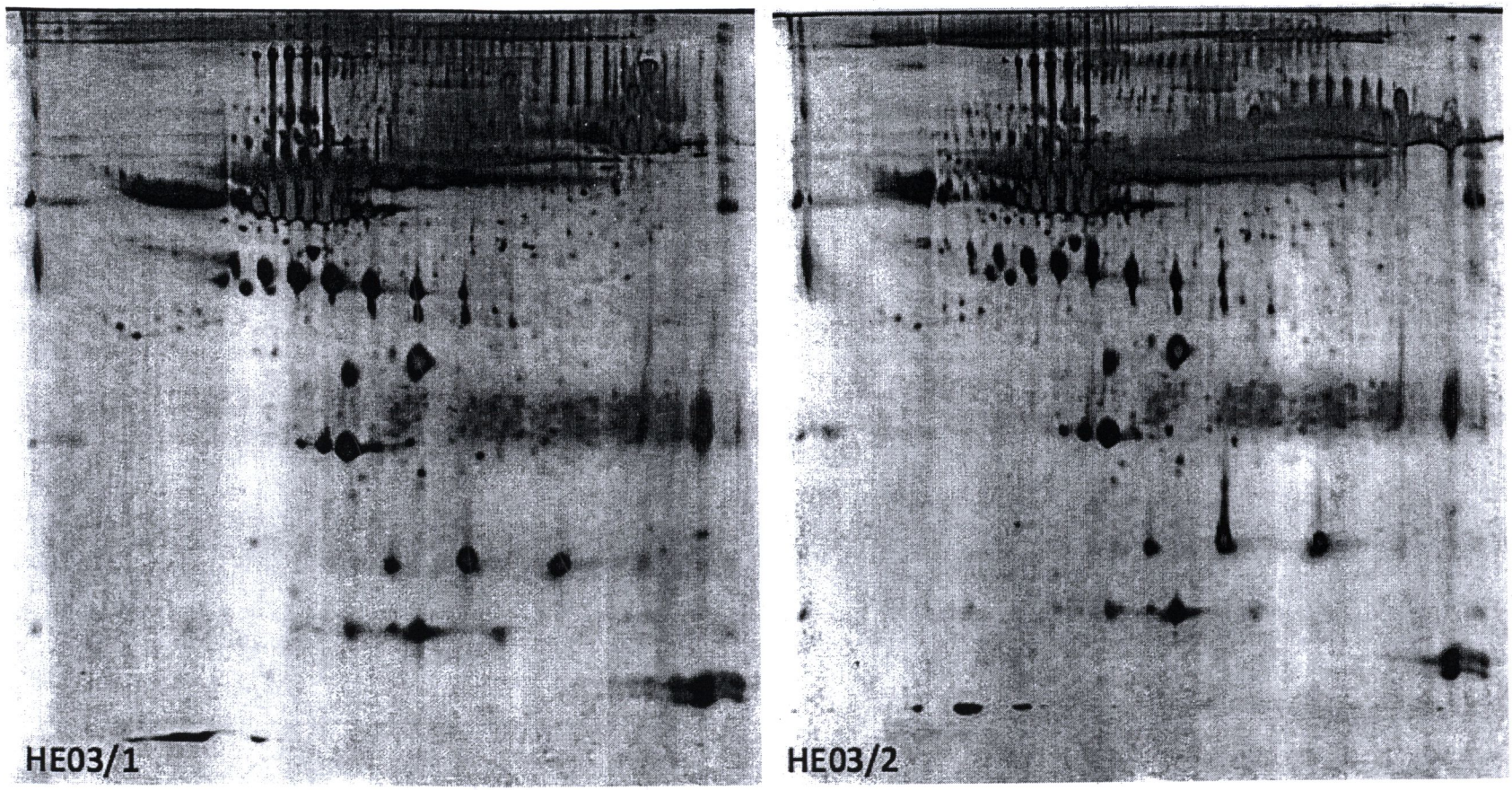

Figure 4-10b Silver stained gels serum 2D pattern of healthy subject HE03: 2D gel of HE03 #1 (left) and 2D gel of HE03 #2 (right).

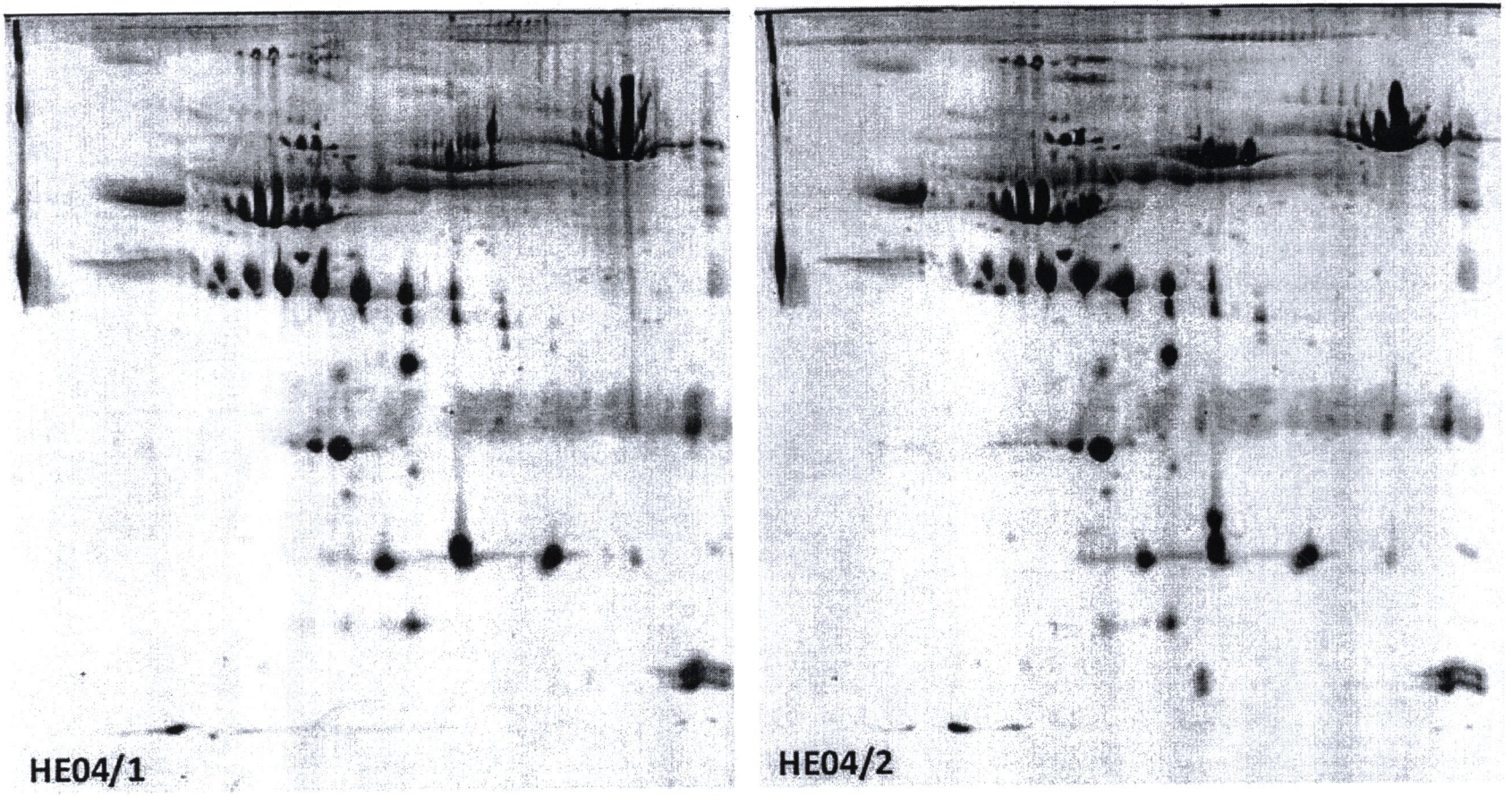

Figure 4-11a Coomassie stained gels serum 2D pattern of healthy subject HE04: 2D gel of HE04 #1 (left) and 2D gel of HE04 #2 (right).

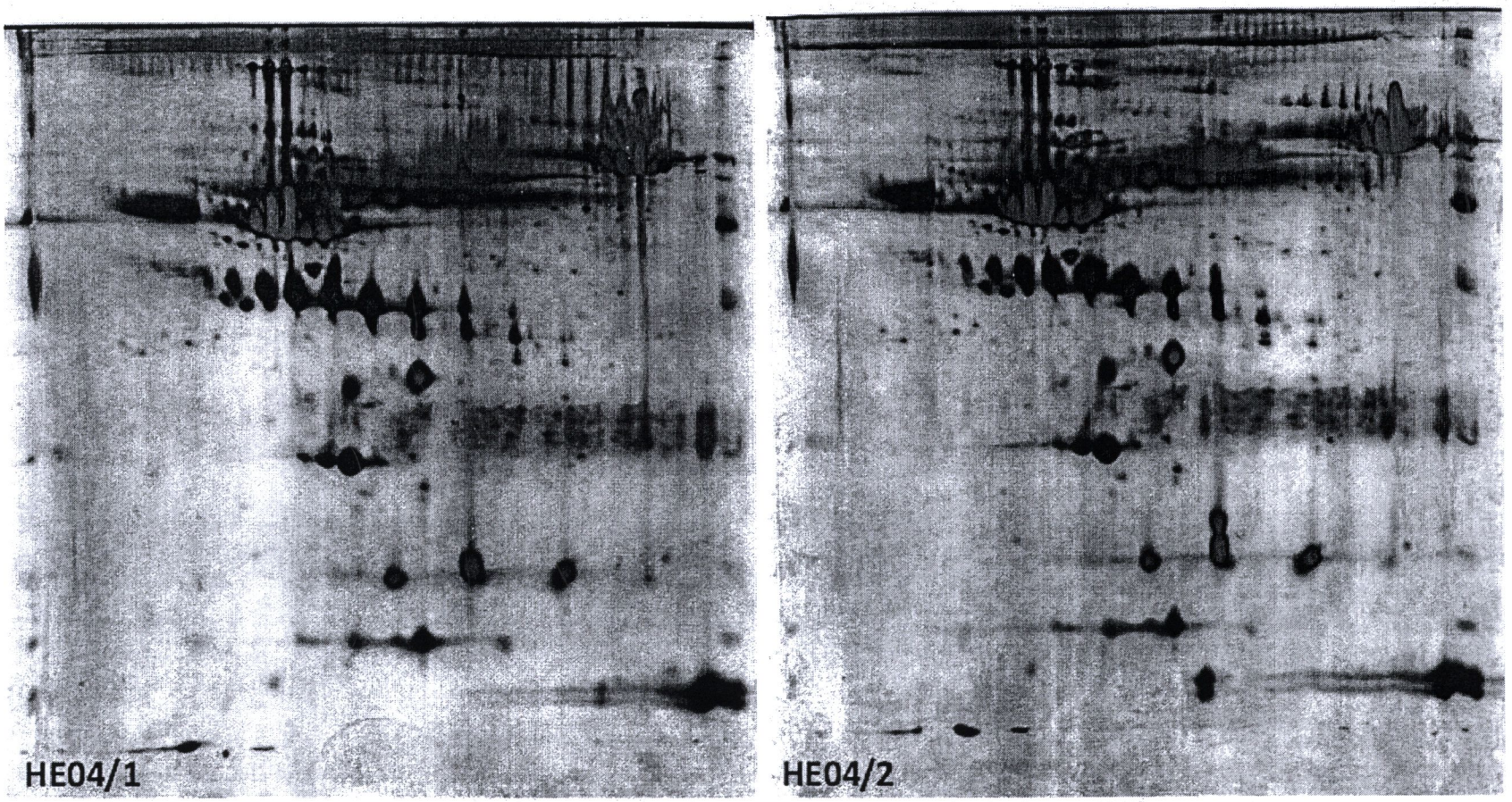

Figure 4-11b Silver stained gels serum 2D pattern of healthy subject HE04: 2D gel of HE04 #1 (left) and 2D gel of HE04 #2 (right).

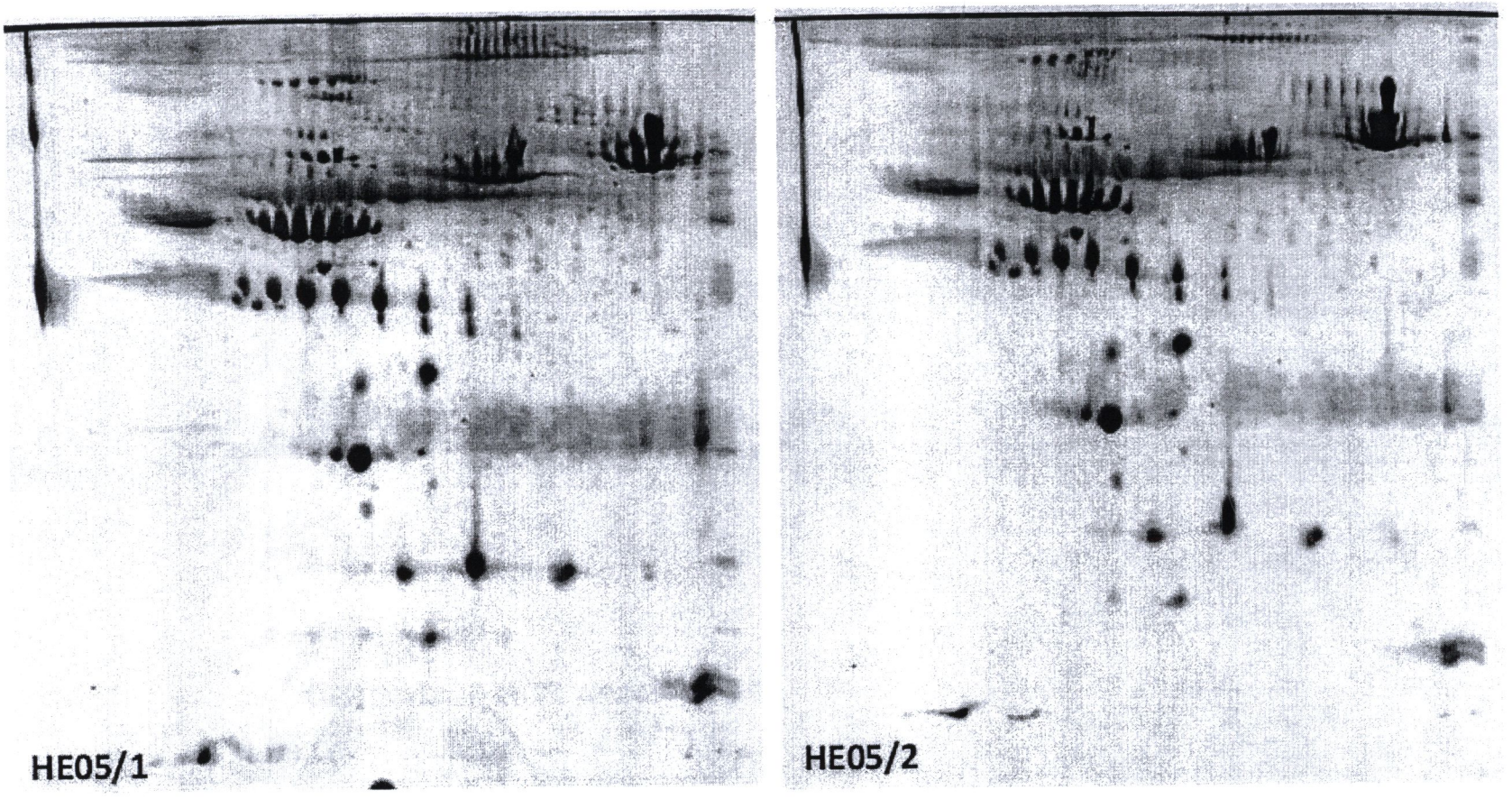

Figure 4-12a Coomassie stained gels serum 2D pattern of healthy subject HE05: 2D gel of HE05 #1 (left) and 2D gel of HE05 #2 (right).

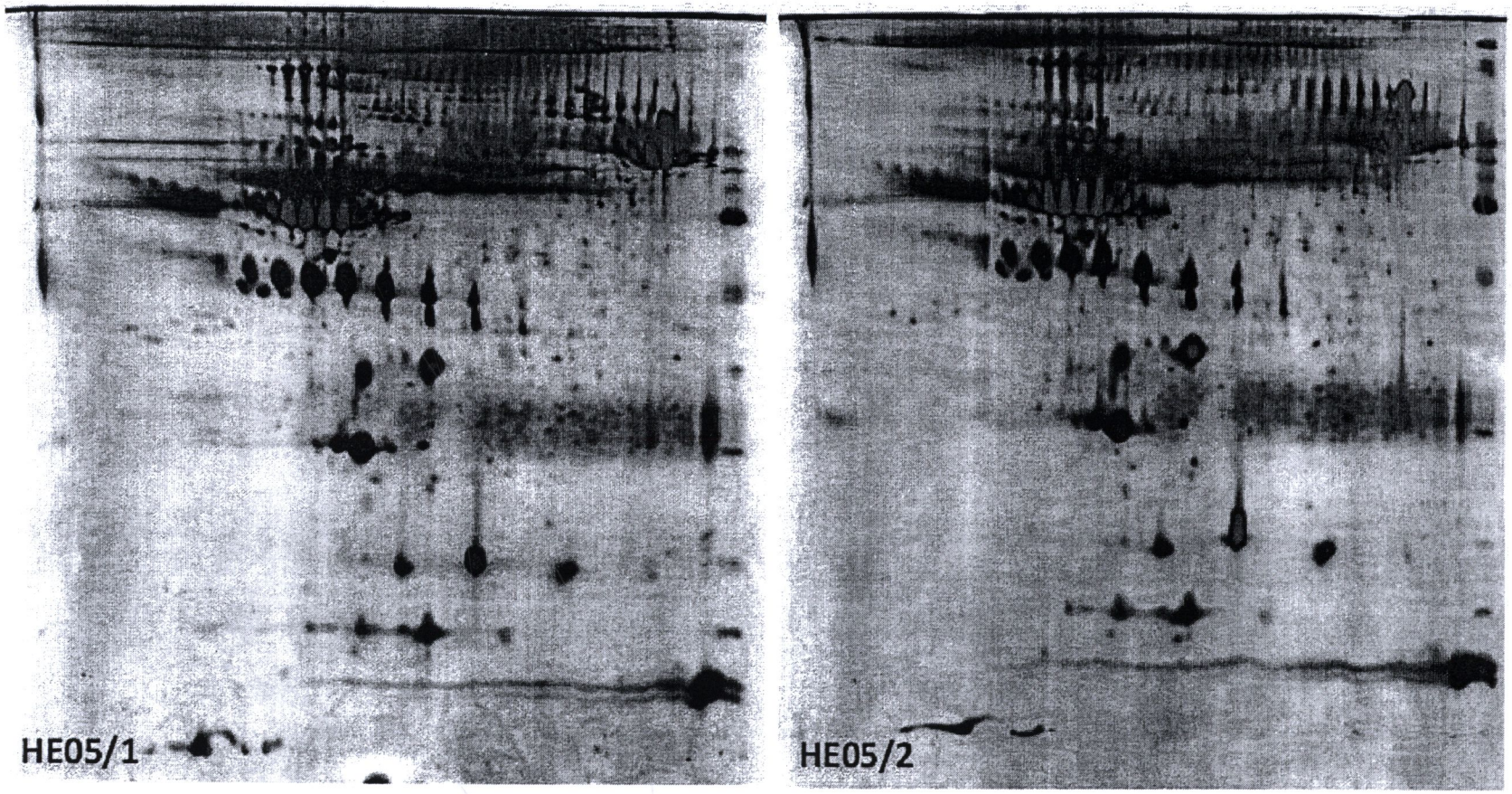

Figure 4-12b Silver stained gels serum 2D pattern of healthy subject HE05: 2D gel of HE05 #1 (left) and 2D gel of HE05 #2 (right).

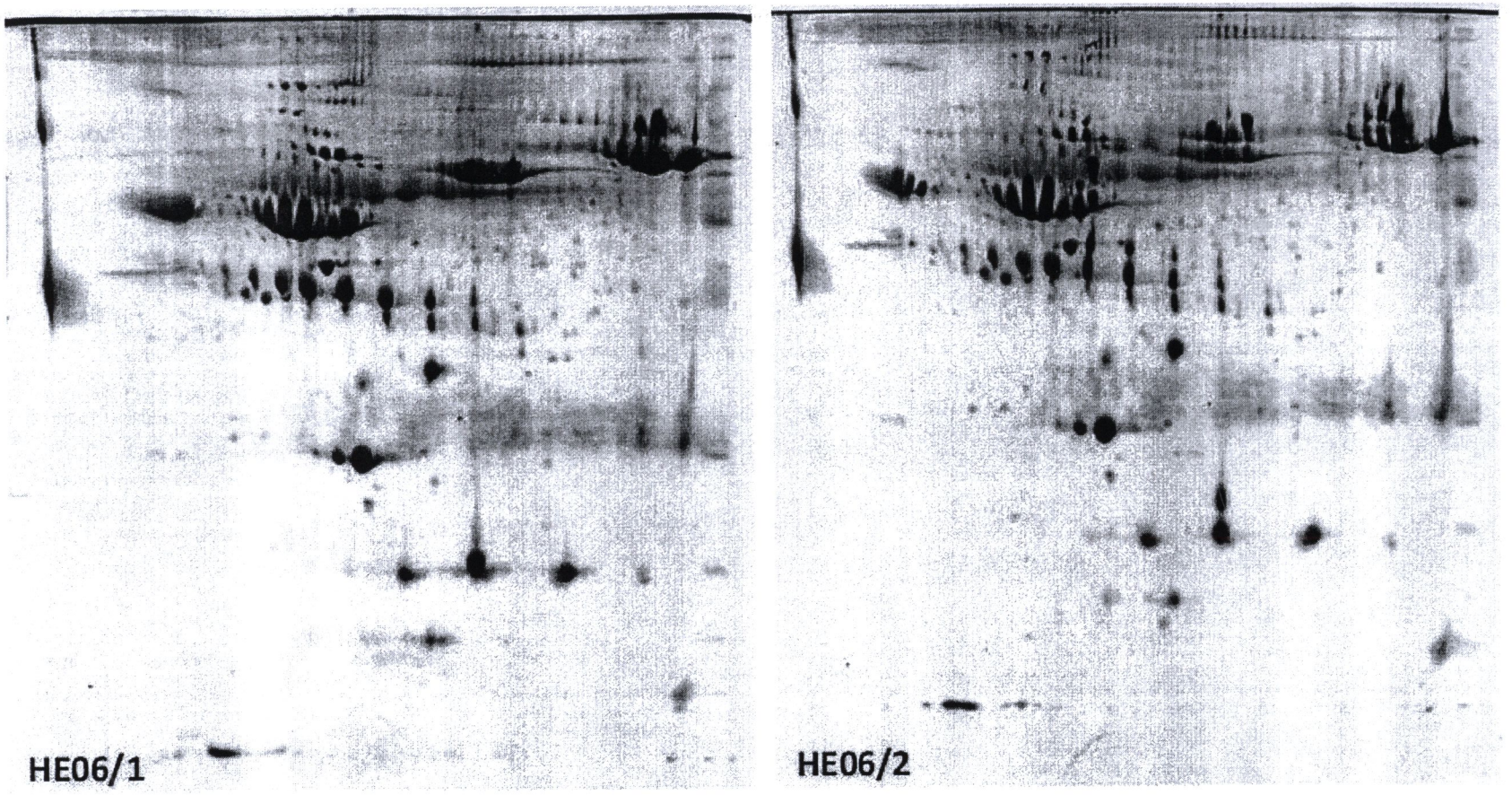

Figure 4-13a Coomassie stained gels serum 2D pattern of healthy subject HE06: 2D gel of HE06 #1 (left) and 2D gel of HE06 #2 (right).

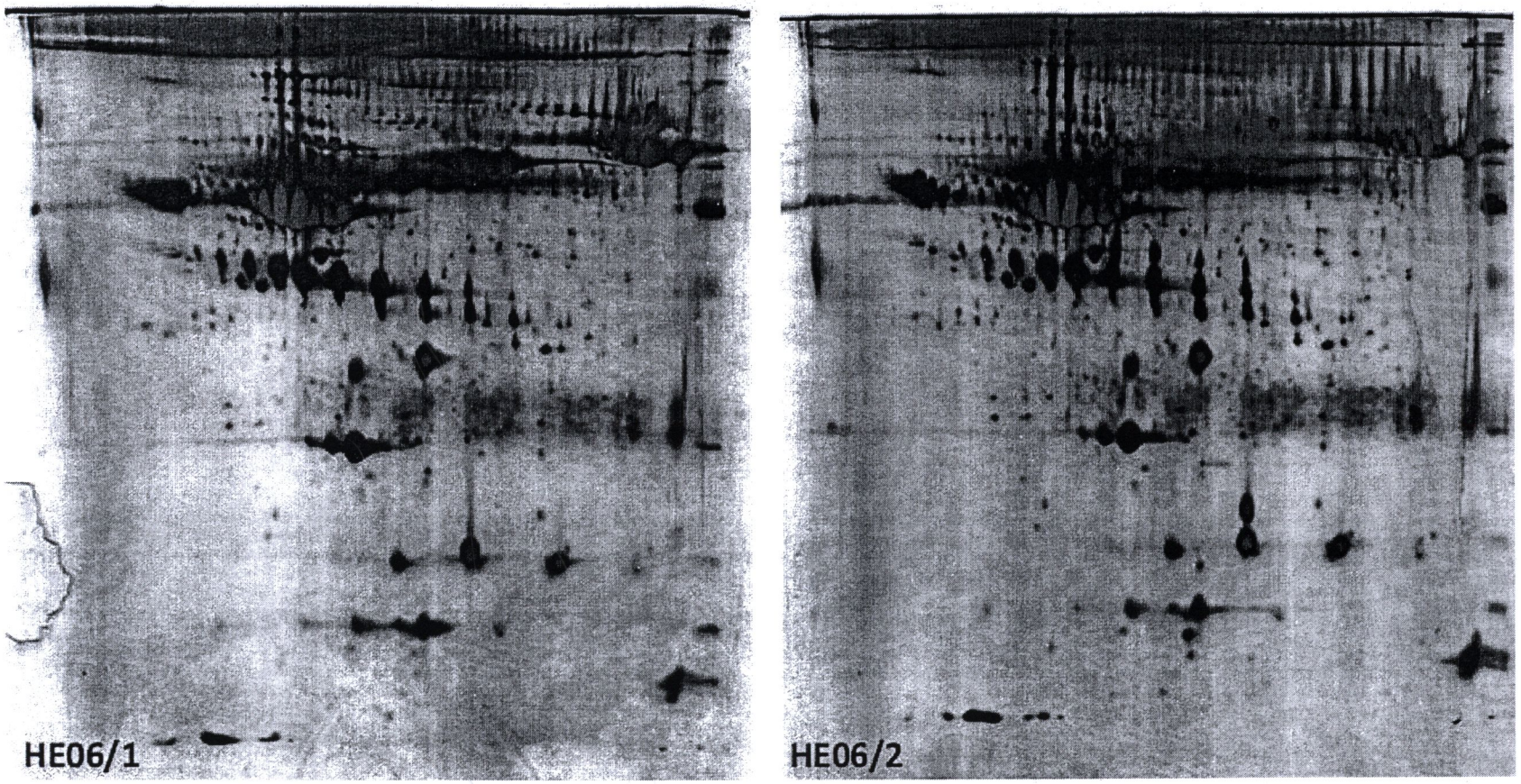

Figure 4-13b Silver stained gels serum 2D pattern of healthy subject HE06: 2D gel of HE06 #1 (left) and 2D gel of HE06 #2 (right).

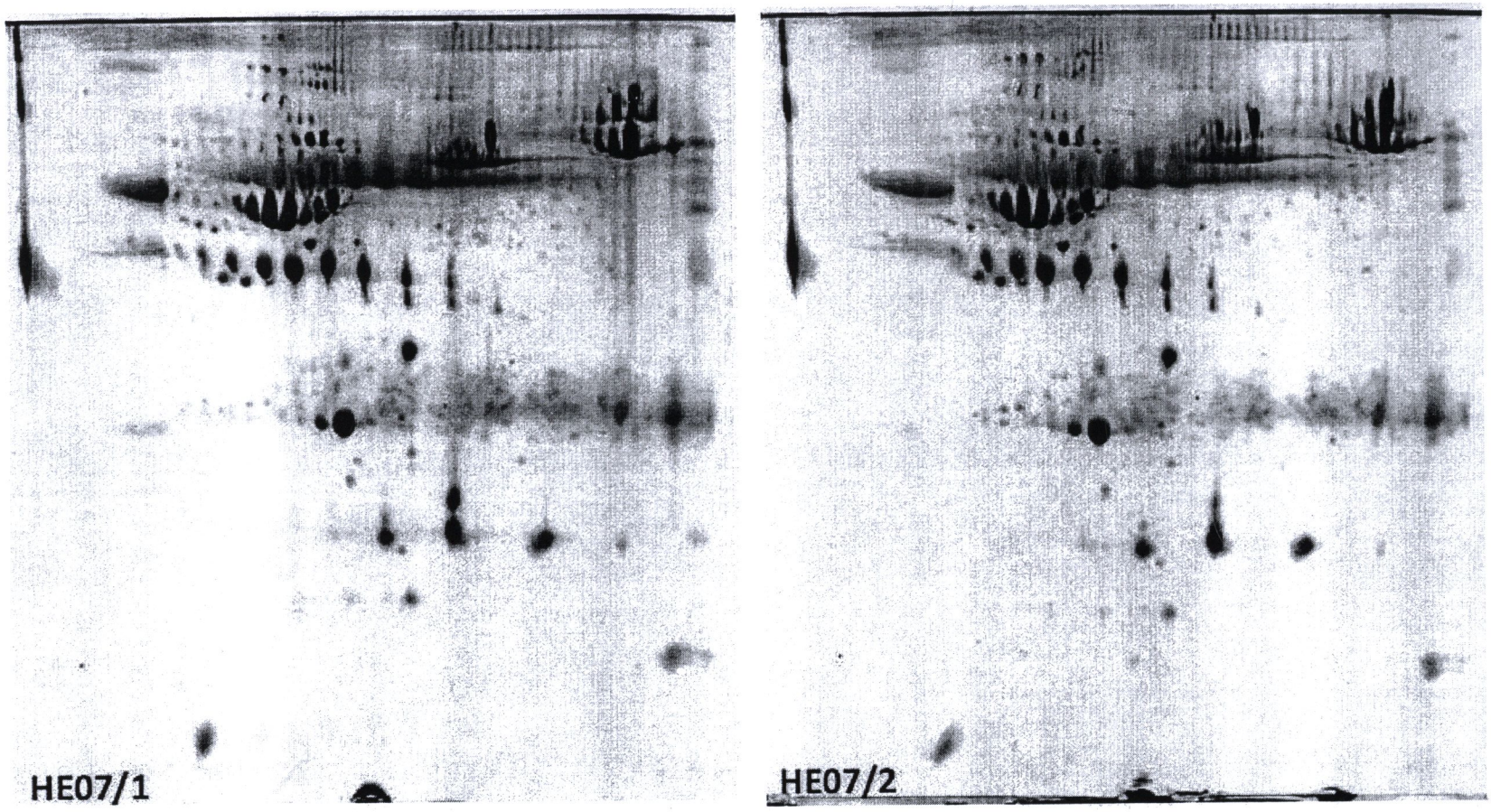

Figure 4-14a Coomassie stained gels serum 2D pattern of healthy subject HE07: 2D gel of HE07 #1 (left) and 2D gel of HE07 #2 (right).

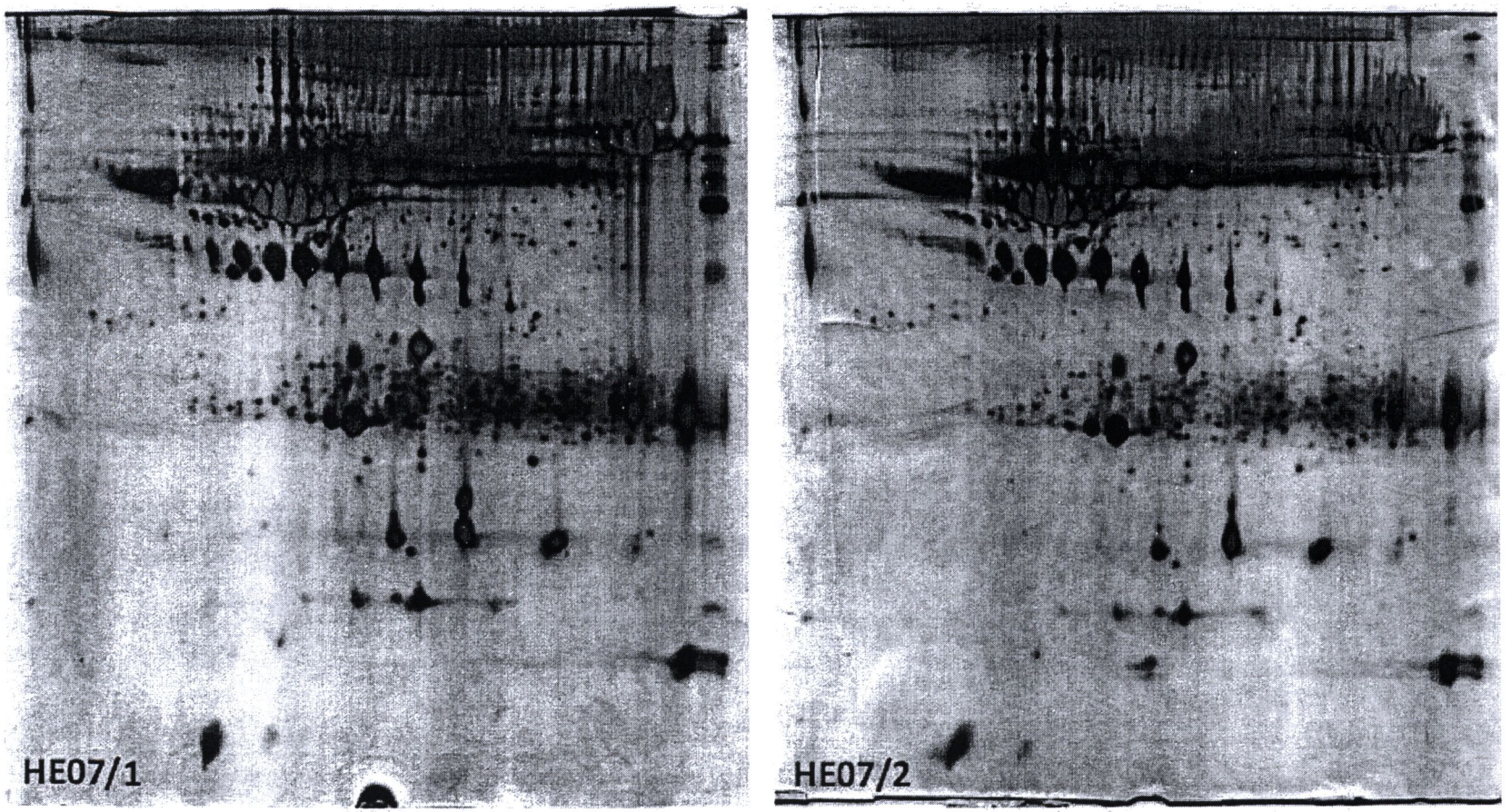

Figure 4-14b Silver stained gels serum 2D pattern of healthy subject HE07: 2D gel of HE07 #1 (left) and 2D gel of HE07 #2 (right).

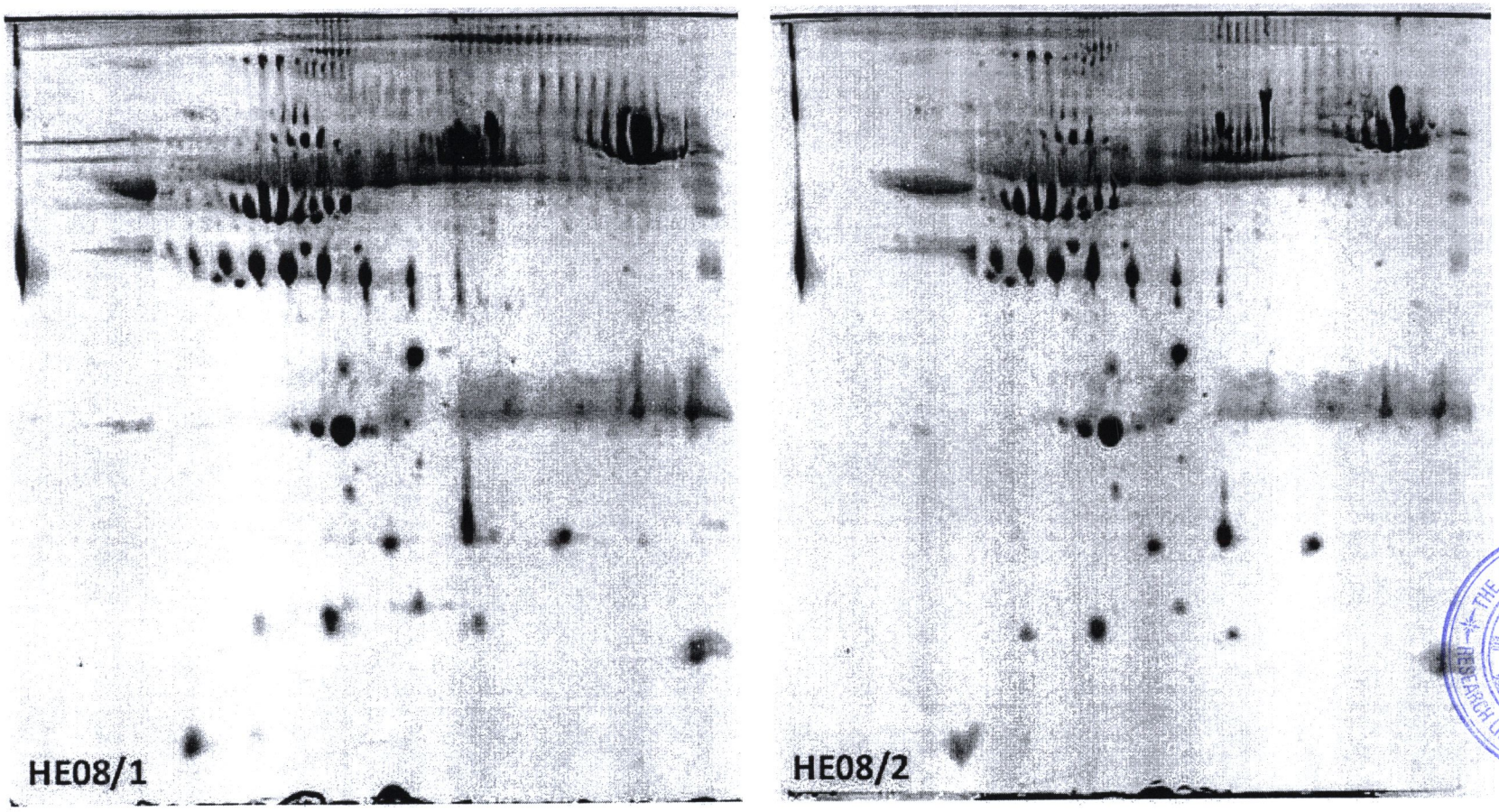

Figure 4-15a Coomassie stained gels serum 2D pattern of healthy subject HE08: 2D gel of HE08 #1 (left) and 2D gel of HE08 #2 (right).

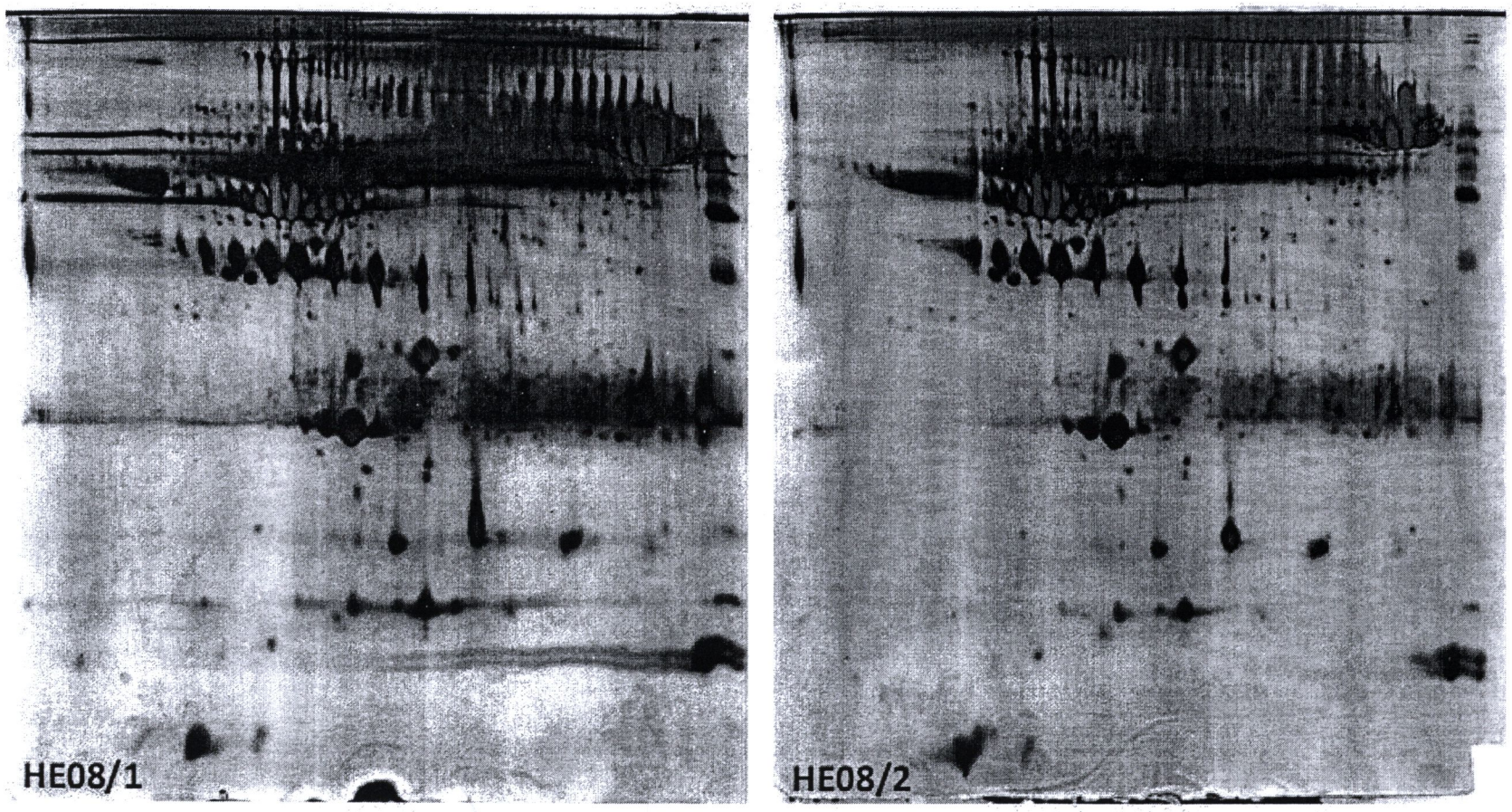

Figure 4-15b Silver stained gels serum 2D pattern of healthy subject HE08: 2D gel of HE08 #1 (left) and 2D gel of HE08 #2 (right).

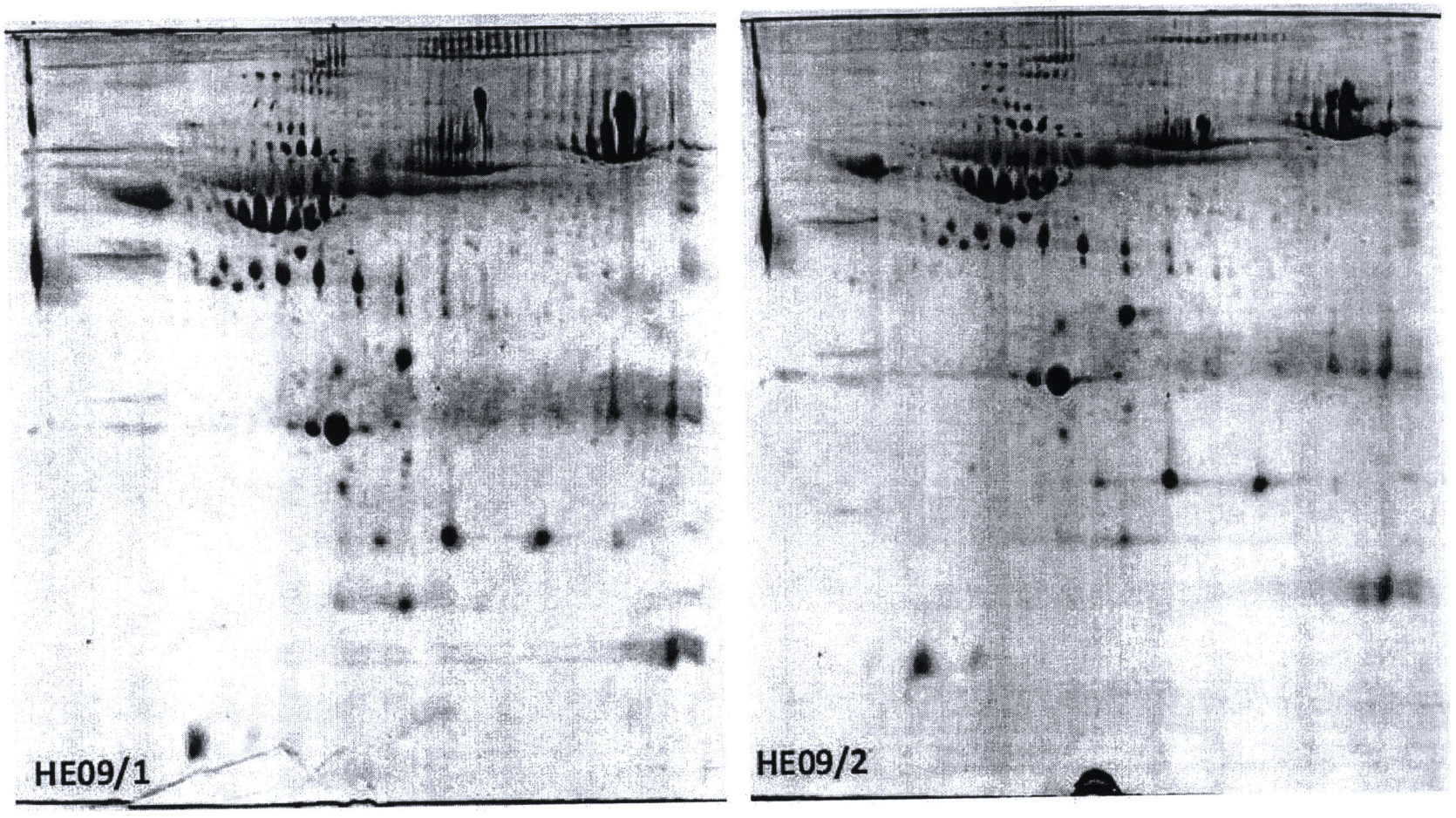

Figure 4-16a Coomassie stained gels serum 2D pattern of healthy subject HE09: 2D gel of HE09 #1 (left) and 2D gel of HE09 #2 (right).

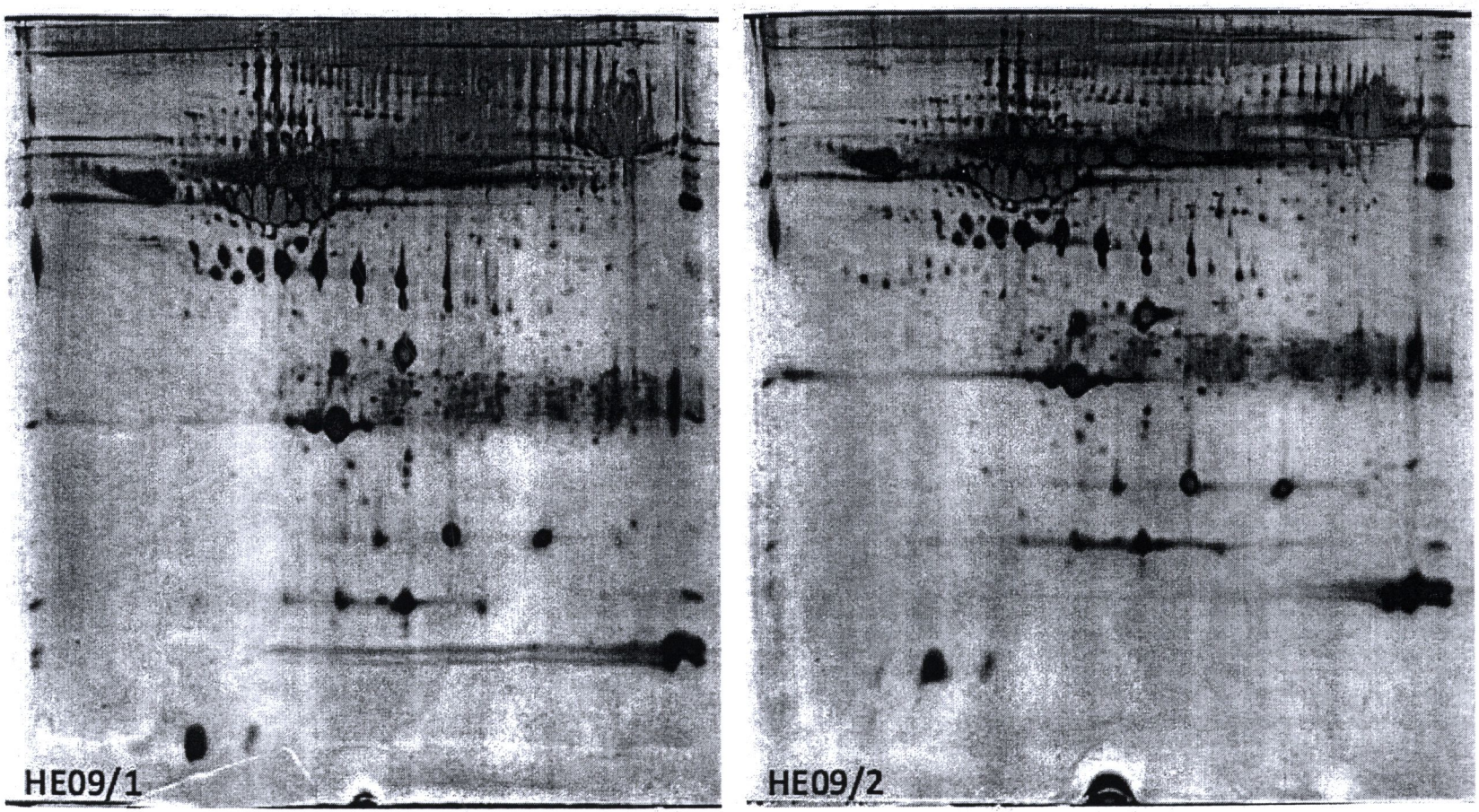

Figure 4-16b Silver stained gels serum 2D pattern of healthy subject HE09: 2D gel of HE09 #1 (left) and 2D gel of HE09 #2 (right).

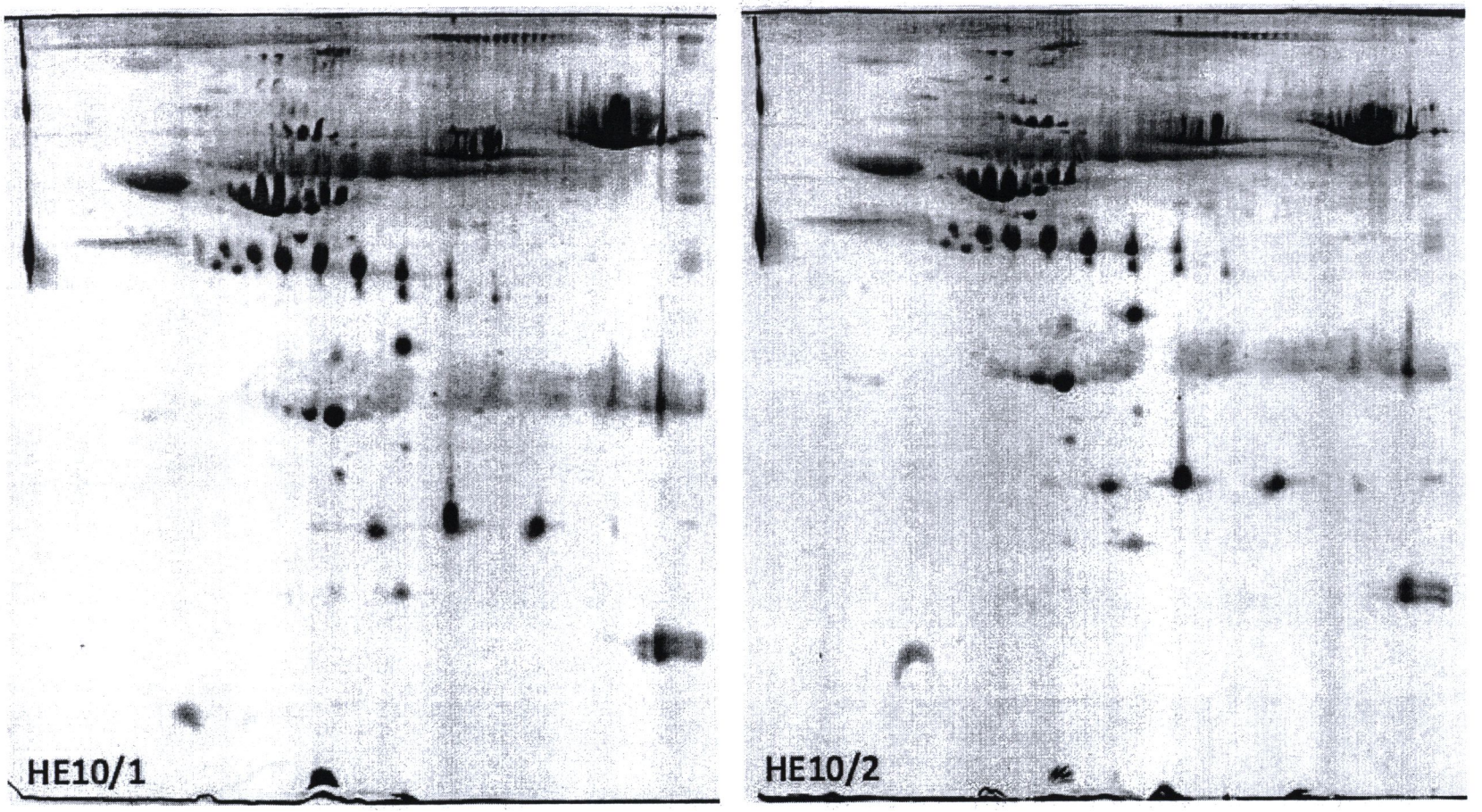

Figure 4-17a Coomassie stained gels serum 2D pattern of healthy subject HE10: 2D gel of HE10 #1 (left) and 2D gel of HE10 #2 (right).

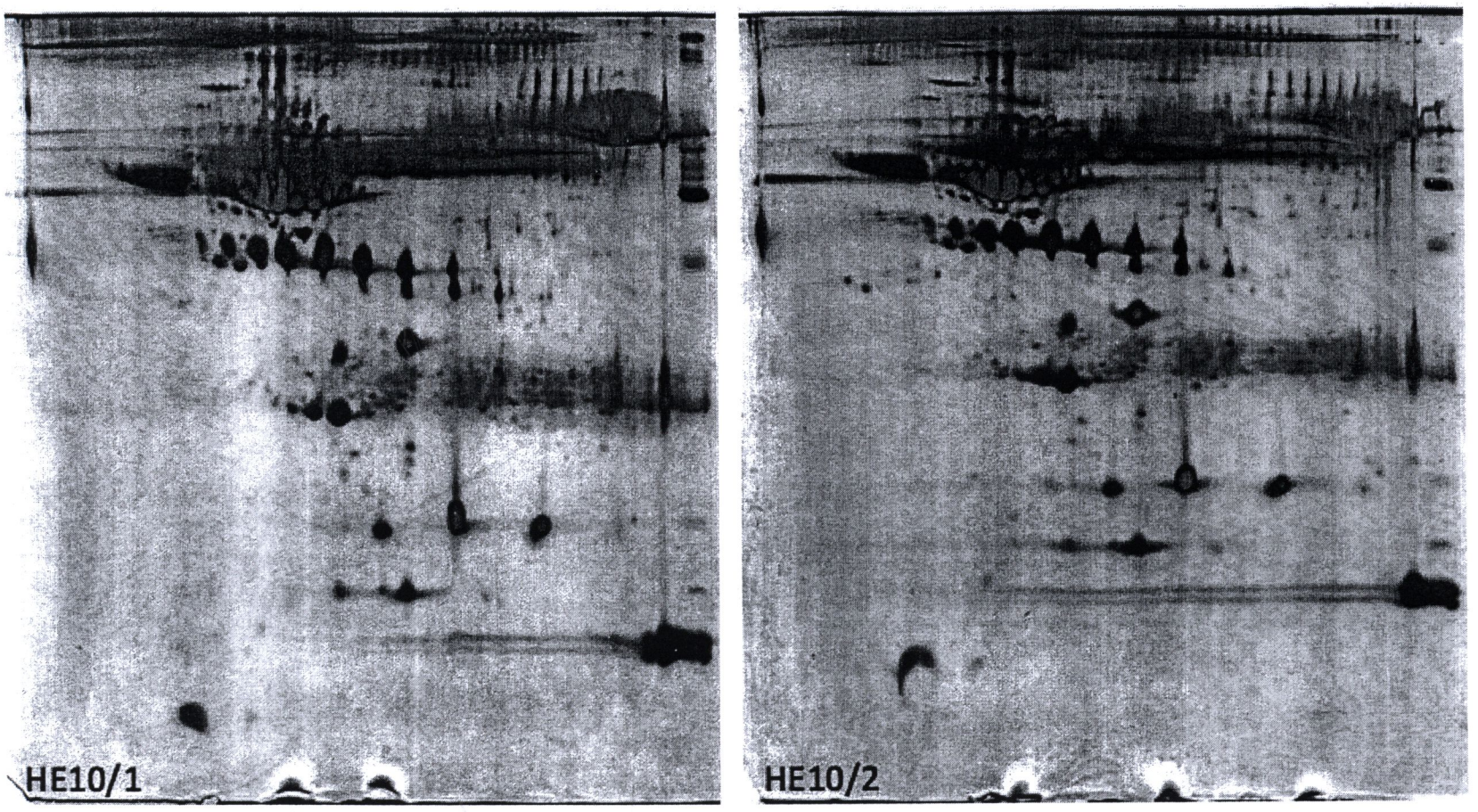

Figure 4-17b Silver stained gels serum 2D pattern of healthy subject HE10: 2D gel of HE10 #1 (left) and 2D gel of HE10 #2 (right).

RESEARCH PUBLICATION

ABSTRACTS AND PROCEEDINGS

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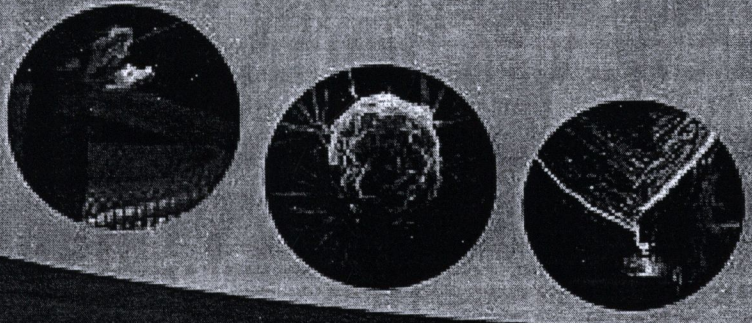

Proceedings 19

DEPLETION OF SERUM ALBUMIN AND IMMUNOGLOBULIN FOR 2-DE ANALYSIS

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ABSTRACT

Serum contains several distinct components including proteins that reflect biochemical and physiological conditions of human body. Many studies suggest that low abundance proteins in serum, such as peptide hormones or small secreted proteins, are associated with pathological conditions and give the opportunity for biomarkers exploration and diagnosis. The serum proteins are extremely diverse in abundance, thus analysis of the serum proteome is challenging. We tried to remove the two most abundant proteins; albumin and immunoglobulin, from human serum by using 2 different HiTrap columns. The method is cheaper comparing to several other depletion kits.

Keywords: serum 2-DE, high abundance protein, albumin and immunoglobulin depletion

INTRODUCTION

Serum is one of the most useful specimens for elucidating biomarkers [1]. Because serum always perfuses tissues, it may indicate the presence of disease by measuring the alteration of specific molecular species in serum [2]. Serum proteome has high protein content, about 60-80 mg/ml that secreted and eliminated from cells and tissues [3-4].

The proteins in serum have a huge dynamic range. There is a difference in the abundance of the most abundant protein, such as albumin, and the lowest abundance proteins that have now been measured clinically for medical use by immunoassays, approximately a 10–100 billion-fold [5]. Most proteomic researchers have tried to avoid using serum because the high abundance of proteins make it so difficult to detect the lower ones. Because the distribution of protein abundance is much more even in tissue and cell culture samples, most of the history of proteomics has been in these samples. There are several procedures and techniques to remove high abundant proteins from serum before studying by proteomics.

Solid phase extraction (SPE) columns are seemingly the method most widely used for depletion of high abundant proteins in serum and they have been widely used as the first step in biomarker-related proteomic studies. Different types of SPE columns based on ion-exchange, metal-chelating, affinity ligands, dye-ligands, bacterial proteins, antibodies or combinations of

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