Proteomic analysis of seminal plasma by 2-D DIGE in men with different spermatogenic impairment

K. Davalieva[1], S. Kiprijanovska[1], T. Plaseski[2], B. Kocevska[2] and D. Plaseska-Karanfilska[1]

[1]Research Centre for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, R Macedonia
e-mail: gde@manu.edu.mk
[2]Endocrinology and Metabolic Disorders Clinic, Faculty of Medicine, Skopje, R. Macedonia

INTRODUCTION

Seminal plasma is a potential source of biomarkers for many disorders of the male reproductive system including male infertility. Proteomic techniques such as 2-D DIGE and MS have the potential to discover links between certain proteins and medical conditions. The identification and characterization of differentially expressed proteins in the seminal plasma can serve as a basis for estimating male infertility.

AIM OF THE STUDY

In this study, we applied 2-D DIGE approach in detection of differential protein expression between four distinct groups of men with normozoospermia, oligozoospermia, asthenozoospermia and azoospermia. The objectives of the present study were to: (I) compare differences in the seminal plasma proteins between fertile, subfertile and infertile males; and (II) to evaluate the presence of seminal plasma proteins associated with different stages of reduced fertility and infertility.

MATERIALS AND METHODS

The 2-D DIGE analysis was performed on IPG strips pH 4-7, 24 cm, with 4 biological replicates per group. DIGE images were analyzed using Image Master Platinum software 7.1 (GE Healthcare). Statistically significant, differentially expressed proteins were selected based on Annova < 0.05 and Ratio > 1.8. Protein identification was done by MALDI-TOF-TOF 4800 (ABI). Database searching was carried out using Mascot version 2.2 via GPS explorer software (ABI) combining MS and MS/MS interrogations on Human proteins from Swiss-Prot databank.

RESULTS

Our results showed that there are no statistically significant differences in protein expression between three groups: normozoospermic, oligozoospermic and asthenozoospermic. However, we found a total of 8 proteins with statistically significant increased expression in azoospermia compared to at least one of the other studied groups (Table 1 and Figure 1). . The identified proteins were: FINC, PAP, PSA3, B2MG, LG3BP, PIP and CNDP2 (Table 2). Notably, PAP was found to be increased in azoospermic patients compared to all other groups.

Table 1. The statistical significance (p<0.05) and calculation of ratios of differentially expressed spots showing change in protein level of more than 1.8 fold.

Classes (A/B)	Match ID	Reference spot[a)]	Annova <0.05	VolRatio Mean (A)	SD (A)	VolRatio Mean (B)	SD (B)	Ratio (A/B)	Fold change[b)]
A=Normozoospermia B=Azoospermia	58	1	0.048	0.650	0.25	2.008	0.80	0.32	-3.1
	59	2	0.031	0.140	0.24	1.589	0.73	0.09	-11.3
	167	3	0.028	0.517	0.47	1.719	0.40	0.30	-3.3
	259	4	0.036	0.498	0.45	1.595	0.41	0.31	-3.2
	446	5	0.037	0.944	0.36	1.959	0.44	0.48	-2.1
A=Oligozoospermia B= Azoospermia	12	6	0.015	0.136	0.24	1.461	0.51	0.09	-10.8
	59	2	0.025	0.080	0.14	1.589	0.73	0.05	-19.9
	167	3	0.035	0.845	0.27	1.719	0.40	0.49	-2.0
	358	7	0.045	0.888	0.22	1.810	0.51	0.49	-2.0
A=Asthenozoospermia B= Azoospermia	146	8	0.011	0.317	0.08	1.572	0.48	0.20	-5.0
	167	3	0.030	0.669	0.38	1.719	0.40	0.39	-2.6
	259	4	0.037	0.335	0.58	1.595	0.41	0.21	-4.8
	446	5	0.047	0.828	0.53	1.959	0.44	0.42	-2.4

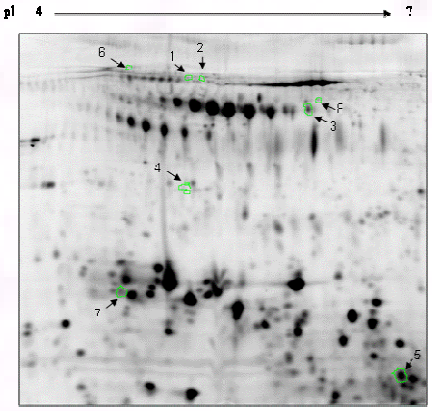

Figure 1. 2-D map of the proteome obtained from seminal plasma by using IEF on pH 4-7 IPG strip (24 cm) and 2-D gel electrophoresis on 12% SDS-PAGE. All the differentially expressed proteins between studied groups are marked with numbered arrows. Details of these proteins identified by MALDI-TOF-TOF are tabulated in Table 2.

Table 2. List of differentially expressed proteins in the seminal plasma between 4 studied groups with different levels of fertility, identified by MALDI-TOF-TOF.

Reference spot[a)]	Name of the protein in SwissProt or NCBI	Number of the protein in SwissProt	Total ion score	Best ion score	No of peptides identified	pI teor	Mw theor (kDa)	pI Exper	Mw Exper (kDa)	% of seq coverage
1	Fibronectin	FINC_HUMAN	93	24	5 / 5	5.5	262	5.3	69	2
2	*no protein identified*	-	-	-	-	-	-	5.8	72	-
3	Prostatic acid phosphatase	PPAP_HUMAN	513	96	9 / 9	5.8	45	6.0	55	24
4	Proteasome subunit alpha type-3	PSA3_HUMAN	99	50	3 / 4	5.2	28	5.3	31	18
5	Beta-2-microglobulin	B2MG_HUMAN	242	94	3 / 3	6.1	14	6.6	9	35
6	Galectin-3-binding protein	LG3BP_HUMAN	468	77	10/10	5.1	65	4.8	72	16
7	Prolactin-inducible protein	PIP_HUMAN	527	109	6 / 6	8.3	17	4.8	14	55
8	Cytosolic non-specific dipeptidase	CNDP2_HUMAN	410	68	9 / 11	5.7	53	6.1	59	33

a) Spot numbers are indicated in Figure 2
b) Numbers correspond to unique peptides identified by MS and MS/MS respectively

CONCLUSION

We suggest that the identified panel of proteins in our study especially PAP, have a strong potential to be used as a azoospermia markers. However, further investigations will be necessary to validate these markers in samples of larger and independent patient cohorts and to clarify their role in the pathogenesis of male infertility.

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