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# P-39 Protein Profiling by SELDI-TOF Mass Spectrometry and Differential Expressions of Apoptosis Regulators in Human Testis with Obstructive and Nonobstructive Azoospermia

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**Background & Objectives:** To provide evidence that a pathological process of apoptosis in testicular cells may participate in developing hypospermatogenesis of the patients with nonobstructive azoospermia and to find the effective biomarkers by protein profiling using surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS).

**Method:** We investigated the differential expressions of Fas/FasL, caspase-3 and Bcl-2 related gene families and provided protein profiling by SELDI-TOF MS in normal testis and in some selected human testicular pathologies. By means of RT-PCR and immunohistochemistry, differential expressions of Fas/FasL, caspase-3 and Bcl-2 related gene families were evaluated in the human testis. The frequency of apoptosis of testicular cells was demonstrated by the in situ 3'-end-labelling method. SELDI analysis was performed by using hydrophobic chips (H4) to compare and identify differences in the protein expression patterns between testicular pathologies and normal testis at the molecular weight from 10 to 100 kDa (i.e., using SPA as a matrix).

**Results:** Fas and FasL mRNAs were increased in testes with Sertoli cell only (SCO) syndrome and Klinefelter's syndrome. Immunohistochemistry demonstrated the intense coexpressions of Fas and FasL in Leydig cells of normal testes, however, this was not associated with the presence of apoptotic interstitial cells. The positive staining of FasL in the seminiferous tubules was also observed in Sertoli cells and Fas expression was detected on occasional spermatocytes in normal testes. In the testes with SCO syndrome, increased immunostaining of Fas and FasL was detected in both Sertoli and interstitial cells and the increased apoptotic cells were also observed in some of SCO patients. In the testes with maturation arrest, the expression of FasL was only increased in/around Sertoli cells. The differential expressions of proteins in testicular pathologies (SCO syndrome, Klinefelter's syndrome, maturation arrest, and hypogonadotropic hypogonadism) and normal cases were examined by SELDI MS using H4 ProteinChip array. In general, there were less peaks in the testicular pathologies than in normal cases. Especially, the 16 kDa peak in the testicular pathologies was lower (around 70%, intensity) in case of SCO or disappeared in cases of maturation arrest, Klinefelter's syndrome and hypogonadotropic hypogonadism.

**Conclusions:** The present study demonstrates that disorders of the control and regulation of apoptosis

may participate in the pathogenesis of deranged spermatogenesis. It also shows that differential expression of protein at the 16 kDa peak could be used as a biomarker. Application of the SELDI-TOF MS technique to study of differential protein expressions may lead to a better understanding of hypospermatogenesis states in human male infertility.

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# P-40 악성 종양 및 재생 불량성 빈혈 치료 전 보관된 정자의 성상 분석 및 치료 후 보조 생식술을 이용한 임신 결과

정미경 · 이숙환 · 한지은 · 김지영 · 신동혁 · 이동률 · 정형민
이우식 · 정태규 · 김현주 · 윤태기

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**Background & Objectives:** 최근 악성 종양 및 재생 불량성 빈혈에 대한 진단과 치료의 발달로 환자의 생존율이 현저하게 증가하였다. 이에 따라 악성 종양 및 재생 불량성 빈혈로 진단 받은 생식연령의 남성 환자에 있어서 고용량 항암제투여와 방사선조사로 초래될 수 있는 영구불임에 대한 관심과 우려가 높아지고 있다. 본원에서도 이들 환자를 대상으로 치료 전 정액을 동결 보존함으로서 치료 후 임신 기회를 제공하고자 하였다. 본 연구는 항암 치료 전 정자 성상이 확인된 냉동 보존 정자를 이용한 시험관아기 시술을 통해 악성종양 및 재생 불량성 빈혈 환자의 정자 냉동 보존 필요성을 확인하고자 하였다.

**Method:** 1998년 7월부터 2004년 5월까지 악성 종양 및 재생 불량성 빈혈로 진단 받아 본원을 내원한 14~41세 까지의 남성 환자 158명 (281례), 만성 골수성 백혈병 (CML) 39명, 급성 골수성 백혈병 (AML) 26명, 급성 림프성 백혈병 (ALL) 18명, 재생 불량성 빈혈 (SAA) 25명, 비 호지킨성 림프종 (NHL) 16명, 골수이형성증 (MDS) 7명, 고환 악성 신생물 (MNT) 7명, 호지킨질환 (HD) 4명, 기타 (골육종, 정상피종 등) 16명을 대상으로 정자의 성상을 확인하고, KS II 배양액을 이용하여 냉동 보존을 실시하였으며, 이들 환자 중 치료 후 임신을 원하여 다시 내원한 환자를 대상으로 시험관 아기 시술을 하였다.

**Results:** 환자의 평균 나이는 25.1±5.6세 (n=158)이었으며 이들 중 37.3% (59/158)가 Oligozoospermia, 77.8% (123/158)가 Asthenozoospermia였으며, 31.6% (50/158)가 Oligo-Asthenozoospermia의 성상을 나타내었고 정상 정액 (WHO manual 기준)의 성상을 나타내는 환자는 12.7% (20/158)였다. 이들 환자들의 평균 정액 성상은 각각 다음과 같았다 (증례, volume; ml, count; $\times 10^6$/ml와 motility; %: Mean ± SD).; CML (76례, 2.9±1.6, 46.7±62.1, 30.9±18.5), AML (36례, 3.0±1.5, 30.3±34.6, 28.6±20.4), ALL (30례, 2.7±1.4, 56.5±105.4, 23.7±20.5), SAA (46례, 3.0±1.7, 51.4±42.4, 38.2±14.6), NHL (21례, 3.4±1.7, 88.4±62.5, 39.0±18.3), MDS (16례, 2.7±1.4, 73.2±76.1, 39.1±18.6), MNT (13례, 2.4±1.7, 33.4±37.4, 18.9±9.1), HD (9례, 4.7±2.5, 70.0±79.5, 22.1±20.4), 기타 (34례, 2.2±1.2, 64.4±43.0, 38.8±15.7). 환자군 별 정상 정액 소