The ratio NGF/proNGF as a biomarker of overactive bladder disease

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INTRODUCTION

Urine components reflect the patient metabolic and physiologic status. 70% of these biomarkers originate from the urogenital tract and are of prime importance for urology diagnostics.

Urinary rise in Nerve Growth Factor (NGF) levels is commonly considered a biomarker for overactive bladder syndrome (OAB). On the other hand, its precursor (proNGF) has been found to accumulate in metabolic stress conditions and to be responsible for degenerative and apoptotic processes through its receptor $p75^{NTR}$.

We measured here NGF and proNGF levels in the urine of an aging female population suffering from overactive bladder syndrome (OAB). We also measured the activities of protein convertases to provide a biochemical mechanism of observed differences.

METHODS

Ethical approval, patient recruitment (Urology clinic, JGH)

Inclusion criteria
Female, 50-80 years
OAB symptoms **OR**
Control (OAB free)

Exclusion criteria
DM, Kidney and liver disease
Malignancy/chemotherapy
Genital prolapse

40 included: 20 control and 20 OAB

Each participant:
- Vital signs and Clinical evaluation
- Validated symptom questionnaires (OABSS, ICIQ-SF, IIQ-7)
- Voiding dairy (24 hours)
- Blood test for electrolytes, lipid profile and glycemic indices
- Two early-morning clean catch urine samples:
  - Culture and analyses

standard care

Analyses: included ELISA and enzymatic kits

RESULTS

- Patients in the OAB group had a **significant higher mean age**, (56.3 years ± 5.2 control vs 68.9 ± 11.4 OAB, p<0.001, t-test).
- Serum analysis showed a **higher insulin resistance index** (HOMA-IR) in the OAB when controlled for age [2.13 (95% CI: 1.48-2.8) in control vs 3.13 (95%CI: 2.47-1.770 in OAB patients, p<0.05, ANCOVA) despite that none of the OAB patients was diabetic at the time of the study. (Table 1)

Table 1: Demographics and blood test variables compared "controlled for age":

	Control group	OAB group
BMI (kg/m²)	26.99 (23.91-30.01)	29.43 (26.68-32.17)
Systolic BP (mmHg)	121.77 (115.0-128.56)	123.17 (116.6-129.7)
eGFR (mL/min/1.73 m2)	91.01 (83.1-99.1)	83.08(75.3-90.9)
Insulin (pmol/L) *	55.22 (39.79-70.65)	79.9 (64.47-95.34)
HBA1c	0.057 (0.054-0.059)	0.054 (0.051-0.057)
HOMA-IR *	2.13 (1.48-2.8)	3.13 (2.47-3.78)
Triglyceride (mmol/L)	1.115 (0.779-1.45)	1.46 (1.14-1.77)
T. Cholesterol (mmol/L)	5.22 (4.7-5.73)	5.23 (4.66-5.8)
LDL	2.82 (2.26-3.37	3.03 (2.5-3.57)
HDL	1.76 (1.5-2.0)	1.57 (0.1.33-1.8)
Total Chol/HDL	3.39 (2.82-3.95)	3.37 (2.8-3.93)

Values are expressed as adjusted mean for age (95% CI), *p<0,05 one-way ANCOVA

RESULTS

Specificity of ELISA kits for NGF has recently been a matter of debate. We assessed the specificity of NGF ELISA kit (from BioSensis) by performing a standard curve in the presence or absence of physiological concentrations of proNGF (500 pg/mL). The two standard curves (3.9 to 250 pg/mL) were plotted and analyzed, NGF alone (red dots) and NGF with addition of proNGF (blue dots). Linear correlation was nearly identical between curves, confirming the specificity of the NGF ELISA kit.

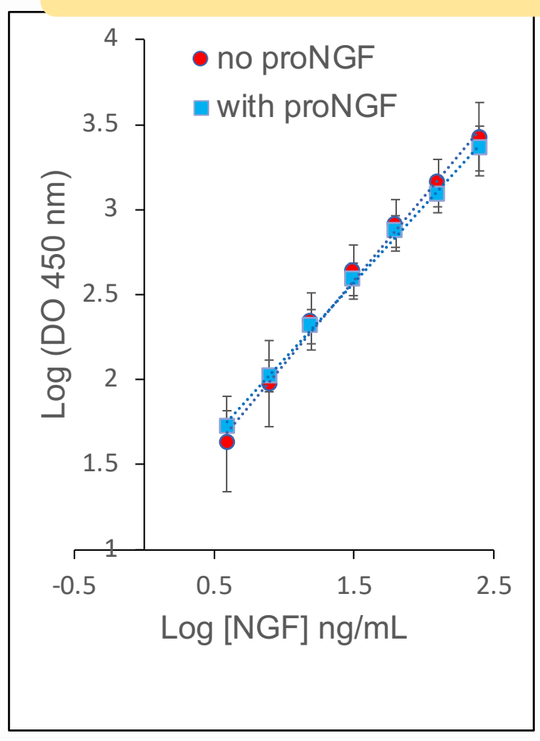

Figure 1: ELISA kit specificity for NGF vs proNGF

Table 2: Correlation between proNGF/NGF ratio and different convertase enzymes and nitric oxide (NO) in the study population

	TIMP-1	MMP-7	proMMP-7	MMP-9	NO
NGF/proNGF	-0.444**	-0.435**	-0.327*	-0.441**	-0.485**
proNGF/NGF	0.588**	0.487**	0.534**	0.528**	0.510**

Data represent the correlation coefficient (*r),* **p<0.001, *<0.05 for the significance of the correlation between the (proNGF to NGF) ratio and the urinary level of each convertase enzyme or NO (Pearson correlation)

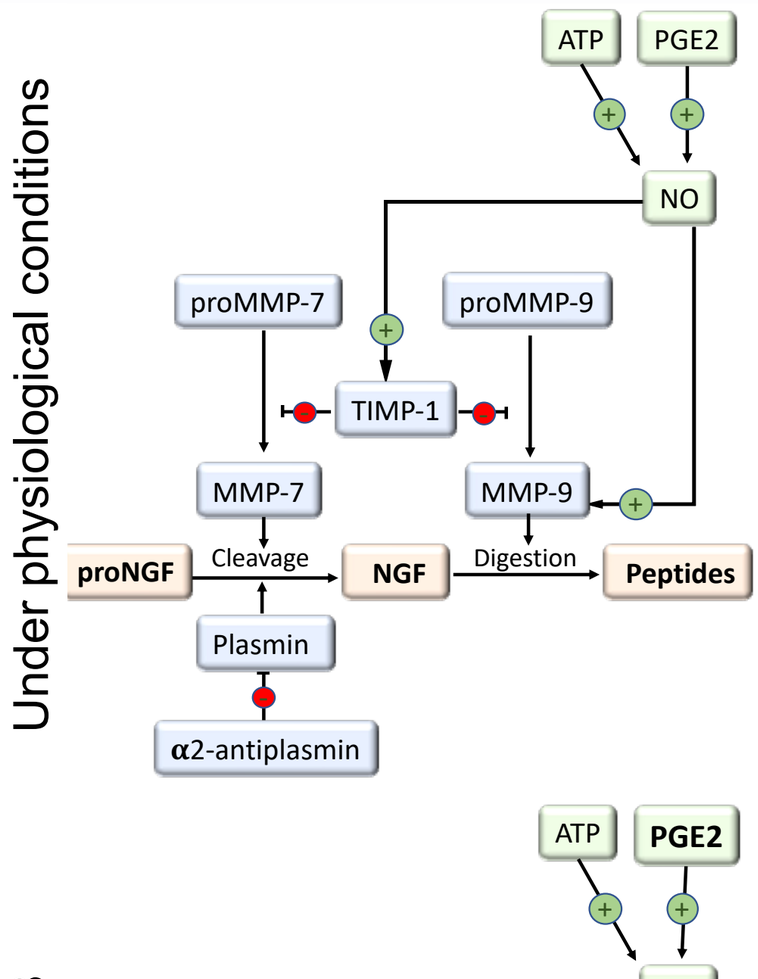

Figure 2: proNGF conversion and NGF digestion by protein convertases in control patients (top) and observed changes in OAB patients (bottom)

Table 3: NGF/proNGF urinary levels and related protein convertase enzymes estimated by ELISA and enzymatic assays, in control and OAB groups:

	Control group	OAB group	P value
proNGF (ng)	0.67 (0.51)	0.67 (0.35)	0.9
NGF (pg)	2.21 (1.2)	1.52 (0.9)	0.060
NGF/proNGF	4.55 (2.56)	2.95 (2.46)	0.053
proNGF/NGF	0.296 (0.17)	0.69 (0.69)	**0.023**
TIMP-1 (ng)	0.99 (0.62)	2.121 (1.8)	**0.015**
Plasmin (ng)	28.75 (22.3)	30.27 (18.3)	0.82
MMP-7 (ng)	0.253 (0.16)	0.55 (0.43)	**0.010**
proMMP-7 (ng)	1.24 (1.8)	3.81 (5.2)	**0.047**
MMP-9 (ng)	0.285 (0.52)	1.69 (2.2)	**0.011**
proMMP-9 (ng)	0.168 (0.3)	0.20 (0.25)	0.731
PGE2 (pg)	280.9 (140.3)	632.9 (581.8)	**0.016**
NO (micromole)	4.55 (1.25)	7.31 (3.99)	**0.011**
ATP (nmol)	11.45 (7.9)	12.21 (8.61)	0.78

Values are presented as mean (SD) of the concentration of each protein corrected to urine creatinine (in mg), independent t-test

Table 4: Correlations between demographics and lab results with the urinary proteins

Age	proNGF/NGF (0.316)*
	PGE2 (0.49)**
	TIMP-1 (0.473)**
	MMP-7 (0.407)*
	MMP-9 (0.33)*
	NO (0.632)**
ATP	BMI (0.33)*
	Cholesterol (0.43)*
	LDL (0.58)**
Glucose	PGE2 (0.49)**
	TIMP-1 (0.39)**
	proMMP-7 (0.32)*
	MMP-9 (0.39)*
	NO (0.45)**
OABSS	PGE2 (0.45)**
	MMP-7 (0.46)*
	NO (0.402)**
ICIQ-SF	MMP-7 (0.462)*
IIQ-7	PGE2 (0.34)**
	MMP-7 (0.454)*

Data are presented as (r), *<p.05, p<0.005 (Pearson correlation)

SUMMARY AND CONCLUSIONS

- OAB increased with age and was associated with impaired insulin sensitivity.
- proNGF/NGF ratio increased with age with concomitant increase in the protein convertase enzymes that are involved in proNGF/NGF maturation and degradation.
- proNGF/NGF ratio was also higher in the OAB patients along with other mediators that are reported in relation to OAB such as PGE2 and NO.
- The levels of convertase enzymes were correlated with the metabolic status parameters such as glucose and lipid serum levels.
- The ratio proNGF/NGF could therefore constitute a more accurate biomarker in the diagnosis of OAB.
- Convertase inhibitors for MMP-9 could constitute a new and promising therapeutic target to restore levels of NGF, to decrease proNGF and to improve OAB.