BANTAO Journal 3 (2): p 106; 2005

Serum Levels of TNFα and IL-6 Versus Urinary Excretion of N-acethyl-β-glucosaminidase and α_1-microglobuline in Patients with Acute Renal Failure

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Introduction

IL-1β,TNFα and IL-6 are the most potent known proinflammatory cytokines[1].Because the effects and concentrations of IL-1β and TNFα are parallel and intercorrelate very match in the *clinical* setting-usually is sufficiently to detect the blood level of one of two cytokines(IL-1β or TNFα;in our case-TNFα[2,3]).IL-6 is known as a cytokine with "endocrine" effect (acts by the distance) in spite of paracrine and autocrine action[4].The experimental and clinical data about IL-6 are controversial (data confirming his proinflammatory or antiinflammatory action!?).In the last few years IL-6 is accepted as a potent proinflammatory cytokine[5].The urinary excretion of N-acethyl -β-glucosaminidase (NAG) and α_1-microglobuline (α_1MG)may be correlated with the serum levels of proinflammatory cytokines (especially TNFα and IL-6[6]).

The newer pathogenetic views in the genesis of acute renal failure

The acute renal failure(ARF) commonly is consequence of ischemic,septic or toxic renal tubular cells injury(for instance-ischemic/reperfusion injury in kidney transplant patients)[7].The proinflammatory cytokines (TNFα,IL-1β,IL-6) are especially activated in sepsis-induced ARF, using the pathways of *oxidative stress* and *endotheline*(ET)-production[8,9].In experimental studies is confirmed the *direct* inclusion of TNFα and IL-6 in the creation of ARF regardless the etiology of this syndrome[10,11].The role of gram-negative bacterial endotoxin (lipopolysacharides/LPS/ detected by Limulus amebocyte lysate-reactive material/LAL-RM/) is certified in septic patients with ARF[12].The production of proinflammatory cytokines from activated monocytes (Mo) and macrophages(MF) is accelerated by LPS and oxidative stress-products[13].The activation of neutrophiles (s.c."enrolling") after P/S-selectins presentation and leucocytes adhesion on the renal endothelial cells-is associated with the proinflammatory cytokines-previous accumu-lation[5].

The membrane biocompatibility and cytokines production

The whole blood (WB) concentrations of two cytokines (TNFα,IL-6) are increased in patients with acute or chronic preterminal, non-jet dialyzed renal failure[14].There are many data covering the problem of membrane biocompatibility or incompatibility in patients suffering from chronic or acute renal failure[15].The hemodialysis *per se* can modify the proinflammatory cytokines production (IL-6,TNFα):the WB concentration of TNFα and IL-6 is increased in uremic,chronically HD patients of each membrane group (biocompatible or bioincompatible) before HD procedure, without differences between two groups.The stimulation of whole blood cytokines synthesis from immunocompetent cells like Mo/MF(using phytohemagglutinine /PHA/for TNFα and LPS for IL-6) after HD session is membrane and type cytokine-dependent process. For example *hemophane* membrane presensitivates only IL-6 synthesis from Mo/MF after LPS exposition,and in the the *polyamide* membrane treated patients there are not presensitisation regarding IL-6 and TNFα[16].There are clinical data confirming the dialysis induced removal of soluble cytokines (f.e. during continuous hemodiafiltration),but the beneficial, clinically approved significance is not confirmed[17].

Our material and methods

We have analyzed 25 (16 males and 9 females;mean age 46.9±18.5 years) patients with different etiology and favourable outcome of ARF (ICU,Clinic for Nephrology,Clinical Centre,Skopje,Macedonia). 15 patients were dialysis dependent (DDP) and the next 10 patients were dialysis independent (DIP) without regard to the sex, age and the etiology of ARF.The whole blood concentration of TNFα,IL-6 (as a "far acting" cytokine) were detected (Institute for Clinical Chemistry, Clinical Centre,Skopje) using commercially prepared kits {RV for TNFα=10.8±6.8 pg/ml,for IL-6=3.6±2.1 pg/ml- ELISA method). The urinary levels of NAG (RV=0.8-1.19 U/mmol/Cr using spectrophotometric method following Peters and al;Boehringer Manheim kits) and α_1MG(RV=8-12mg/l following immunoturbidometric method using DAKO ready tests) were measured at the same time with the serum concentration of proinflammatory cytokines (namely, in the first 10 days of hospitalization).

Results

Our results are presented at the folowing tables:

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Table 1. Differentiation of our patients following sex,age, hospitalization-time,basic disease/type of ARF and urinary excretion of proteins/creatinine (first 10 days of recovery phase)

Patient	Sex (M,F)	Hospitalization (days)	Age (years)	Basic disease Type of ARF	Urinary proteins (gr/l)	Urinary Creatinine (mmol/l)
NR	M	63	57	ischemic	1.21	05.03
NT	M	23	63	ischemic	1.25	19.49
RN	M	12	31	Sepsis,TTP	0.35	05.49
GM	M	11	19	Sepsis	0.05	03.03
SR	M	23	43	IBD,ischemic	1.82	02.19
IN	F	07	28	ischemic	0.18	05.28
AM	M	15	77	Enterocolitis,ischemic	0.94	07.55
KLj	F	11	47	ischemic	0.21	05.81
TV	M	22	75	Sepsis	0.10	04.44
SDz	F	28	34	Urosepsis,HRS	0.40	03.14
SB	M	13	39	Sepsis,Ags-toxicity	1.14	04.21
GA	F	15	30	Pregnancy,hypochloremic alkalosis,ischemic	0.14	05.65
JM	M	10	44	Malignant HTA,ischemic	0.10	07.89
PM	M	11	64	Sepsis,Cardiac arrest	0.96	07.55
KN	F	32	16	Acute allergic TIN	1.47	05.79
SDz	M	15	54	GITbleeding,Shock,ischemic	0.29	03.96
GB	F	07	40	Endocarditis bact.,Sepsis	0.38	02.82
NJ	M	14	67	Enterocolitis,ischemic	0.20	14.30
IA	F	14	43	Sheean sy,DI,ischemic	0.28	04.71
BB	M	21	55	Sch,acute medicam.TIN	0.46	08.92
VDz	M	07	55	Ischemic	0.97	09.30
II	M	12	24	SLE,ischemic	0.21	06.07
JF	M	12	24	Rhabdomyolysis,TTP	1.86	06.74
MF	F	17	75	Enterocolitis,ischemic	0.44	05.87
MD	F	17	69	Cholelithiasis,Sepsis	0.18	05.73
TOTAL	16M+9F	X=17.28±11.46	46.92±18.49	Septic ARF = 8 cases	0.62±0.56	6.44±3.71

Legend:TTP-thrombotic thrombocytopenic purpura;TIN-tubulointerstitial nephritis; SLE-systemic lupus erythematodes;DI-diabetes insipidus;HTA-arterial hypertension;GIT-gastrointestinal;IBD-inflammatory bowel disease;Ags-aminoglycosides;HRS-hepatorenal syndrome

Table 2. Urinary excretion of α_1MG/NAG and serum concentration of IL-6/TNFα in our patients with ARF (first ten days of recovery phase,X±SD)

α_1MG(mg/l)	NAG(mmol/grCr)	IL-6(pg/ml)	NFα(pg/ml)	Use of HD
88.74	2.12	33.14	69.89	+
39.80	1.27	18.63	15.20	+
33.50	4.00	25.07	76.33	+
04.70	1.11	07.73	12.20	-
120.00	2.31	48.33	86.90	+
25.24	0.96	10.20	29.27	-
86.09	0.88	76.98	245.40	+
14.67	0.92	-	14.33	-
12.10	5.62	-	-	+
41.75	2.97	44.35	212.56	+
77.75	3.62	42.25	170.83	+
24.23	2.37	13.25	18.54	-
08.95	1.73	-	17.17	-
37.20	3.90	13.33	47.70	+
15.19	6.03	-	-	-
15.61	1.88	05.40	16.86	+
23.67	1.17	-	-	-
84.10	0.82	36.30	130.67	+
38.82	2.08	18.33	59.33	+
76.50	1.21	33.00	22.80	-
177.25	2.35	37.75	220.33	+
06.07	0.59	-	-	+

α_1MG(mg/l)	NAG(mmol/grCr)	IL-6(pg/ml)	NFα(pg/ml)	Use of HD
86.00	2.86	44.67	124.83	+
14.60	2.52	-	-	-
21.27	1.01	-	-	-
Total:46.95±42.39	2.25±1.46	29.92±18.67	83.74±77.99	DDP=15,DIP=10

Legend:HD-hemodialysis;DDP-dialysis dependent patients;DIP-dialysis independent patients

Table 3. Plasma cytokines (TNFα,IL-6) an urinary NAG,α_1MG in patients with ARF in the first ten days of recovery phase

	Plasma cytokines (pg/ml)		Urinary proteins excretion	
	TNFα	IL-6	NAG(mU/mmol Cr)	α_1MG(mg/l)
RV	10.8 ± 6.8	3.6 ± 2.1	0.8 - 1.19	8-12
Day: 2-5		48.0 ± 8.7		
			3.8 ± 2.0	
Day:4-7	108.8 ± 17.5			137.4 ± 12.4
Day:1-10	83.74±77.99	29.92±18.67	2.25 ± 1.4	46.95 ±42.39

Table 4. Correlation between investigated factors

Correlation between:	Coefficient of correlation(R)
Age and urinary proteins excretion	**- 0.07***
Age and urinary creatinine excretion	**0.37****
Urinary excretion of α_1MG and NAG	**- 0.05***
Serum levels of IL-6 and urinary α_1MG	**0.65*****
Serum levels of TNFα and urinary NAG	**0.21****
Serum levels of IL-6 and TNFα	**0.83*****
Serum levels of αMG and TNFα	**0.66*****
Serum levels of IL-6 and urinary NAG	**- 0.01***

Legend: * - no correlation; ** - weak to middle correlation; *** - strong to very strong correlation

Comment of our results

Urinary excretion of NAG and serum level of IL-6 present the maximal values and very strong positive correlation between 2nd and 5th day of polyuric (recovery) phase of ARF (8 DIP) , inversely,urinary presence of αMG and plasma concentration of TNFα have demonstrated a highest positive correlation and maximal values a litle bit later, namely between 4th and 7th days (11 DDP included).An obvious overlap is present in the days 4th and 5th of recovery phase estimating the investigated proteins (two DIP and four DDP included).

Conclusion

The serum concentration of proinflammatory cytokines (TNFα,IL-6) may be a useful biochemical tool in differentiating the etiology (f.e.septic genesis) and evolution of recovery phase of ARF (especially non-differentiated cases).The urinary detection of NAG and α_1MG may be a cheaper and reliable demonstrator in differentiation of dialysis requesting from dialysis non-requesting ARF patients.

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