

Original article

Indicators of sepsis: Microalbuminuria and serum Nitric oxide

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Abstract

Introduction: Sepsis is one of the challenges for the doctors who treat critically ill patients. Delay in diagnosis and late administration of antibiotics have been shown to increase the mortality in this cohort. In the present study microalbuminuria was used as early marker of sepsis and nitric oxide(NO) as a marker of oxidative stress in sepsis.

Material and Methods: 30 patients from MICU and surgical ICU showing signs of SIRS were included in the study in the case group. 30 healthy age and sex matched individuals served as controls. Both the groups were analyzed for microalbuminuria in terms of ACR(urinary albumin creatinine ratio) and serum nitric oxide and compared statistically. A correlation between microalbuminuria and nitric oxide was established.

Observation and results: There was a significant rise in ACR whereas a significant fall in the NO levels in the septic patients when compared with control group. There was a negative correlation between ACR and NO though not significant.

Conclusion: ACR and serum NO can be used for early diagnosis of sepsis in the critically ill patients.

Keywords: Sepsis, microalbuminuria, nitric oxide, albumin creatinine ratio

Introduction

Sepsis is defined as the presence of the Systemic Inflammatory Response Syndrome (SIRS) and a presumed or confirmed infection. Sepsis remains a major global healthcare concern, owing to high morbidity and mortality, despite the advances in medical therapeutics.^{1,2} It is a condition which is difficult to diagnose as the clinical and laboratory signs are similar to those presented in different severities of SIRS. So the mortality due to severe sepsis remains high³. Sepsis is marked by a severe host defense response that involves triggering of potent inflammatory cascades which release a plethora of pro-inflammatory molecules into the circulation⁴. The endothelium becomes dysfunctional due to the sustained onslaught of the inflammatory molecules and the simultaneous oxidative stress. An early event is the loss of barrier integrity leading to systemic capillary leak⁵. The glomerular manifestation of this enhanced capillary permeability is increased excretion of albumin in the

urine⁶. Microalbuminuria (MA) is known to be a sensitive expression of the increased permeability of the systemic microcirculation^{7,8}. Microalbuminuria, typically defined as albumin excretion in urine of 30-300 mg/24 hrs, occurs rapidly after acute inflammatory insult and is shown to be associated with outcomes in many clinical settings, including sepsis, multiple trauma and intracranial hemorrhage⁹⁻¹¹. The mechanisms of development of microalbuminuria, have been extensively studied but remain elusive¹¹.

In the past decade, lot of research on Nitric Oxide (NO) is being carried out. NO is believed to play a key role in the pathogenesis of sepsis, although many aspects of involvement of NO remain poorly defined¹². NO has profound biological effects in the cardiovascular, nervous and immune system and derangements in NO homeostasis have been found in many pathological conditions. NO is a highly reactive molecule and is synthesized from L-

Arginine by the enzyme Nitric Oxide Synthase (NOS)¹³.

In view of all these facts the present study was planned to estimate the serum NO and urinary microalbumin levels in patients admitted in ICU having SIRS.

Aims and Objectives

To evaluate whether microalbuminuria and NO can be used as a marker of sepsis and the correlation between the levels of microalbumin and NO (the inflammatory marker) on the day of admission. The aim was achieved by following objectives; estimation of urinary microalbumin and creatinine levels and their ratio. Microalbumin was expressed as the Albumin/creatinine ratio(ACR) to correct for variations in urinary flow rate and estimation of serum NO levels in the patients admitted to ICU with SIRS and the healthy subjects. To see whether there is any correlation between urinary microalbumin and NO levels in the ICU patients.

Material and methods

The study was carried out in department of Biochemistry, B.J. Medical College, Pune. Patients admitted in medical & surgical ICU were screened for signs of SIRS and then 30 adult patients (Age >18 yrs) with SIRS from Sassoon General hospitals Pune were included in the study (Case group). 30 age and sex matched healthy controls were analyzed for comparison (Control group).

On admission, the following data was collected for each patient: age; gender; date and time of admission, provisional diagnosis; co-morbid conditions such as diabetes, hypertension and chronic kidney disease. Clinical and laboratory data was collected; cultures sent and antibiotics administered within 24 hours of admission were noted.

Exclusion criteria: Patients having anuria, macroscopic hematuria [confirmed with dipstick], female patients with menstruation or pregnancy

were excluded. Retrospectively, patients with significant proteinuria [more than 1+ protein on dipstick] due to renal and post renal causes, for example urinary tract infection, were excluded.

Inclusion criteria- Patients admitted in medical & surgical ICU (Age >18 yrs) with SIRS were included in the case group.

The study was carried out after the approval from institutional ethical committee.

5ml of intravenous blood samples of the subjects were collected for NO analysis.

Estimation of Urinary microalbumin (ACR)

Spot urine samples were collected for quantification of ACR . Urinary microalbumin was measured by the immunoturbidimetric method and urinary creatinine by modified kinetic Jaffe reaction .The methods covered an analytical range of 1.3–100 mg/L for microalbumin and 0-20 mg/dl for creatinine. Microalbuminuria was defined by ACR values between 30 and 299 mg/g. ACR of > 300 mg/g is considered as clinical proteinuria. ACR < 30 mg/g is normal for a healthy population⁹. However, to obtain comparable data, microalbumin was expressed as the microalbuminuria/creatinine ratio to correct for variations in urinary flow rate¹⁴. These threshold values are well accepted for clinical use and have been predefined on the basis of published literature.

Estimation of nitric oxide (NO₂ +NO₃) concentration by Cortas and Wakid method

Nitric oxide concentration was measured as total nitrates and nitrites (NO₂ +NO₃) by the Cortas and Wakid method. Absorbance was read at 545nm. Concentration was determined using standard graph¹⁵.

Statistical analysis

Results are presented as mean ± standard deviation and statistically compared by Student unpaired 't' test. A 'p' value of 0.05 or less was considered

significant. ACR and NO were correlated using pearson's correlation coefficient.

Observations and results

Table 1 shows significant increase in the levels of ACR whereas the serum NO levels were significantly decreased in cases as compared to controls (p<0.000).

Table 1: Comparison between levels of ACR and Nitric oxide in controls and patients

	Controls (n=30)	Patients of Sepsis (n= 30)	P- value
ACR (mg/gm)	9.84 ± 2.87	68.27 ±39.66	< 0.001*
Nitric Oxide (µmole/L)	65.10 ± 21.40	44.92 ± 10.43	< 0.001*

* p value is highly significant

Table 2 shows a negative correlation between the ACR and serum NO which was not statistically significant.

Table 2: Correlation of ACR and Nitric Oxide

Group	Correlation R value	P-value
ACR Vs Nitric oxide	-0.29	0.601

Discussion

Diagnosing sepsis early is vital for patient management and outcome but there is no reliable way of doing it yet. Culture of body fluids, the gold standard, are not always positive, and yield results not earlier than 24 h, which may be too late for administering targeted therapies⁹. This study reveals that sepsis itself is associated with induction of a “systemic inflammatory response”, which is characterized by the release of pro-inflammatory mediators and the activation of different types of cellular elements^{10, 16}. The endothelial cells themselves may be injured by an intense inflammatory reaction, and one of the main effects of this injury is increase in endothelial permeability¹⁰. The reason for increased incidence of microalbuminuria in critically ill patients is probably the result of widespread endothelial dysfunction arising from the effects of cytokines, and other inflammatory mediators, released during the intense inflammatory responses that are associated with critical illnesses¹⁷. The effects of disruption of the integrity of the endothelial barrier

is manifested as altered glomerular endothelial permeability in the kidneys, allowing increased amounts of albumin to escape into the glomerular ultrafiltrate. The tubular reabsorptive mechanism for albumin from the ultrafiltrate is exceeded beyond its threshold capacity, leading to increased excretion of albumin in the urine¹⁸. Several studies in various groups of critically ill patients have unequivocally established microalbuminuria as a significant prognostic marker of morbidity and mortality in the ICU¹⁹. The study of Thorevska et al²⁰ and Gosling et al²¹ also showed similar type of results with specific cut off values on ICU admission. The rapid increase in renal permeability to plasma proteins after trauma, surgery or ischemia, which is proportional to the severity of the insult, led to the suggestion that increased renal and vascular permeability occur simultaneously, and may share common pathways during the early stages of the acute disease process²².

Microalbuminuria would be an ideal tool for the early and accurate identification of patients with

high risk of morbidity and mortality. This would allow the intensivist to triage and optimize aggressive and sometimes expensive therapeutic interventions in patients most likely to benefit and to curb use in those who are unlikely to do so, especially in situations of financial constraints. Measurement of ACR can guide optimum allocation of resources, counselling of family and/or patient and opportune triaging to the wards, too¹⁸. Generally oxidative stress is also in the foreground of sepsis. This oxidative stress causes uncoupling of NOS resulting in decreased NO levels. Along with arginine availability, endogenous inhibitors of NOS like ADMA (asymmetric dimethylarginine), may affect NO synthesis. Arginase can compete with NOS for their common substrate, L-arginine, and thus inhibit NO production. This regulatory mechanism may be

particularly important when the extracellular supply of L-arginine is limited^{23,24}. Citrulline the precursor for arginine synthesis is a nonprotein amino acid, it is formed along with NO by the action of NOS, as NOS activity is decreased formation of citrulline is also decreased hence arginine synthesis may also be affected. Even though Nitric oxide is responsible for immune response, there is a thin line of demarcation to show whether nitric oxide is friend or foe in human health and disease²⁵.

Conclusion:

Diagnosis of sepsis early in the critically ill patients is of utmost importance. Microalbuminuria and NO are the parameters which can be used as markers of sepsis so that early intervention is made possible. Further studies with a large sample size are to be done in this field.

References:

1. Angus DC, Linde-Zwirble WT, Lidicker J. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Crit Care Med.* 2001;29:1303–10.
2. Todi S, Chatterjee S, Bhattacharyya M. Epidemiology of severe sepsis in India. *Crit Care.* 2007;11:65
3. Angus DC, Pereira CA, Silva E : Epidemiology of severe sepsis around the world. *EndocrMetab Immune Disord Drug Targets* 2006,6:207-212
4. Hotchkiss RS, Karl I. The pathophysiology and treatment of sepsis. *N Engl J Med.* 2003;348:138–50.
5. Aird William C. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood.* 2003;101:3765–77.
6. Gosling P. Microalbuminuria: A marker of systemic disease. *Br J Hosp Med.* 1995;54:285–90.
7. Yoshiaki Terao, Masafumi Takada, Takahiro Tanabe, Yuko Ando, Makoto Fukusaki, Koji Sumikawa : Microalbuminuria is a prognostic predictor in aneurysmal subarachnoid hemorrhage : *Intensive Care Med ; 2007; 33; 1000-1006.*
8. Nakamura M, Onoda T, Itai K, Ohsawa M, Satou K, Sakai T, Segawa T, Sasaki J, Tonari Y, Hiramori K, Okayama A, (2004). Association between serum C-reactive protein levels and microalbuminuria: A population based cross-sectional study in northern Iwate, Japan. *Intern Med.* 43: 919-925.
9. Basu S, Bhattacharya M, Chatterjee TK, Chaudhari S, Todi SK, Majumdar A. Microalbuminuria: A Novel biomarker of sepsis. *Indian J Crit Care Med.* 2010; 14(1): 22-28.
10. De Gaudio AR, Adembri C, Grechi S, Novelli GP. Microalbuminuria an early index of impairment of glomerular permeability in post-operative septic patients. *Intensive Care Med.* 2000; 26(9) 1364-1368.
11. Zhongheng Zhang, Baolong Lu, Hongying Ni, Xiaoyan Sheng, Ni Jin: Microalbuminuria can predict the development of acute kidney injury in critically ill septic patients. *J Nephrol* 2013; 26(4):724-730.
12. Abhijit Chandra, Perenlei Enkhbaatar, Yoshimitsu Nakano, Lillian D Traber and Daniel L. Traber. Sepsis: Emerging role of nitric oxide and selectins *Clinics.* 2006; 61 (1):71-76.

13. Markus Munder. Arginase: an emerging key player in the mammalian immune system. *British Journal of Pharmacology*, 2009; 158, 638 – 651
14. Dezier JF, Le Reun M, Poirier JY (1988) Usefulness of the urinary albumin / creatinine ratio in screening for microalbuminuria. *La Presse Medicale* 17/18: 897-900
15. Najwa KC, Nabil WW. Determination of inorganic nitrate in serum and urine by kinetic reduction method. *Clinchem* 1990; 36 (8):1440-43.
16. Parillo JE (1993) Pathogenic mechanisms of septic shock. *N Engl J Med* 328: 1471-1477.
17. Dziedzic T, Slowik A, Szczudlik A. Urine albumin excretion in acute ischaemic stroke is related to serum interleukin-6. *Clin Chem Lab Med* 2004; 42(2):182-5.
18. S Basu, S Chaudhuri, M Bhattacharyya, T K Chatterjee, S Todi, A Majumdar. Microalbuminuria: An Inexpensive, Non Invasive Bedside Tool To Predict Outcome In Critically Ill Patients. *IJCB*: 2010 / 25 (2) 146-152
19. Gopal S, Carr B, Nelson P. Does microalbuminuria predict illness severity in patients on the intensive care unit? A systematic review. *Crit Care Med*. 2006; 34:1805-10.
20. Thorevska N, Sabahi R, Upadya A, Manthous C, Amoateng-Adjepong Y. Microalbuminuria in critically ill medical patients: Prevalence, predictors, and prognostic significance. *Crit Care Med* 2003; 31:1075-81
21. Gosling P, Czyz J, Nitingale P, Manji M. Microalbuminuria in the intensive care unit: Clinical correlates and association with outcomes in 431 patients. *Crit Care Med* 2006; 34(8):2158-66
22. Omar Abid, Qinghua Sun, Kerji Sugimoto, Dany Mercan, and Jean Louis Vincent: Predictive value of microalbuminuria in medical ICU patients. *Chest* 120/6 December, 2001; 1984-1988.
23. Boger R.H. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the 'L- Arginine Paradox' and acts as a novel cardiovascular risk factor. *J.Nutr.* 2004; 134, 2842S – 2847S
24. Tran, C.T., Leiper, J.M. and Vallance P. The DDAH/ADMA/NOS pathway. *Atherosclerosis suppl.* 4, 2003; 33 – 40.
25. Jung C, Gonon AT, Sjöquist PO, Lundberg JO, Pernow J. Arginase inhibition mediates cardioprotection during ischaemia-reperfusion. *Cardiovasc Res.* 2010 Jan 1; 85(1):147-54.