

Evaluation of Arginase and Nitric oxide levels in Sepsis

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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 mortality in this cohort. In the present study CRP is used as a traditional marker of an acute inflammatory state and infection. In last couple of years use of Arginase as immune marker has increased enormously. An interplay between Arginase and Nitric oxide has also been reported in immune cells including macrophages, lymphocytes etc. due to limited availability of Arginine in sepsis. Keeping all these facts in mind, present study was designed. **Aims and Objectives:** To find out the diagnostic role of Arginase and Nitric oxide in sepsis and to compare with CRP level. **Material & Methods:** Thirty patients admitted in MICU of Sassoon Hospital, Pune having SIRS and thirty age, sex matched healthy controls were included in this study. Intravenous blood samples were obtained and analyzed. CRP was done by kit method, Arginase activity was estimated by Roman and Ray method while NO levels were measured by Cortas & Wakid method. **Results and Conclusion:** The results of this study showed significant increase in the levels of CRP. Serum arginase was also increased significantly with concomitant decrease in the levels of nitric oxide when compared with healthy controls. So we can conclude from the results that nitric oxide and arginase levels along with CRP may be useful in diagnosis of sepsis.

Key words: Arginase, Nitric Oxide, CRP, Sepsis

Introduction

Sepsis is defined as the presence of the Systemic Inflammatory Response Syndrome (SIRS) and a presumed or confirmed infection. Even today, this life threatening complication of infection is associated with significant morbidity and mortality in the Intensive Care Unit (ICU), where it is the most common cause of mortality [1]. 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. Thus, the importance of infection as an inducer of a systemic inflammatory response is underlined. A variety of inflammatory mediators are released by activated blood cells, vascular cells and different parenchymal cells during sepsis [2]. On this background some inflammatory markers like C-Reactive Protein (CRP), Nitric oxide (NO) and Arginase were studied.

CRP is used as a marker of an acute inflammatory state and infection. In spite of being a traditional marker of

sepsis it is not specific enough for clinicians to give their clinical judgment. In past decade lot of research on 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 defined [1]. 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). L-Arginine is a common substrate for two enzymes viz; NOS and Arginase. Use of Arginase as an immune marker has also increased enormously in the last couple of years. This is due to the fact that the enzyme is crucially involved in various aspects of inflammation [3].

The literature regarding Arginase both as a marker of sepsis or as a therapeutic agent is increasing and gaining importance day by day. So the present study was undertaken to see changes in levels of NO and the efficacy of Arginase activity as a marker of sepsis.

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Aims and objectives

The aim of the study is to find out the diagnostic role of Arginase and Nitric oxide in sepsis and to compare with CRP level. The aim will be achieved by following objectives; estimation of activity of arginase, nitric oxide and CRP levels in patients and controls.

Material and Methods

The study was carried out at the department of Biochemistry, B. J. Medical College after the approval from institutional ethical committee.

Study group

30 adult subjects (age above 18 years) with SIRS. All patients admitted in MICU irrespective of the cause, having SIRS were included in the study.

Control group

30 age, sex matched healthy adults were included.

Exclusion criteria

Paediatric patients below 18 years of age were excluded.

Collection of serum

5ml of intravenous blood samples of the subjects was collected, centrifuged to separate the serum and stored at -20°C till the analysis was done.

Results

The present study aimed to evaluate the levels of Nitric Oxide and Arginase activity and also to see whether these 2 parameters can help in the diagnosis of sepsis. CRP was done as a routine marker of inflammation.

The results showed a significant increase in the Arginase activity and at the same time nitric oxide showed a concomitant decrease as compared to controls ($p < 0.001$) [Table no.1]. CRP levels were elevated in patients as compared to controls ($p < 0.001$) [Table no.1].

CRP also showed a positive correlation with Arginase which is statistically significant (p value 0.002) whereas it showed a negative correlation with NO but was not statistically significant [Table no.2]. The probable mechanism of these changes may be due to many reasons.

Table 1: Comparison between different levels of CRP, Arginase, Nitric oxide in controls and patients

	Controls (n=30)	Patients of Sepsis (n= 30)	P- value
CRP (mg/L)	5.2 ± 2	40.166 ± 11.47	< 0.001*
Arginase (IU/L)	3.89 ± 4.08	48.99 ± 9.99	< 0.001*
Nitric Oxide (µmole/L)	65.10 ± 21.40	44.92 ± 10.43	< 0.001*

* **p value is highly significant**

Table depicts that there is increase in the Arginase activity and at the same time nitric oxide showed a concomitant decrease as compared to controls ($p < 0.001$).

Estimation of CRP

Estimation of CRP was done by Turbilatex kit method. The reagent CRP-Turbilatex agglutination assay is a quantitative turbidimetric assay for measurement of CRP in human serum [4].

Estimation of arginase activity by Roman and Ray method

Ninhydrin reacts with ornithine formed by arginase action in the presence of $MnCl_2$ giving a pink coloured ornithine-ninhydrin complex which is read spectrophotometrically at 530nm. Concentration was determined using standard graph. [5].

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 graph [6].

Statistical analysis

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

Table 2: Correlation of CRP with Arginase and Nitric Oxide

Group	Correlation R value	P-value
CRP Vs Arginase	0.499	0.002*
CRP Vs Nitric oxide	-0.091	0.601

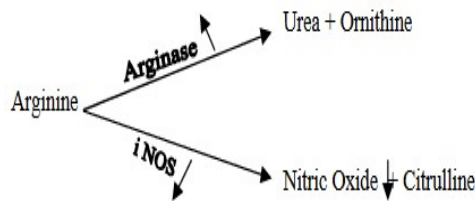
* denotes statistically significant

CRP also showed a positive correlation with Arginase which is statistically significant (p value 0.002) whereas it showed a negative correlation with NO but was not statistically significant

Discussion

Arginine is derived from the diet, de novo synthesis and protein breakdown [7]. Arginine is a sole source of NO, if Arginine is diverted towards Arginase then the substrate availability is decreased for Nitric oxide synthase (NOS) resulting in decreased NO levels (Fig 1). Keita Miki et al reported an interplay between Arginase and cellular NOS in vascular endothelium, smooth muscle cells, immune cells including macrophages, lymphocytes, and the respiratory system [8,9,10,11,12]. According to Chiung-I Chang et al Arginase can compete with NOS for their common substrate and thus inhibit NO production. This regulatory mechanism may be particularly important when the extracellular supply of L-Arginine is limited [13]. So we can say that the bioavailability of Arginine decides which pathway is to be taken. We also observed that CRP levels increased according to the severity of the disease and along with it Arginase activity is also increased. So we can say that Arginase can be used as a marker of severity in sepsis.

Fig 1: Fate of Arginine and interplay between Arginase and iNOS



In addition to this 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 [14]. Dimethylarginines are synthesized by methylation of arginine residues in proteins and are subsequently released by proteolysis [15].

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 disease. On the other hand Arginase activity gives a better picture about the disease as it increases with the severity

of disease. Arginase is not only a good marker, but now-a-days it is also a therapeutic target in some diseases such as Myocardial Infarction. [16]

Conclusion

So it can be concluded from the results that Arginase can be used as a good marker in diagnosis of sepsis. Further studies with a larger sample size are required to show the role of Arginase as a diagnostic marker.

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