SERUM ADENOSINE DEAMINASE LEVELS IN ACUTE KIDNEY INJURY

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Abstract:
Acute kidney injury describes a sudden decline in kidney function over hours and days. Acute kidney injury is frequently caused by renal ischemia and represents an important cause of morbidity and mortality of hospitalised patients. Ischemic tissue damage involves several different mechanisms including renal inflammation, direct tubular damage, and alteration of vascular response. Inflammation leads to leucocytes infiltration and free radicals release which result in damage to cell membrane and the release of membrane associated enzymes like ADA. ADA is a marker of the T cell activation and hence important in acute and protracted inflammatory response.

AIMS AND OBJECTIVES-The aim of the study is to evaluate the serum adenosine deaminase activity in patients with acute kidney injury and to establish a relationship between ADA activity and acute kidney injury.

MATERIALS AND METHODS-Study group included 50 individuals aged 20 to 60 years who were clinically diagnosed as acute kidney injury with serum creatinine level greater than 1.5 mgdl and the control group included 50 healthy individuals.

ADA activity was measured by the method of Guisti and Glantis using semi-autoanalyser.

RESULTS-The mean ADA activity in the study group (47.53 plus or minus 8.81) was significantly high when compared to the control group (22.06 plus or minus 5.94) and the P value is statistically significant. ADA activity was significantly correlated with Serum creatinine (P value less than 0.01) and blood urea (P value less than 0.01).

CONCLUSION-The present study showed an increased level of ADA in acute kidney injury. Further elevated ADA indicates that there is increase in release of free radicals and an oxidative damage. There is also increased metabolism of adenosine and loss of beneficial effects of adenosine in acute kidney injury. Hence ADA would be a novel therapeutic intervention in acute kidney injury and can be used as a routine marker of acute kidney injury.

Keyword: Adenosine deaminase, Acute kidney injury, Serum creatinine, Blood urea.
SERUM ADENOSINE DEAMINASE LEVELS IN ACUTE KIDNEY INJURY INTRODUCTION

The kidneys are vital organs that perform a variety of important functions such as elimination of non protein nitrogenous substances from plasma, homeostasis of the body water and electrolytes and acid base status, excretion of drugs and toxins and participation in hormonal regulations (1).

Acute kidney injury, previously known as acute renal failure is characterized by the sudden impairment of kidney function resulting in the retention of nitrogenous and other waste products normally cleared by the kidneys, disordered hydrogen ion homeostasis and disturbance of ECF volume and composition. The sequelae of above consequences are difficult to control and hence, it is potentially a life threatening condition despite the widespread availability of effective renal replacement therapy (2). AKI is currently defined as a rise of serum creatinine, at least 0.3 mg/dl or 50% higher than the base line within a 24-48 hours period or reduction in urine output to 0.5ml/kg/hr for longer than 6 hours. AKI is not a single disease but rather designation for heterogenous group of conditions that share common diagnostic features; specially the blood urea concentration or increase in serum creatinine concentration, often associated with reduction in urine volume (3).

The distinction between acute and chronic renal failure or even acute on chronic renal failure may not be readily apparent and hence ADQI group proposed RIFLE criteria. The acronym RIFLE defines AKI with grades of increasing severity (Risk, Injury, Failure) and outlines two outcome variables (Loss and End stage), utilizing either increase in serum creatinine or decrease urine in output (4) (5).

Acute kidney injury can be divided into three categories:

- Pre renal (renal hypo perfusion leading to lower GFR)
- Intrinsic renal disease
- Post renal cause (obstructive uropathy)

Pre renal azotemia is mainly due to renal hypoperfusion that can occur in several ways such as decrease in intravascular volume, change in vascular resistance or low cardiac output. If hypoperfusion persists, ischemia can result and cause intrinsic kidney injury. The sites of injuries are the tubules, interstitium, vasculature and glomeruli. Several indices have been used to differentiate prerenal azotemia from intrinsic AKI like BUN: creatinine ratio, urinary sodium, fractional excretion of sodium, urine osmolality and urinary sediment (6) (7). Adenosine deaminase (EC 3.5.4.4) activity was first noticed by Gyorgy and Rothler. ADA is an ectoenzyme that is found in surface of many cells. The enzyme is widely distributed in human tissues and is found in kidney, intestinal mucosa, spleen, skeletal muscle, liver, serum, lymphocytes, leucocytes and erythrocytes (8) (20). Adenosine deaminase is an enzyme of purine metabolism which catalyses the deamination of adenosine to inosine and ammonia (9) (10). Human ADA consists of three isoenzymes ADA1, ADA1 CP, ADA2 distinguished from each other by electrophoretic technique. ADA1 is a monomeric protein with a
molecular mass of approximately 35 KDa (gene assignment, chromosome 20). ADA₁ + CP is composed of two ADA₁ molecules connected via a combining protein with a molecular mass of approximately 280 KDa (gene assignment, chromosome 2 & 6). ADA₂ is coded by a separate gene locus of unknown chromosomal position (11). ADA₁ exists in all human tissues and accounts for the main ADA activity in tissues. ADA₂ is the main isoenzyme in the serum and it originates from monocyte macrophage system (12). ADA induces T-cell dependent differentiation of monocytes into macrophages and stimulates macrophage proliferation (13). ADA, a polymorphic enzyme also has a role in proliferation and differentiation of epithelial cells, lymphoid cells and stimulates lymphocyte maturation (14) (15).

ADA also contributes to the regulation of intracellular and extracellular concentration of adenosine. Adenosine acts as a “Retaliatory metabolite” that has organ and cell protective function (16). It also decreases liberation of superoxide anion and H₂O₂ from stimulated neutrophils and thus protects vascular endothelium. Increased ADA activity is considered to be a sign of decreased adenosine level. It is known that adenosine can attenuate the ischemic/reperfusion injury. ADA is a marker of T cell activation and hence important in acute and protracted inflammatory responses (17).

AIMS AND OBJECTIVE:
The aim of the study is to evaluate serum adenosine deaminase activity in patients with acute kidney injury and to compare the same with normal healthy adults taken as controls and correlate the ADA level with other markers of AKI like blood urea and serum creatinine.

MATERIALS AND METHODS:
The study was conducted at our medical college hospital. Written informed consent was obtained. Study group included 50 individuals aged from 20-60 years who were clinically diagnosed as acute kidney injury of various etiologies (sepsis, snake bite and dehydration) with serum creatinine level > 1.5 mg/dl. Control group included 50 healthy individuals free from any major illness. Control and study group are age and sex matched. Both the groups were analyzed for ADA and blood urea and creatinine.

INCLUSION CRITERIA: Patients with Acute Kidney Injury

EXCLUSION CRITERIA: Individuals with other acute infections, tuberculosis, liver diseases and chronic inflammatory diseases were excluded from the study.

SAMPLE COLLECTION: 2ml of venous blood was collected. Blood samples were allowed to clot for half an hour and then centrifuged to get the serum. Hemolysed samples were excluded because erythrocytes contain adenosine deaminase and can falsely elevate ADA levels in serum.

ESTIMATION OF SERUM ADA:
ADA was estimated by the method of Guisti and Gianni's using semiautoanalyzer.

PRINCIPLE:
Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of bluecoloured Indophenol complex formed is directly proportional to the amount of ADA in the sample and it is measured at 620nm at 37° C in the semiautoanalyzer. One unit of ADA is defined as the amount of enzyme required to release 1 mol of ammonia per minute from adenosine at standard conditions. Normal reference range of Serum ADA: <30U/L
OTHER PARAMETERS:
1. Serum creatinine was estimated by the Jaffe’s method. 2. Blood urea was estimated by DAM acid mixture method.

RESULTS: TABLE: 1 STUDENT’S t-TEST ANALYSIS OF SERUM ADA BETWEEN CONTROL AND STUDY GROUP

![Table 1]

STATISTICAL ANALYSIS:
Results are shown as mean ± SD. Student’s t-Test was employed to determine statistical significance. P Value less than 0.05 was considered statistically significant. Pearson’s correlation was done in the study group between serum ADA with serum creatinine and blood urea. P value less than 0.01 was considered statistically significant.

![Table 2]

![Table 3]

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DISCUSSION:
In our present study, we measured serum ADA in patients with acute kidney injury and compared the same with normal healthy individuals. The mean ADA level was significantly high in the study group (47.53 ± 8.81) when compared to the control group (22.06 ± 5.94) and the p value is statistically significant.

Our study also showed an increased level of serum creatinine in the study group (4.29 ± 1.92) when compared to that of control group (0.82 ± 0.15). This fact clearly supports the pathogenesis that in acute kidney injury, the ability of the kidney to excrete creatinine is reduced when compared to healthy individuals. Pearson’s correlation studies revealed a positive correlation between serum ADA and serum creatinine and blood urea. The kidney plays a central role in body homeostasis. Systemic neurohumoral control system & intrarenal regulation are important for proper renal function. Various autacoids are potential candidate to contribute to the signaling cascade involved in the local regulation and adenosine is one among them (18). In acute kidney injury reduction in blood flow represents a common pathological pathway for decreased glomerular filtration rate. Prolonged ischemia leads to inflammation and infiltration with leukocytes. The activated leucocytes release free radicals which inturn damage the cell membrane and leads to the release of ADA. Recent studies implicate the role of endogenous signaling molecule, adenosine in kidney protection from ischemia. It plays a critical role in attenuating renal inflammation & preserving kidney function during episodes of renal ischemia (19).

Increased levels of ADA drive the metabolism of adenosine towards inosine and ammonia. Inosine is further degraded into uric acid by xanthine oxidase (20). If adenosine is metabolized by high level of ADA, the beneficial effects of adenosine will be lost and by the same time the production of superoxide radicals exacerbate the acute kidney injury.

CONCLUSION:
To conclude, our study showed an increased level of serum ADA in acute kidney injury when compared to normal healthy individuals. Elevated ADA indicates that there is an oxidative damage and decreased adenosine in acute kidney injury. At present, therapeutic modalities to prevent or treat AKI are limited and the association of AKI with ADA implies, it would be a novel therapeutic intervention for acute kidney injury.

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