# Quantitative determination of endogenic human Pseudomonas aeruginosa exotoxin A (PEA) concentrations in serum of renal failure patients by ELISA.

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## Abstract

P.aeruginosa is one of the most danger cause of morbidity and mortality in patients in nosocomial infection and renal failure. ELISA method this assay has high sensitivity and excellent specificity for detection ofhuman PEA. The medical records of 64 patients (42 males and 22 females of renal failure patients ) undergoing hemodialysis at the Department of Nephrology–hemodialysis of the Tikrit Teaching Hospital from (12 -2012 to 12-2013 ) were retrospectively reviewed. Control of exotoxin concentration is zero because the minimum detectable dose of human PEA concentration is typically less than 0.039ng/ml depend on sensitivity of ELIZA kit.Detectable exotoxin A in serum of 64 patients The result showin this study maximum concentration was 3.5 ng/ml .positive result was 37 males of 42 and 17 females of 22.and no significant exotoxin A concentration between males and females.

Key word: P.aeruginosa , Renal failure , Exotoxin A ,Patients ,Serum ,ELISA.

## INTRODUCTION

Patients with end-stage renal disease requiring dialysis are at increased risk for bloodstreaminfection ,this type of infection representsa main cause of morbidity and cause death. (1,2,3,4) . Virulence of P. aeruginosa is multifactorial and has been attributed to cell associated factors like exotoxin A (5,6). pathogenic p. aureoginosa strains possess a type III secretion system that allow them to deliver toxins directly into the cytoplasm of a host cell. ExotoxinA which causes tissue necrosis since it block protein synthesis(7) . The most important factor in the pathogenicity of P. aeruginosa, ETA consists of two subunits; fragment A is catalytic, and fragment B is responsible for interaction with eukaryotic cell receptors. ETA is cytotoxic to numerous mammalian cells tubular necrosis of kidneys.(8,9,10)

## MATERIALS AND METHODS

Patients : The medical records of 64 patients (42 males and 22 females of renal failure patients ) undergoing hemodialysis at the Department of Nephrology–hemodialysis of theTikrit Teaching Hospital from (12 -2012 to 12-2013 ) were retrospectively reviewed.

Control of exotoxin concentration is zero because the minimum detectable dose of human PEA concentration is typically less than 0.039ng/ml depend on sensitivity of ELIZA kit ,that kit use in this method [human pseudomonas exotoxin A (PEA) ELISA Kit / Cusabio- china]

The free EDTA tubes (non-EDTA blood) was left about 15 min. in room temperature, the blood clot was detached from tubes surface sides to free the clot and let serum to accumulate at the clot surface. Centrifuged at 400 rpm (18 C°) for 10 min., the supernatant (serum was aspirated by Pasteur pipette, and re-centrifuged the supernatant in the same manner to sediment any erythrocytes may cause serum contamination, the supernatant was aspirated, store at -20 C° in tubes labeled with patient's number.

❖ Human pseudomonas exotoxin A (PEA) ELISA Kit

- Principle of the assay : This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for PEA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PEA present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for PEA is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PEA bound in the initial step. The color development is stopped and the intensity of the color is measured.
- Procedure :Kit were prepared as instructed by manufacturing Companies.

## CALCULATION OF RESULTS

Using the professional soft "Curve Expert 1.4" to make a standard curve is recommended, which can be downloaded fromCusabio web site.

## Results

PEA concentrations in 64 renal failure patients were estimated in serum by using ELISA .

Table (2 ) , show the mean concentration of exotoxin A of Pseudomonas for 42 males and 22 females of renal failure patients . there was no significant in exotoxin A concentration between males and females . Control of exotoxin concentration is zero because the minimum detectable dose of human PEA concentration is typically less than 0.039ng/ml depend on sensitivity of ELIZA kit ,that kit use in this method [human Pseudomonas exotoxin A (PEA) ELISA Kit / Cusabio- china]

Figure (2) show detectable exotoxin A in serum of 64 patients .The result showin our study maximum concentration was 3.5 ng/ml .

Rational Function: y=(a+bx)/(1+cx+dx^2) (manual of kit )

Coefficient Data:

a = -0.2258472

b = 1.4496171

c = -0.10786801

d = -0.043816625

X = OD(absorbtion)

Y = Concentration of exotoxin A

Also , in our study show no significant exotoxin A between males and females who have toxin in serum. Show in table (3) positive result was 37 males of 42 and 17 females of 22.

## Discussion

In this study , show the result of 64 renal failure patients (37 males and 17 females )were

have exotoxin A (PEA) concentration average from (0.039 to 3.5 )ng/ml in serum produced by causative agent Pseudomonasaeruginosa. Saroj Sharma, Ramanjeet Kaur, Vanashree Yadav, Kusum Harjai and Kusum Joshi.2004 show the importance of this organism is of special relevance since it is UTIs third leading cause, accounting for about 11% of nosocomial UTIs . In the present study, an exotoxin A-producing strain of P. aeruginosa PAO, and its mutant lacking this ability were employed to study the possible role of exotoxin A in acute as well as in chronic pyelonephritis.(11)

P. aeruginosa exotoxin A (toxin, lethal toxin), which is produced by over 90% of P. aeruginosa clinical isolates, However, some strains of Pseudomonas do not produce good yields of exotoxin A when grown in chemically defined medium (12)

Our study the first attempted to evolution exotoxin A in human serum , all previous studies on serum of laboratory animals , or determinate exotoxin A in broth media and most of studies determinate antibodies in serum against exotoxin A(13,14)and show in this result no different in exotoxin concentration between males or females .This result agreed with ,the pathogenic substances of Pseudomonas aeruginosa include chemoattractant factor,(15) numerous exoproducts such as exotoxins, proteases ,phospholipase and leucocidin In disease states such as cysticfibrosis, many clinical isolates are positive for alkaline protease, elastase and exotoxin A production (16,17)These enzymes have been implicated as important factors contributing directly or indirectly to the pathogenicity of microbes .Patients with UTI and kidney disease have a high prevalence of Pseudomonas aeruginosa colonization(18,19), which rapidly causes a chronic infection of the mucosal surface of the This was also tested for exotoxin A by using two culture supernatants at concentrations of 5.4 and 10.8 µg/l for different strains .Also, agree with the study Acute Pseudomonas aeruginosa was established in guinea pigs by intratracheal instillation of bacteria. Challenge strains included PAO-1, a strain known to produce exotoxin A, alkaline protease, and elastase, and several PAO-1 mutants deficient in either biologically active exotoxin A or elastase production. Survival, intrapretoneal killing of bacteria, and blood cultures were compared among the groups. Strains of P. aeruginosa deficient in active elastase production appeared to be less virulent than the parent strain and were more easily cleared from the lung. Opposite results were obtained for the exotoxin A-deficient mutants. These data suggest that elastase, but not exotoxin A, was an important virulence factor during acute disease due to P. aeruginosa. Experimental data show that elastase and exotoxin A elicit high levels of antibodies both in experimental animals and in patients. These results suggest that these proteins should be considered for use in a prophylactic Pseudomonas vaccine.(20,21,22)

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**Table(1)Materials provided in ELISA kit**

| Quantity | Reagents |
|---|---|
| 1(96 wells) | Assay plate (12 x 8 coated Microwells) |
| 2 | Standard (Freeze dried) |
| 1 x 120 µl | Biotin-antibody (100 x concentrate) |
| 1 x 120 µl | HRP-avidin (100 x concentrate) |
| 1 x 15 ml | Biotin-antibody Diluent |
| 1 x 15 ml | HRP-avidin Diluent |
| 1 x 50 ml | Sample Diluent |
| 1 x 20 ml | Wash Buffer (25 x concentrate) |
| 1 x 10 ml | TMB Substrate |
| 1 x 10 ml | Stop Solution |
| 4 | Adhesive Strip (for 96 wells) |
| 1 | Instruction manual |

**sample: Serum Separation**

**Table (2) Toxin concentration in renal failure patient according to gender**

| Gender | No. | Mean±SD |
|---|---|---|
| **Male** | **42** | **0.814±0.95** |
| **Female** | **22** | **0.436±0.48** |

**t=1.744, df=62, P > 0.05 not significant**

**Table (3) The positive renal failure patient distribution according to serum toxin level and gender**

| Toxin in serum | Male | | Female | |
|---|---|---|---|---|
| | Frequency | Percent | Frequency | Percent |
| Positive (>0.039)ng/ml | 37 | 88.1 | 17 | 77.3 |
| Negative | 5 | 11.9 | 5 | 22.7 |
| Total | 42 | 100 | 22 | 100 |

**Yates' chi-square=0.593, df=1, p > 0.05 not significant**

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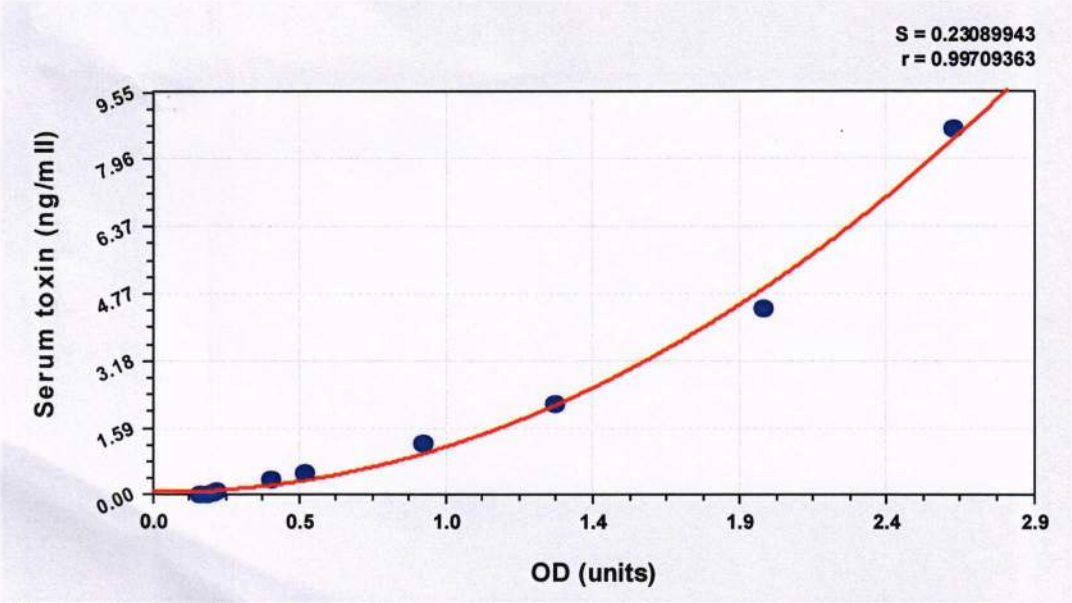


**Figure (1): Standard curve of concentration Pseudomonasaeruginosaexotoxin A**

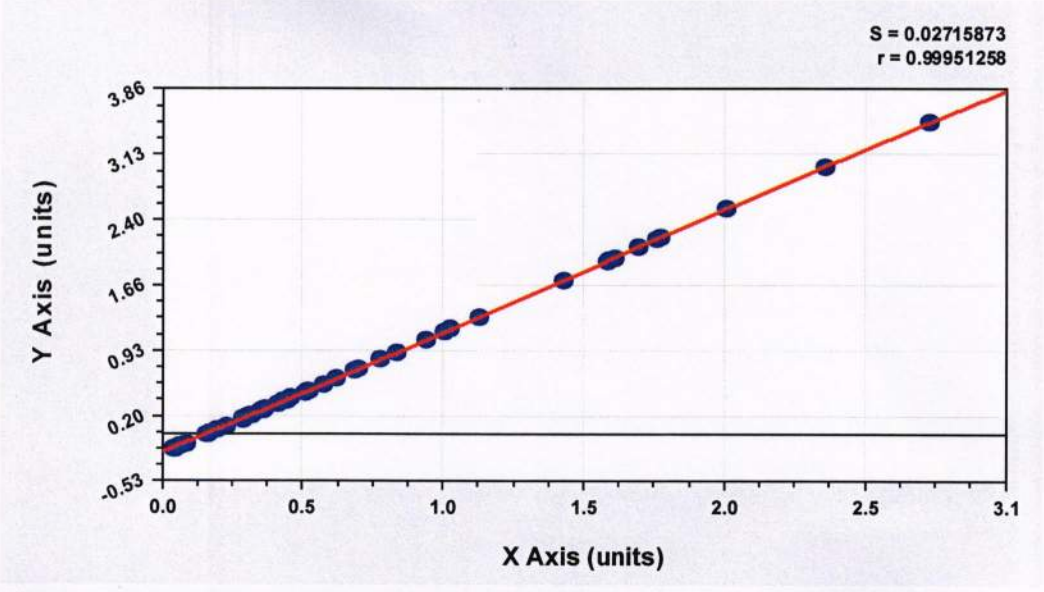


**Figure (2) Quantitative determination of endogenic human Pseudomonas exotoxin A (PEA) concentrations in serum of renal failure patients by ELISA.**