Effect of Radio Contrast Agents on Release of Urinary Endothelin in Diabetic Patients with or Without Renal Impairment

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Abstract

Radiocontrast media (CM) is one of the most common causes of hospital-acquired acute renal failure. The proposed mechanisms include direct nephrotoxicity and renal ischemia. Endothelin-1 has potent vasoconstrictor effects on various vessels, including the renal vasculature. Patients with pre-existing renal insufficiency, diabetes mellitus, congestive heart failure or dehydration are at high risk of radio contrast nephropathy.

Aim of the Study: Was to examine the role of Endothelin in radiocontrast nephropathy in diabetic patients with or without renal impairment.

Material and Methods: Forty patients who have been scheduled for examination using radiocontrast agents were enrolled in the present study. They were collected from cath lab and hemodialysis units in Kasr EL Aini hospitals. They were divided into 4 groups according to their renal function:

Group A: 10 patients with DM but normal renal function.
Group B: 10 patients with DM and impaired renal function.
Group C: 10 patients with DM and advanced kidney disease.
Group D: 10 healthy subjects of matched age and sex.

Exclusion Criteria: Dehydration and pregnant women.

All subjects were subjected to thorough clinical examination, All of them were scheduled for coronary angiography.

All subjects undergone the following investigations before infusion of the contrast media (CM) and 48 h after infusion of contrast media:

1- Measurement of serum creatinine and blood urea concentration
2- Estimation of glomerular filtration rate (GFR).
3- Measurement of plasma endothelin (ET) and urinary endothelin concentrations by radio immunoassay.

Results: As regards urinary level of ET before radio contrast infusion in different subjects groups the highest levels of ET before radio contrast was for groups B and C (ET levels were 3.64 and 3.76 respectively).

Urinary ET was higher in control subjects (group D) than diabetic patients with normal kidney functions (group A).

By comparing urinary ET before and after radio contrast infusion; there was no significant difference in groups A and C. While there was a significant rise in urinary ET in groups B and D. With the highest levels of urinary ET noted in group B.

There was a positive correlation between urinary ET and blood urea and serum creatinine 48 hours after radiocontrast.

Key Words: Contrast – Nephropathy – Diabetes – Endothelin.

Introduction

RADIO contrast media (CM) is one of the most common causes of hospital-acquired acute renal failure. Although the precise mechanisms of radio contrast-induced nephropathy remain unclear [1].

The proposed mechanisms include direct nephrotoxicity and renal ischemia. Endothelin-1 has potent vasoconstrictor effects on various vessels, including the renal vasculature and is reported to induce hemodynamic changes similar to those induced by CM when infused into the renal artery.

Furthermore ET antagonists such as BQ123 and FR 13917 can inhibit CM induced vasoconstriction in the kidney. Therefore, it is conceivable that ET is one of the important mediators of RCN.

Patients with pre-existing renal insufficiency, diabetes mellitus, congestive heart failure or dehydration are at high risk of radio contrast nephropathy.
The aim of study: Is to examine the role of Endothelin in radiocontrast nephropathy in diabetic patients with or without renal impairment.

Material and Methods

Forty patients (24 male and 16 females, their age group 39-70 years) who have been scheduled for examination using radiocontrast agents were enrolled in the present study. They were collected from cath lab and hemodialysis units in Kasr El Aini Hospitals. They were divided into 4 groups according to their renal function.

**Group A:** 10 patients with DM but normal renal function (serum creatinine concentration 1±0.2mg/dl).

**Group B:** 10 patients with DM and impaired renal function (mean serum creatinine concentration 1.9±0.5mg/dl).

**Group C:** 10 patients with DM with advanced kidney disease (serum creatinine 6.2±1.36 mg/dl).

**Group D:** 10 healthy subjects of matched age and sex (serum creatinine concentration 0.9±0.1mg/dl) with no history of diabetes mellitus and normal body weight.

Exclusion criteria: Dehydration and pregnant women.

All subjects were subjected to thorough clinical examination. All of them were scheduled for coronary angiography.

All patients were properly hydrated before radiocontrast infusion.

All subjects undergone the following investigations before infusion of the contrast media (CM) and 48 h after infusion of contrast media:

1. Measurement of serum creatinine and blood urea concentration.
2. Estimation of glomerular filtration rate (GFR).
3. Measurement of plasma endothelin (ET) and urinary endothelin concentrations by radio immunoassay before infusion of (CM) and 48 h after infusion of CM.

Venous Blood samples were placed into test tubes containing sodium-EDITA and immediately centrifuged at 4C, the plasma was extracted plasma and stored at-20C. Urinary samples were obtained and stored then used without pretreatment.

Radio immunoassay for endothelin were performed by standard double antibody precipitation technique [4].

All reagents and samples must be at room temperature (18-26°C) before use in the assay. Take microtiter strips out of the aluminum bag. Store unused strips with desiccant at 2-8°C in the aluminum bag.

Add 50 µL (Standard/Sample/Control) in duplicate into respective well, except blank.

Add 200 µL (Detection antibody) into each well, except blank, swirls gently.

Cover tightly and incubate overnight (16-24 hours) at room temperature (18-26°C).

Aspirate and wash well with 300 µL diluted wash buffer, remove remaining wash buffer by hitting plate against paper towel after the last wash. Add 200 µLCNJ (Conjugate) into each well. Cover tightly and incubate 1 hour at room temperature (18-26°C).

Add 200 µL substrate into each well. Incubate for 30 minutes at room temperature (18-26°C) in the dark.

Add 50 µL stop solution into each well, shake well.

Measure absorbance immediately at 450nm with reference 620nm, if available [4].

Statistical methods:

Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 10.

Parametric data was expressed as mean ± SD.

Comparing the mean ± SD of 2 groups was done using the student’s t test.

\[ t (df) = \frac{X - Y}{SDp \sqrt{\frac{1}{nX} + \frac{1}{nY}}} \]

Where, \( t (df) \): Value at the degrees of freedom. 
\( df \): Degrees of freedom. 
\( X \): Mean of sample X. 
\( Y \): Mean of sample Y. 
\( nX \): Number of sample X. 
\( nY \): Number of sample Y. 
\( SDp \): Pooled SD (SD of both samples).

\( p \) value ≤0.05 is considered significant & if ≤0.01 is considered highly significant.

Results

Group A (diabetics with normal kidney functions) showed no significant difference in all variables even ET after radio contrast infusion \( p >0.05 \) (Table 1).
As regards group B (patients with renal impairment), there was a significant difference in urinary ET, GFR, and serum creatinine levels after radio contrast infusion: 4.69 ± 1.57, 24.9 ± 8.7 and 3.25 ± 0.88 respectively than before CM infusion where these parameters were 3.64 ± 0.71, 28.3 ± 8.31 and 2.83 ± 0.52 respectively (p < 0.05) Table (2).

In group C, there was only a significant decrease in GFR level where it was 8 ± 2.36 before CM infusion and became 7.2 ± 1.87 after radio contrast infusion (p < 0.05), with no significant difference as regard other variables (Table 3).

The control subjects (group D), showed a significant increase in urinary ET after CM 1.79 ± 0.64 than before infusion where it was 1.54 ± 0.59 (p < 0.05) (Table 4).

Diabetic patients of group B had the highest levels of urinary Endothelin after infusion of contrast media compared to other groups with significant difference (Table 5).

They also showed a significant difference in plasma ET level (significantly higher in group B rather than other groups (p < 0.05).

There was a significant positive correlation between Urinary ET and urea, creatinine (after 48 h) of radiocontrast infusion in groups A & B, 0.772 & 0.785 respectively.

### Discussion

With increasing use of contrast media in diagnostic and interventional procedures, contrast media induced nephropathy has become the third leading cause of hospital acquired ARF [5].

The risk of contrast medium nephropathy continue to be considerable despite the use of newer and less nephrotoxic contrast agents in high risk patients in recent years [1].

Contrast medium nephropathy is usually defined as impairment of renal function occurs within 48 hours after administration of contrast media [5].

The rate of contrast medium nephropathy in patients with pre existing renal dysfunction or DM in whom standard hydration protocol was not administrated is between 12 and 26% [6].

Experimental studies suggest that contrast medium nephropathy result from combination of Renal

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<th>Table (1): Comparison between laboratory data before and after among group A.</th>
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<th>Table (5): Urinary ET levels among different groups after CM.</th>
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ischemia and direct toxic effect on renal tubular cells regardless of the underlying cause [2].

Endothelin (ET)-1 is an endothelium-derived peptide with potent vasoconstrictor and proliferative properties [7].

CM has been shown to induce an increase in ET mRNA [8].

Pre-treatment with BQ123 (endothelin antagonist) in vitro significantly inhibited the effects of various contrast media on GFR [3].

In a large multicenter trial, Wang and co-workers gave a non-selective ET antagonist to patients prior to CM exposure. However, after 24 hours an increase in creatinine was seen, suggesting a reduction in GFR. While this may indicate that other peptides are important in mediating RCIN in humans, the use of a selective ETA-receptor antagonist may have produced different results as the dilatory effect of ET through ETB receptors would have been conserved. This is a controversial area that requires further research [9].

However, regardless of the mediating substances, the haemodynamic changes we have discussed are responsible for the ischaemic damage induced by CM. In the present study, we measured the dynamic changes in the plasma and urinary excretion of ET after the infusion of CM and investigated the role of ET in radio contrast nephropathy by comparing diabetic patients with renal dysfunction and those with normal renal function.

Clark, et al. [10] reported significant increase in plasma ET after administration of CM in normal rat.

Although, Masanri and co-workers, reported that no significant change in plasma ET or urinary ET levels after CM infusion in patients with normal renal function [11]. This goes with the results of our study that revealed non-significant change in plasma ET, urinary ET after infusion of CM in patients with normal renal function.

As regards diabetic patients, recent study by Heymen and colleagues, reported rising of plasma ET shortly after administration of CM in diabetic patients [12].

Another study found that diabetes and radio contrast agents are associated with increased ET-1 level, and ET is believed to participate in pathogenesis of diabetic nephropathy [13].

Also, it was found that endothelin play an important role in radio contrast nephropathy in diabetic patients [14].

In contrast, our study revealed, non significant increase in plasma ET or urinary ET after radio contrast infusion in diabetic patients with normal kidney functions (p >0.05).

However, chronic renal impairment was an important risk factor for contrast induced nephropathy so patients with renal impairment or those on regular dialysis showed the highest levels of urinary ET before and after CM infusion with declining of GFR.

This goes with Masanori, et al. [11] who reported a significant increase in urinary ET after radio contrast infusion in patients with impaired renal function whereas no significant changes was observed in level in plasma endothelin after radio contrast infusion.

In conclusion: Endothelin especially urinary endothelin is considered positive test and may be used in early prediction of radiocontrast nephropathy in patients with chronic renal impairment.

References


