**The Study Relation of Endothelin-1 and Malondialdehyde with Chronic Kidney Disease**

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**Abstract**

Chronic kidney disease is a worldwide public health problem with an increasing incidence and prevalence. A cross-control study was carried out for estimation of endothelin-1 and MDA and some parameters in patients with chronic renal disease.The number of patients under the study were 40 patients with chronic renal disease (under hemodialysis) their ages were between 20-75 years old. The study included 40 healthy control group who apparently haven’t any disease, with the same demographic properties.The study showed that the highest mean level of endothelin-1 and MDA were found in patients with chronic renal disease as respectively (102.6±15.1 pg/ml) and (12.03±3.94 nmol/ml). The lowest mean of the two markers were in the control group as respectively (39.03±5.94 pg/ml) and (7.56±2.25nmol/ml). The current study showed that the mean age of patients enrolled in the study was 51.7 years, the highest mean of endothelin-1 in HD patients was recorded within the age group >50 year with hypertension (P<0.05). The study showed that the highest means level of B. urea, S. creatinine and Cystatin were found in patients with chronic renal disease (156.3±22.4 , 7.22±2.91 mg/dl and 12.88±2.16 mg/dl) respectively, and the lowest means of the tests above were noted in the control group with highly significant relation (P. value <0.01). The study showed positive correlation of ET-1 and MDA with B. urea, S. creatinine and cystatin c. The study showed significant positive correlation of MDA with ET-1 in CKD patients (r: 0.57, P<0.01).

***Keywords:*** *Endothelin\_1, Malondialdehyde, Chronic kidney disease*

1. **Introduction**

Chronic kidney disease is a very important worldwide public health problem with an increasing incidence and prevalence, that cause poor outcomes, and high cost. Outcomes of chronic kidney disease include not only kidney failure but also complications of decreased kidney function and cardiovascular disease [1].

In recent years, a pivotal role for the Endothelin system (ETs) has been documented in normal renal function, and also in renal disease. Endothelin-1 (ET-1) acts as an autocrine and paracrine manner in renal vessels and nephron segments, it is modulating renal hemodynamics by tubular water and minerals reabsorption [4]. ET-1 has been implicated in pathological conditions such as renal fibrosis, glomerular sclerosis which leads to the progressive decline in renal function, development of hypertension and cardiovascular hypertrophy.

On the otherwise renal disease is associated with a graded elevation in oxidative stress (OS) markers even in early CKD [2]. Oxidative stress can enhance renal injury progression and contribute to increased cardiovascular risk. Some studies have documented that peritoneal dialysis is associated with decreased levels of OS and inflammatory markers more than hemodialysis [3]. Malondialdehyde (MDA) is the marker of OS produced by peroxidation of unsaturated fats, it is associated in atherosclerosis progression in patients with chronic kidney disease (CKD) not yet on dialysis compared to patients on peritoneal dialysis is less known, particularly concerning cardiometabolic syndrome [5].

**2. Material and Methods**

**2.1** **Study Design**

The study is performed in Kirkuk city from 27th of October 2020 to 30th of November 2020. For the study, the relationship between Endothelin-1 and Malondialdehyde with the chronic kidney disease we depended on the samples taken from CKD patients on hemodialysis and other samples were taken from healthy people as the control group. The number of patients under study reached 40 patients with chronic kidney disease (under dialysis). The study included also 40 healthy control group who haven’t any disease, with the same demographic properties (healthy individuals). Five ml of the blood sample was taken by vein puncture without using tourniquet from each subject registered in this study. Blood samples were placed into sterile test tubes, after blood clotting, centrifuged at 3000 rpm for 15 minutes then clot removed and remain re-centrifuged at 3000 rpm for 10 minute and the obtained serum were aspirated using a mechanical micropipette and transferred into clean test tubes which labelled and stored in a deep freeze at -20 °C for biochemical measurement of blood urea, serum creatinine, serum (MDA), as well as estimation of human Endothelin-1 by ELISA technique.

**2.2 Estimation of Human Endothelin-1 by ELISA**

For estimation of human Endothelin-1 by ELISA kit Sandwich-ELISA method is used. The Microelisa strip plate provided in the kit has been pre-coated with an antibody specific to ET-1. Each of the standards and samples were added to the appropriate Microelisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for ET-1 is added to each well and incubated. Then TMB, substrate solution was added to each well. The wells which contain ET-1 and HRP conjugated ET-1 antibody will appear blue and when added the stop solution it will turn to yellow. The optical density (OD) is measured by the spectrophotometer at a wavelength of 450 nm. The concentration of ET-1 is proportional to the OD value. We can calculate the concentration of ET-1 in the prepared samples by comparing the OD of the samples to the standard curve.

**2.3 Determination of Serum Malondialdehyde**

The level of malondialdehyde was determined by the reaction of MDA with thiobarbituric acid –TBA, forming an MDA-TBA2 according to the modified method described by Schmedes and Holmer.

**Manual procedure**

In the table 2.1. below the details of the manual procedure for the determination of serum malondyaldehyde is showed.

|  |  |  |
| --- | --- | --- |
| **Pipette into well identified test tubes:** | **Test** | **Standard** |
| **Serum**  | 150 µl | - |
| **Distilled** **water** | - | 150 µl |
| **TCA(17.5%)** | 1 ml | 1 ml |
| Mix well |
| **TBA (0.6%)** | 1 ml | 1 ml |
| The tubes were mixed well by vortex, incubated in boiling water bath for 15 minutes, and then allowed to cool.  |
| **TCA(70%)** | 1 ml | 1 ml |
| The mixture was let to stand at room temperature for 20 minutes, The tubes were centrifuged at 2000 rpm for 15 minutes, and the supernatant was taken for measurement of the absorbance at 532nm.  |

**3. Results and Discussion**

**3.1. General Characteristics of the Studied Groups**

**Table 3.1**. General characteristics of the studied groups

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters**  | **CKD patient****(n:40)** | **Control group****(n:40)** | **P. value** |
| **Age (Mean±SD)** | 51.7±3.9 | 50.3±3.8 | NS |
| **BMI** | 27.5±3.8 | 27.2±4.2 | NS |
| **Sex** | 25 male/ 15 female | 24 male/ 16 female | NS |
| **Residence (urban)** | 55% | 56% | NS |

**3.2 Relation of Endothelin-1 with CKD**

The study showed that the highest mean level of endothelin-1 was found in patients with chronic renal disease (102.6±15.1 pg/ml) and the lowest mean was in the control group (39.03±5.94 pg/ml), (P< 0.01), Table 3.2.

|  |  |  |
| --- | --- | --- |
| **Studied groups** | **N** | **Endothelin-1 level (pg/ml)** |
| **Mean** | **SD** | **SE Mean** |
|  CKD | 40 | 102.6 | 15.1 | 8.5 |
| Control group | 40 | 39.03 | 5.94 | 1.1 |
| **P. value: 0.001** |

**3.3 Level of Malondialdehyde (MDA) in Patients with CKD and The Control Group.**

The study showed that the highest mean level of MDA was found in patients with chronic renal disease (12.03±3.94 nmol/ml) and the lowest mean was recorded in the control group (7.56±2.25nmol/ml), (P< 0.01), Table 3.3

|  |  |  |
| --- | --- | --- |
| **Studied groups** | **N** | **MDA level (nmol/ml)** |
| **Mean** | **SD** | **SE Mean** |
| CKD | 40 | 12.03 | 3.94 | 1.1 |
| Control group | 40 | 7.56 | 2.25 | 0.80 |

**3.4 Relation of ET-1 with Age of CKD Patients**

The current study showed that the mean age of patients enrolled in the study was 51.7 years, the highest mean of endothelin-1 in HD patients was recorded within the age group >50 year (P<0.05), Table 3.4.

|  |  |
| --- | --- |
| **Age groups (years)** | **Endothelin 1 level (pg/ml) (Mean±SD.)** |
| No. | Chronic renal disease (n:30) |
| **<30** | 8 | 88.5±22.5 |
| **30-50** | 18 | 109.1±31.5 |
| **>50** | 14 | 115.4±29.4 |
| **P. value:** 0.014 |

**3.5 Correlation of MDA with ET-1 of CKD patients**

The study showed significant positive correlation of MDA with ET-1in CKD patients (r: 0.57, P<0.01), Figure 3.5



**Figure 1.** Correlation of MDA with ET-1 of CKD patients

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