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THROMBOMODULINE IN CORRELATION WITH SYSTEMIC INFLAMMATION AND ATHEROSCELEROSIS IN CHRONIC RENAL DISEASE PATIENTS

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Abstract: Background: Chronic kidney disease (CKD) is a global health burden with high economic costs and an independent risk factor for cardiovascular disease in healthy systems (CVD). Nevertheless, in the development of atherosclerosis, chronic inflammation and endothelial dysfunction are critical events; both are present in CKD and HD patients. Thrombomodulin (TM) is a transmembrane glycoprotein originally located on the vascular endothelium that is expressed on the cell surface. In natural human anticoagulation systems, TM on vascular endothelial cells is a significant molecule. In atherosclerosis, TM has specific impacts on cell proliferation, adhesion and inflammation, all of which are essential measures. Aims: The aim of the present study is to detect the relationship between thrombomodulin and atherosclerosis in chronic kidney disease patients. Materials and Methods Thrombomodulin (TM) was assessed in blood specimens of 38 patients with CKD (19 patients with CKD who did not begin HD (Group 2) and 19 patients with CKD who were on regular HD for more than 6 months (Group 1) and compared to 19 control subjects (Group 3) (Control Group). Results: The mean value of Thrombomodulin (TM) in the control group (430.2±102), the mean value in the CKD predialysis group (521.1±198) and The mean value in the CKD on HD group (729.4±513),thus The mean values Thrombomodulin (TM cells in CKD on HD group was significantly higher than that of the control group (P=0.00), and The mean value of Thrombomodulin (TM) was higher in the CKD group for HD than in the CKD group for predialysis, and there was a substantial difference among the CKD group for predialysis and the CKD group for HD (P=0.00), but there was no substantial difference among the CKD group for predialysis and the control group (p=0.4). There is a high significant direct correlation between Thrombomodulin (TM) and CIM in the studied groups r=(0.618)and p(<0.01). .Conclusions: In CKD patients, not only is Thrombomodulin (TM) strongly extended, but this expansion is correlated with preclinical atherosclerosis. In the CKD (predialysis and regular HD) groups, there was an independent association between thrombomodulin (TM, age, hsCRP) and IMT.

Keywords: Cardiovascular disease, Atherosclerosis, Inflammation, CIMT, Thrombomodulin (TM), CKD (predialysis or on regular HD).

INTRODUCTION

Chronic kidney disease(CKD) characterized as the existence of renal damage, evidenced by abnormal excretion of albumin or reduced renal function, quantified by the measured or predicted glomerular filtration rate (GFR) remaining for more than 3 months**. (1)**. Chronic kidney

disease(CKD) is an independent risk factor for cardiovascular disease (CVD) and is a global health burden with a large economic cost to healthy systems. All CKD stages are correlated with elevated rates of cardiovascular morbidity, early death, and/or reduced life quality **(2**). In patients with CKD, cardiovascular disease (CVD) is twice as common as in the general population **(2)**.

Cardiovascular disease (CVD) is closely correlated with end-stage renal disease (ESRD) and chronic kidney disease (CKD) and has been shown to be the main trigger of morbidity and mortality in end-stage renal disease patients (ESRD) **(3**).

Thrombomodulin (TM) is a transmembrane glycoprotein, originally recognized on vascular endothelium that is expressed on the cell surface **(4)**. A major molecule in the natural human anticoagulation system is TM on vascular endothelial cells. TM functions as a thrombin receptor on the surface of vascular endothelial cells **(4)**. The binding of thrombin with thrombomodulin activates protein C and the thrombin thrombomodulin complex functions as an anticoagulant and anti-inflammatory stimulus **(5**). Localized TM loss from arteriosclerotic lesions overlaying endothelium contributes to a focal weakness of activation of Protein C **(6).** Studies showed expansion of thrombomoduliin end stage renal disease (ESRD) patients especially those with cardiovascular disease. The uraemia-induced proinflammatory conditions are thought to be responsible for the expansion of these cells. **(7)**

SUBJECTS AND METHODS

The ethical standards were followed by the protocol for this research and accepted by our institution's ethical committee and all participants provided informed consent to take part in this research. This research was conducted out on 38 patients with CKD (16 women and 22 men). In additionally, as a control group, 19 healthy participants (10 men and 9 women) of matched age and gender were included. Participants were classified into three groups: group I (N=19); group II, CKD patients with regular HD for more than six months; patients with CKD who did not begin HD (predialysis group) and group III (control group) (N=19); apparently normal individuals.

The research omitted patients with active infection, autoimmune disease history, hepatitis c virus infection, malignancy, immunosuppressant drug patients, ischaemic heart disease and smokers. All participants have undergone a complete history and clinical review. The Cockcroft-gault formula was used to estimate GFR:(140-age in years) x weight (kg)/ 72 X plasma creatinine (mg/dl).

Laboratory assessment

Sterile venipuncture blood samples were obtained and divided into 3 parts; on the dipotassium ethelenediamine tetra-acetic acid (EDTA) tube, the first part was collected for CBC. The second component was delivered to a single tube in which the serum was separated by centrifugation for 10 mins at 3000 rpm and used to evaluate renal function, serum calcium, lipid profiles, phosphorus, albumin and turbidimetry for highly sensitive CRP evaluation. The third component was delivered using a cytometer to the EDTA tube for the Flow-Cytometric Analysis of T Cell Subsets (BECTON DICKINSON.(

Sample collection and preparation

Blood samples were collected in a steady state condition after 20 minutes of resting in semi recumbent position. Samples were collected before the dialysis session for hemodialysis patients. Samples were centrifuged and serum was kept at -70°C .

Assay Procedure of thrombomodulin

Before usage, the ABC work solution and TMB colour development agent must be stayed warm at 37 ° C for 30 minutes. They must be mixed thoroughly and uniformly when diluting samples and reagents. For every experiment, the standard thrombomodulin detection curve must be

prepared. By crude estimate of the quantity of Thrombomodulin in specimens, the user can decide the specimen dilution fold.

Radiological investigations;
Calculation of intimal media thickness of the common carotid artery with ultrasound system, 12 leads (ECG).

Statistical analysis
To conduct the analysis, we used the statistical package of social signs (SPSS, version 16). The number and percentages of categorical data and continuous variables were described as mean ± standard deviation (SD). The ANOVA or Kruskalwalis test was used as one way to compare more than two independent groups of quantitative variables as necessary. The Chi-Square Test, T-Test and Correlations of Spearman (r) have been used.

RESULTS

1: Biochemical parameters

Biochemical parameters are recorded in the 57 participants (19 controls and 38 patients) (Table 2). In both groups (CKD predialysis and HD group), the average value of hemoglobin, serum calcium and HDL was substantially lower than in the control group. And their average value for CKD was substantially lower in the HD group than in the CKD predialysis group, whereas their average value for serum urea, creatinine, phosphorus, cholesterol and LDL was substantially higher in the CKD (predialysis and HD) group than in the control group, and their average CKD values for the HD group were substantially higher than those for the CKD predialysis group, but there was no substantial variation among the three groups studied with respect to the average triglyceride value.

2: Values of highly sensitive CRP and CIMT

Regarding inflammatory marker (hsCRP),There is significant variation among group 1 (haemodialysis) and group III (control) (p=0.00) also there is significant variation among group 1 (haemodialysis) and group II (predialysis) (p=0.00), but there is no significant difference between group II (predialysis) and group III control) (p=0.1).
Regarding CIMT there is significant variation among group 1 (haemodialysis) and group III (control) (p=0.00) also there is significant variation among group 1 (haemodialysis) and group II (predialysis) (p=0.00), but there is no significant difference between group II(predialysis) and group III (control) (p=0.13)

3: Thrombomodulin (TM)

As regarding Thrombomodulin (TM of the studied groups ,the mean Thrombomodulin (TM ±SD within the haemodialysis group(group 1)was(729.4±513) compared to predialysis group(group 2) (521.1±198) and the control group(group3)(430.2±102) which is a substantial correlation among the three groups(p=0.000).

There is substantial variation among group A (haemodialysis) and group C(control) (p=0.000) also there is significant variation between group A (haemodialysis) and group B(CKD) (p=0.000), but there is no significant variation between group B(CKD) and group C(control) (p=0.4).

4: The correlation among CIMT, **Thrombomodulin, CRP, and GFR All Cases**

From the table correlation analysis reveal a high significant direct correlation between Thrombomodulin (TM) and CIM in the studied groups.

There is also a high significant direct association among CRP and Thrombomodulin (TM) in studied groups.

There is also a high significant indirect association among Thrombomodulin (TM)and GFR in the studied groups.

Also There is a high significant direct association among CRP and CIMT in studied group

6: Correlation between Thrombomodulin (TM)T cells and other parameters There is high significant direct association among Thrombomodulin (TM)and cholesterol level(r=0.539, P<0.001)

There is non-significant direct association among Thrombomodulin (TM) and triglycride level(r=0.129, P>0.05)

There is non-significant direct association among Thrombomodulin (TM) and HDL level(r=0.034, P>0.05)

There is non-significant indirect association among Thrombomodulin (TM)and LDL level(r=-0.112,P>0.05)

There is a significant indirect association among Thrombomodulin (TM)and CA level(r=-0.469,P<0.001)

There is a significant direct association among Thrombomodulin (TM) and PHOSPHORUS level(r=0.464,P<0.00

Table1 Lipid Profile ,CA,PHOSPHORUS, HB AND GFR Among studid groups

	1	2	3	F	P
Cholesterol X± SD	**146.6±14.66**	**138.13±22.41**	**116.83±20.48**	**18.62**	**0.00**
Triglycride X ±SD	**110.21±12.61**	**139.3±18.39**	**88.03±13.15**	**88.5**	**0.00**
HDL X±SD	**52.40±22.21**	**50.67±14.65**	**51.83±21**	**0.061**	**0.94**
LDL X ±SD	**98.11±27.88**	**102.85±31.39**	**111.97±26.75**	**1.80**	**0.17**
CA	**8.10±.93**	**8.23± .71**	**9.68 ±.69**	**37.67**	**0.00**
PHOSPHORUS	**5.46± 1.31**	**5.42± 1.26**	**3.23± .79**	**37.32**	**0.00**
HB	**9.83± 1.68**	**9.67± 1.41**	**12.22± 1.34**	**27.82**	**0.00**
GFR	**5.87± 1.43**	**36.30 ±11.82**	**99.93 ±18.31**	**434.51**	**0,00**

Table2: **Thrombomodulin, Carotid Intima Media Thickness (CIMT) among studied group**

variable	**Group 1**	**Group 2**	**Group3**	**F**	**P**
Thrombomodulin pg/dl	*****				
X±SD	**729.4±513**	**521.1±198**	**430.2±102**	**4.27**	**0.018**
Range	**128.1-2486.2**	**326-1029**	**320-609.1**		
CIM cm	*****		**+**		
X±SD	**1.05±.15**	**0.98±0.15**	**0.82±0.08**	**15.1**	**<0.001**
Range	**0.75-1.35**	**0.8-1.2**	**0.68-0.93**		

Table3: Least significant difference (LSD) for comparison between groups according to X±SD of Thrombomodulin

	Group 1 729.4±513	**Group 2 521.1±198**
Group 3 430.2±102	**P<0.05**	**P>0.05**
Group 2 521.1±198	**P<0.05**	

Table4: Least significant difference (LSD) for comparison between groups according to X±SD of CIMT (cm)

	Group 1 1.05±.15	Group 2 0.98±0.15
Group 3 0.82±0.08	P<0.001	P<0.001
Group 2 0.98±0.15	P<0.001	

Table 5: Correlation among CIMT, Thrombomodulin , CRP,and GFR All Cases.

n = 90	CIMT	Thrombomodulin	CRP	GFR
CIMT				
Thrombomodulin	0.618			
CRP	0.600	0.625		
GFR	0.741	0.837	0.665	

Table 6:The correlation between Thrombomodulin, and other variables.

Thrombomodulin	R
cholesterol	0.539
Triglycerides	0.129
LDL	-0.112
HDL	0.034
CA	-0.469
PHO	0.464

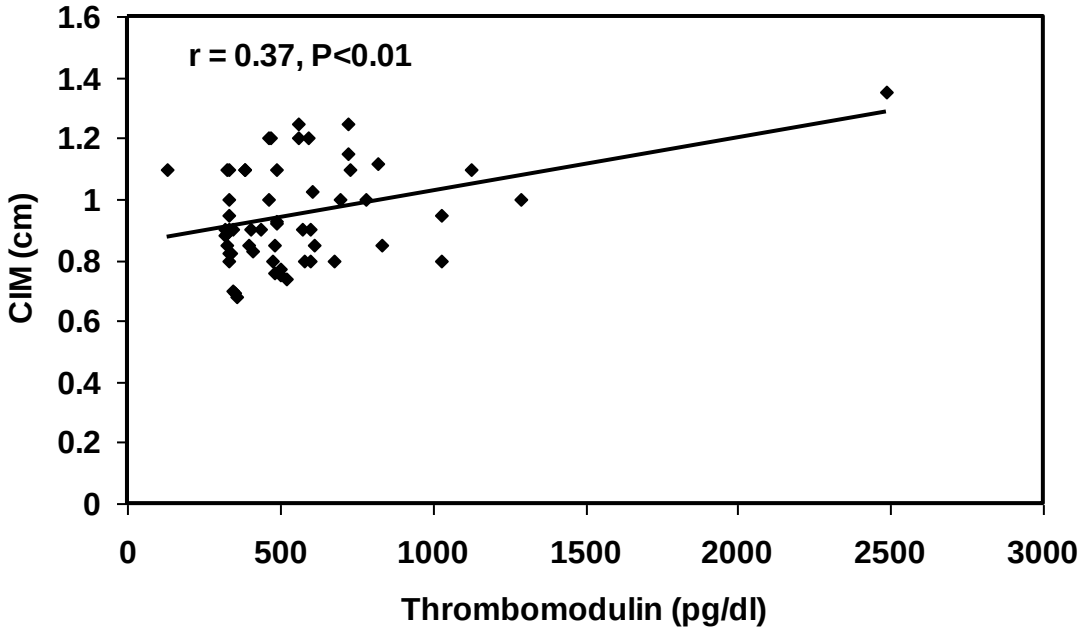

Fig. (1): Correlation between Thrombomodulin and Carotid intima media thickness (CIM).

DISCUSSION:

Chronic kidney disease (CKD) becomes a worldwide health burden and an independent risk factor for cardiovascular disease with a high economic cost to healthy systems (CVD). The higher risk of cardiovascular morbidity, early death, and/or reduced quality of life is correlated with all stages of CKD **(2**). In patients with CKD, cardiovascular disease (CVD) is twice as common as in the general

population **(2**). One of the main causes of morbidity and mortality among CKD patients is atherosclerotic cardiovascular disease **(3).**

The current study was performed to clarify the relation between Thrombomodulin and atheroscelerosis and systemic inflammation in patients with chronic kidney disease (CKD) in Theodor Bilharz Research Institute . It included 19 patients on regular hemodialysis group (1), 19 patients with renal impairment (on conservative treatment) group (2), in addition to 19 normal individuals group (3), matched for age and sex, as control. All patients and healthy control subjects were subjected to full medical history , clinical examination ,estimation of serum urea, serum creatinine,GFR ,serum calcium , phosphorous ,serum albumin , AST,ALT cholesterol, triglycerides , HDL ,LDL ,blood picture, CRP and serum Thrombomodulin levels , in addition to ECG and Dopller on the carotid arteries .

A significant raise in CRP was observed in the predialysis versus control group and a significant raise in CRP was observed in the hemodialysis versus predialysis versus control group (P< 0.001). In line with our findings (8), which have reported evidence that CKD patients are in a state of chronic inflammation with activation of C-reactive protein and proinflammatory cytokines, related to increased oxidative stress and endothelial dysfunction. The inflammatory nature of atherosclerotic plaques is confirmed by substantially elevated serum CRP in groups of patients with elevated CIMT, but atherogenesis remains a complex chronic inflammatory process

There is a significant increase in carotid intima thickness in the hemodialysis group versus the predialysis group and the control group (p<.001) with respect to carotid intima thickness.

In line with our research (9), (10) and (11), this observed that the average IMT was substantially higher than the control group in HD patients. These results are often due to the accelerated atherosclerosis process that is typically seen in patients with ESRD.

Also **(12) (13**) and observed that the frequency of atherosclerotic plaques and the IMT value of the carotid artery increased substantially compared to healthy volunteers in CKD patients.

We found in our study that there is a significant increase in thrombomodulin level in haemodialysis group compared to control group (p<0.05) also in chronic kidney disease on conservative treatment group relative to control group(p<0.05).

In agreement with our study **(14)** observed that the levels of soluble TM were substantially higher in all CKD patients than those of healthy controls (p<0.001). The concentration of soluble TM rises significantly in patients with CKD and is correlated with disease severity. Significant associations among serum soluble TM and disease severity (r= 0.714, p<0.001) were observed in the association analysis. It was found that serum soluble TM was associated with eGFR (r= -0.766, p<0.001) and creatinine serum (r= 0.778, p<0.001). Soluble TM can play a critical role in CKD development as a biomarker of damage to endothelial cells, anticoagulation, and anti-inflammation.

It was also found **(15)** that even small decreases in eGFR are correlated with substantially higher sTM and sTF levels. These findings indicate that homeostasis dysregulation could play a significant pathological role in CKD.

We find in our study a significant direct relation between TM And carotid intima media thickness (CIM).r=(0.618) .

In agreement with our study (**12)** and **(16)** who found in their studies that thrombomodulin is positively correlated with IMT in CKD patients

CONCLUSION:

From our work, we showed that thrombomodulin in patients with CKD is not only substantially expanded, but that this expansion is correlated with preclinical atherosclerosis and that HD is not an independent risk factor for such expansion. In CKD (predialysis and regular HD) patients, there is an independent correlation among thrombomodulin, age, hsCRP and IMT, and these results suggest that thrombomodulin may serve as a new risk factor for CVD in patients with CKD.

ABBREVIATIONS:

CIMT (carotid intima-media thickness), CKD (chronic kidney disease), CVD (Cardiovascular Disease), HD (hemodialysis), hsCRP (highly sensitive C-reactive protein), eGFR (estimated glomerular filtration rate)

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CONFLICT OF INTEREST
No conflict of interest.

APPENDIX A. SUPPLEMENTARY DATA
Supplementary data to this article can be found online at https:// doi.org/10.1016/j.humimm.2019.03.008

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