Research Article

Trefoil 2 level in chronic kidney disease

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Abstract

Objective: To detect the role of trefoil factor (TF2)(in both serum and urine) in different stage of CKD. **Study design**: A total of 40 patients with CKD and 20 apparently healthy volunteers (as control group) were eligible for the study. Patients group was subdivided into 3 subgroups {Mild (13), Moderate (13) and late group (14)}.Complete Blood Count(CBC),kidney function tests,Urine Protein /creatinine ratio (P/C Ratio),Estimated glomerular filtration rate (eGFR), Serum and urine TF2 were done to all subjects. **Results:** Patients groups exhibited higher seruumTF2 concentrations (371.5 pg/ml) than control group (2 pg/ml). A significant increase in serum.TF2 level in group III when compared to group I, group II and group IV (p value= <0.001). Also, serum TF2 was significantly higher in group II when compared to group I and group IV (P value= <0.001). In addition there was significant increase in group I when compared to group IV (p value= <0.001).As regard urine TF it was higher in patients groups (460 pg/ml) than control group (2 pg/ml).A significant decrease in urine.TF2 level in group III when compared to group I, group II and group IV (p value= <0.001). Also, urine TF2 was significantly decrease in group II when compared to group I and group IV (p value= <0.001)**. But** urine TF2 was significant increase in group I when compared to group IV (p value= <0.001). **Conclusion:** The data suggest the role of TF2 concentration (in urine and serum) to detect the stage of CKD. Serum TF2 concentrations increased progressively in later stages than early and moderated stage. Urine TF2 levels were significantly higher in early and mid CKD stages as compared to later stages.

Keywords: Chronic kidney disease (CKD), Estimated glomerular filtration rate (eGFR), Serum TFF2,Urine TFF2

Introduction

Chronic kidney disease (CKD) is the 12th cause of death, and 1.1 million deaths worldwide every year. The role of CKD as a cause of death is where renal replacement therapy (RRT) is not available, it was found that RRT consumes about 3–5% of the global healthcare where dialysis is available without restrictions[(1)]**.** CKD can progress to kidney failure that known as the end stage renal disease (ESRD)[(2).]

ESRD is one of the main health problems in Egypt. Hemodialysis is the main mode for treatment of CKD stage 5. According to 9th Annual Report of The Egyptian Renal Registry provided by Egyptian Society of Nephrology and Transplantation (ESNT), prevalence of ESRD in Egypt raised to 483 patients per million. Mean age is about 49.8 ± 19 years[(3)]**.**

The progression of CKD can proceeds silently, so patients diagnosed at a state where most therapeutic options to prevent adverse outcomes are insufficient. The early detection of patients at risk is highly desirable to initiate early treatment to prevent disease progression[(4)]**.**

Trefoil factor family peptide (TFF) was discovered as a new marker in diagnosis of (CKD), it consist of a three-looped structure of cysteine residues, known as the trefoil domain, and the family comprises three members in mammals: TFF1, TFF2, and TFF3. TFF1 and TFF3 contain one trefoil domain, while TFF2 contains two[(5)]**.**

TFF2 facilitate epithelial regeneration processes by the induction of cell migration, angiogenesis, and raising cell resistance to proapoptotic stimuli. TFF peptides are secreted by most epithelial tissues that contain mucus secreting cells including renal tubular epithelial cells in the kidney[(6)]**.**

TFF2 in CKD can be detected in urine and serum which indicate changes in renal functions and can predict different stages of CKD[(7)]**.**

The aim of this work is to evaluate the diagnostic utility of serum and urine TF2 in chronic kidney disease.

Subjects and Methods

Subjects

The present study was carried out at the Clinical Pathology Department, Faculty of Medicine, Minia University. It was conducted on 40 patients diagnosed with chronic kidney disease (CKD) were selected from in-patient and out-patient clinics of Minia nephrology and urology university hospital, through the period January 2019 to December 2019. They were 25 males and 15 females, their ages ranged from 28 to 71 years and subdivided into 3 subgroups {Group I (Mild stage of CKD 13 patients), Group II (Moderate stage of CKD 13 patients) and Group III (Late stage of CKD14 patients)}. The study also included 20 apparently healthy subjects with matched age and sex as the control group (Group IV).

Laboratory methods

Blood samples: About 7ml of venous blood were withdrawn from each subject by sterile venipuncture. This sample was divided as follows: 1ml in EDTA containing tube for determination of CBC (using automated cell counter, **Celltac alpha, Japan),** 3 ml on plain tube. Blood was left to clot in the incubator then centrifuged. The expressed serum was used for determination of renal functions. (using fully automated clinical chemistry auto-analyzer system, **(Selectra-Pro xl, Netherlands)**. And 3 ml of blood on plain tube was put at room temperature for 10-20 minute, centrifuged at the speed of 2000-3000rpm for 20-min and expressed serum was stored at -20℃ for further estimation of T.F2 by EIA method.

Urine samples:

1) For assessment Protein/creatinine ratio (P/C Ratio) in urine. (using fully automated clinical chemistry auto-analyzer system,**(Selectra-Pro xl, Netherlands)**.

2) For measurement of T.F2 in urine by EIA method. Urine was collected in a sterile container centrifuged at the speed 2000-3000 3) rpm for 20 minute, supernatant was removed.

Statistical analysis

All analyses were performed with version 19 of Statistical Package of Social Science (SPSS). Qualitative data were expressed as proportions, while quantitative data were expressed as mean + standard deviation (SD). Qualitative data were analyzed by Chi square ($\chi 2$) test. Comparisons between groups for normally distributed quantitative data were performed by Student's t-test. Correlations between variables were obtained by Pearson's test. For all analyses, statistical significance was defined as p values less than 0.05.

Results

This study was carried out on 60 subjects divided into:

Group I (Mild stage CKD group): Thirteen patients included 8 males (61.5%) and 5 females (38.5%). Their age ranged from 32 to 70 years (mean±SD 51.5±12.2). Body mass index (BMI) ranged from 15 to 37 (mean±SD 25.1±6.4).

Group II (Moderate stage CKD group): Thirteen patients included 9 males (69.2%) and 4 females (30.8%). Their age ranged from 28 to 70 years (mean ±SD 51.2±13.9). BMI ranged from 20 to 38 (mean±SD 28.5±4.8).

Group III (Severe stage CKD group): Fourteen patients included 8 males (57.1%) and 6 females (42.9%). Their age ranged from 35 to 71 years (mean ±SD 53.1±12.1). BMI ranged from 19 to 32 (mean±SD 26.1±4.5).

Group IV (Control group): Twenty apparently healthy control subjects included 11 males (55%) and 9 females (45%). Their age ranged from 30 to 77 years (mean±SD 50.9±13.6). BMI ranged from 18 to 22 (mean±SD 20.1± 1.4). A total of 40 patients with CKD and 20 controls participated in this study. Demographic and biochemical characteristics of all patients and controls are summarized in **(Table-1).**

Table 1: Demographic, Hb, Urea, Creatinine, p/Cr ratio and eGFR of the study groups.

	Early stage CKD (I)	Mild stage CKD (II)	Late stage CKD (III)	Control (IV)	P value						
	N=13	N=13	N=14	N=20	Among 4 groups	I vs II	I vs III	I vs IV	II vs III	II vs IV	III vs IV
Age in year *Range* *Mean ± SD*	(32-70) 51.5±12.2	(28-70) 51.2±13.9	(35-71) 53.1±12.1	(30-77) 50.9±13.6	*0.970*	*1*	*0.990*	*0.999*	*0.981*	*1*	*0.966*
Sex *Male* *Female*	8(61.5%) 5(38.5%)	9(69.2%) 4(30.8%)	8(57.1%) 6(42.9%)	11(55%) 9(45%)	*0.866*	*0.860*	*0.816*	*0.710*	*0.516*	*0.414*	*0.901*
Hb (g/dl) *Range* *Mean ± SD*	(10-14) 11.8±1.2	(8.9-11.7) 10.1±0.	(6.5-10.5) 8.4±1.1	(11-14) 12±0.8	*<0.001**	*<0.001**	*<0.001**	*0.954*	*<0.001**	*<0.001**	*<0.001**
Creatinine (mg/dl) *Range* *Mean ± SD*	(0.9-1.4) 1.1±0.1	(1.1-2.2) 1.6±0.	(2.9-5.5) 4.1±0.9	(0.5-0.9) 0.7±0.1	*<0.001**	*0.035*	*<0.001**	*0.045*	*<0.001**	*<0.001**	*<0.001**
Urea(mg/dl) *Range* *Mean ± SD*	(22-37) 28.2±5.4	(33-59) 43.8±8.2	(31-89) 62.4±21.1	(18-22) 20±1.3	*<0.001**	*0.004*	*<0.001**	*0.176*	*<0.001**	*<0.001**	*<0.001**
P/Cr ratio(mg/g *Median* *IQR*	2 (1-3)	4 (2-5)	6.5 (4.8-7.3)	14 (9.5-17)	*<0.001**	*0.030**	*<0.001**	*<0.001**	*0.004**	*0.001**	*0.002**
eGFR (ml/min/1.73m2) *Range* *Mean ± SD*	(61.8-89.6) 75.5±9.	(31.4-58.6 45.1±8.3	(8.1-24.1) 14.9±5.2	(93-131) 109.9±12.1	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

The comparison between the studied groups regarding to routine laboratory data demonstrated in **(Table 2).**

	Mild stage CKD (I)	**Moderate stage CKD (II)**	**severe stage CKD (III)**	**Control (IV)**	**P value**						
	N=13	**N=13**	**N=14**	**N=20**	**Among 4 groups**	**I vs II**	**I vs III**	**I vs IV**	**II vs III**	**II vs IV**	**III vs IV**
Hb (g/dl) *Range* *Mean ± SD*	(10-14) 11.8±1.2	(8.9-11.7) 10.1±0.9	(6.5-10.5) 8.4±1.1	(12-16.5) 13.2±1.1	<0.001*	<0.001*	<0.001*	0.954	<0.001*	<0.001*	<0.001*
TLC ($x10^3$/cmm) *Range* *Mean ± SD*	(3.9-12.8) 7.4±3.2	(5.5-12) 8.3±2.1	(4-11) 7.2±2.1	(5-10) 7.3±1.7	0.555	0.747	0.996	0.998	0.595	0.574	1
Platelets ($x10^6$/cmm) *Range* *Mean ± SD*	(145-285) 228.2±46.1	(145-270) 208.3±46.6	(145-264) 212.1±49.3	(150-280) 208.1±35.7	0.581	0.654	0.776	0.571	0.996	1	0.993
Creatinine (mg/dl) *Range* *Mean ± SD*	(0.9-1.4) 1.1±0.1	(1.1-2.2) 1.6±0.3	(2.9-5.5) 4.1±0.9	(0.5-0.9) 0.7±0.1	<0.001*	0.035	<0.001*	0.045	<0.001*	<0.001*	<0.001*
BUN(mg/dl) *Range* *Mean ± SD*	(22-37) 28.2±5.4	(33-59) 43.8±8.2	(31-89) 62.4±21.1	(18-22) 20±1.3	<0.001*	0.004	<0.001*	0.176	<0.001*	<0.001*	<0.001*
Na(mmol/l) *Range* *Mean ± SD*	(134-147) 140.7±4.2	(135-149) 140.8±4.2	(133-152) 140.6±4.7	(137-144) 140.1±1.8	0.927	1	1	0.962	0.999	0.931	0.968
K(mmol/l) *Range* *Mean ± SD*	(3.1-5) 3.6±0.5	(2.6-4.5) 3.6±0.5	(2.9-5.7) 4.3±1	(3.7-5) 3.8±0.4	0.009*	0.985	0.011	0.574	0.028	0.802	0.121
I.Ca(mmol/l) *Range* *Mean ± SD*	(0.9-1.2) 1.05±0.09	(0.63-1) 0.89±0.12	(0.6-0.8) 0.71±0.06	(1-1.2) 1.1±0.1	<0.001*	<0.001*	<0.001*	0.651	<0.001*	<0.001*	<0.001*

***: Significant level at P value < 0.05**

Additionally, The mean ±SD of serum trefoil factor 2 (S. TFF2) was 15 pg/ml in group I,70 pg/ml in group II, 371.5 pg/ml in group III and 2 pg/ml in group IV. There was statistically significant increase in serum. TFF2 level in group III when compared to group I, group II and group IV (p.value= <0.001). Also, there was statistically significant increase in group II when compared to group I and group IV (P.value= <0.001). In addition there was statistically significant increase in group I when compared to group IV (p.value= <0.001) **(Fig1).**

The mean ±SD of urine trefoil factor 2 (U. TFF2) was 460 pg/ml in group I, 245 pg/ml in group II, 94.5 pg/ml in group III and 2 pg/ml in group IV. There was statistically significant decrease in urine. TFF2 level in group III when compared to group I, group II and group IV (p.value= <0.001). Also, there was statistically significant decrease in group II when compared to group I and group IV (p.value= <0.001). But there was significant increase in group I when compared to group IV (p.value= <0.001) **(Fig 2)**.

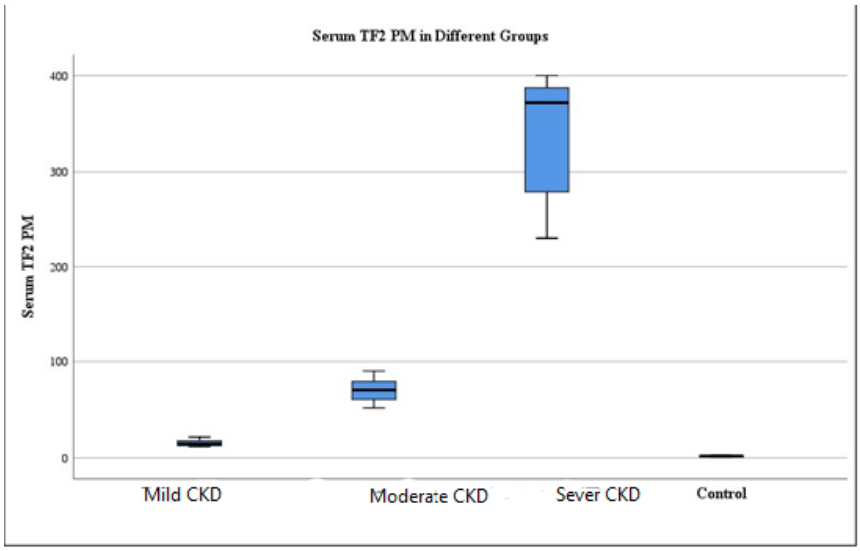


Fig (1): Bar charts showing Comparison between the studied groups as regard S. TFF2 PM

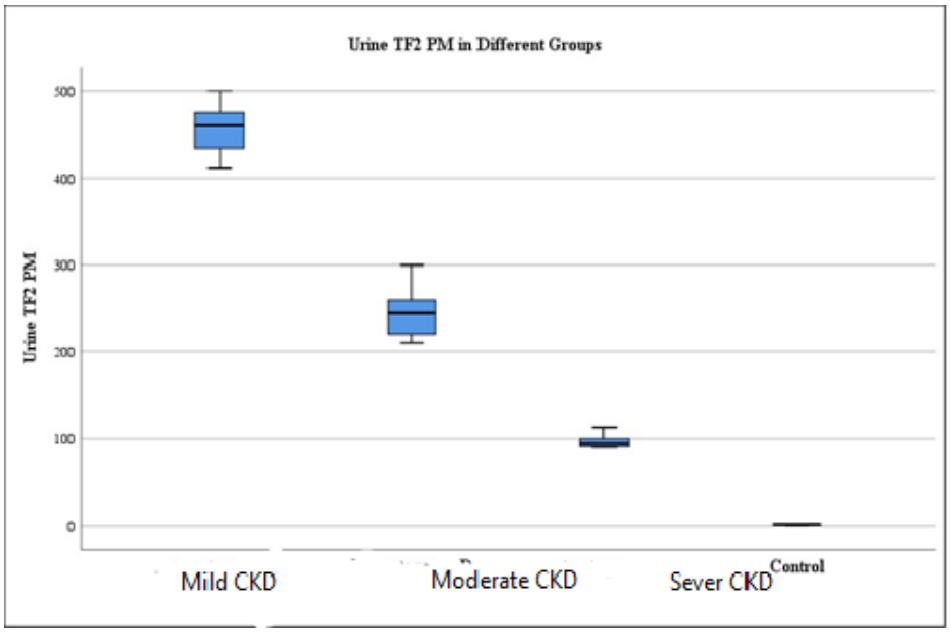
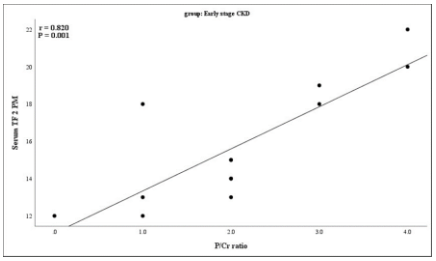
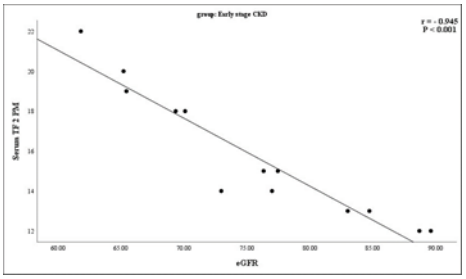


Fig (2): Bar charts showing Comparison between the studied groups as regard U. TFF2 PM

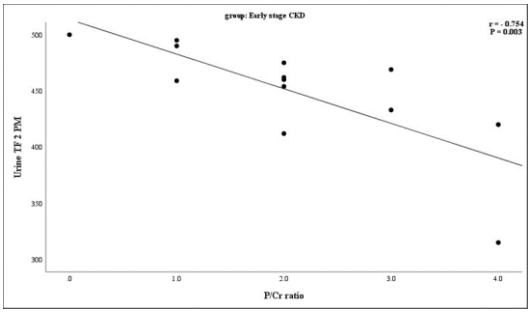
a- Correlation between different parameters and serum and urinary TFF2 in mild stage CKD:
In serum TFF2, there was significant positive strong correlation between serum TFF2 and P/C ratio (r=0.820, p=0.001) **(fig 3)**, and significant negative strong correlation between serum TFF2 and eGFR (r=-0.945, p=<0.001) **(fig 4).** While in urine there was significant positive moderate correlation between urine TFF2 and eGFR (r=0.748, p=0.003) . Moreover, there was significant negative strong correlation between urine TFF2 and P/C ratio (r=-0.754, p=0.003) **(fig5).**



(Fig 3); Positive correlation between serum TFF2 and P/C ratio in mild stage CKD

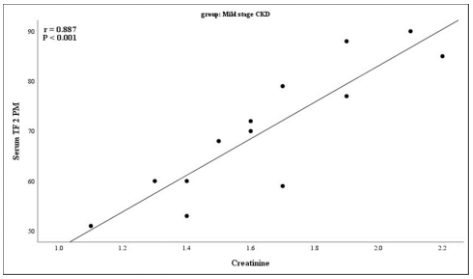


(Fig 4): Negative correlation between serum TFF2 and eGFR in mild stage CKD

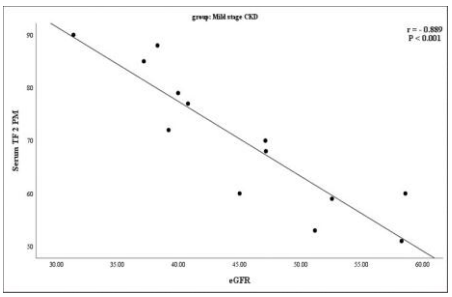


(Fig 5): Negative correlation between urine TFF2 and P/C ratio in mild stage CKD

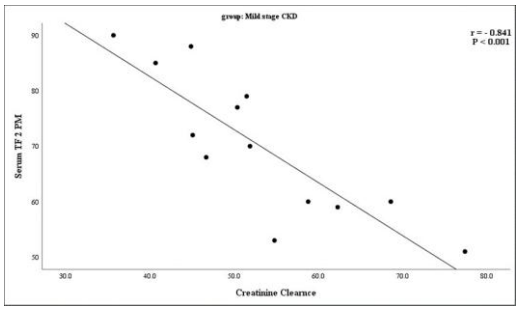
b- Correlation between different parameters and serum and urinary TFF2 in moderate stage CKD:
In serum TFF2, there was significant positive strong correlation between serum TFF2 and creatinine (r=0.887, p=<0.001) **(fig 6).**
There was significant positive moderate correlation between serum TFF2 and P/C ratio (r=0.669, p=0.012).
There was significant negative strong correlation between serum TFF2 with eGFR and creatinine clearance (r= -0.899, p=<0.001)(r=-0.841, p=<0.001) respectively **(fig 7), (fig 8).** While in urine there was significant positive moderate correlation between urine TFF2 with eGFR (r=0.850, p=<0.001), creatinine clearance (r = 0.903, p=<0.001) and Na (r=0.804, p=0.001) .
There was significant negative moderate correlation between urine TFF2 and creatinine (r=-0.691, p=0.009)



(Fig 6): Positive correlation between serum TFF2 and creatinine in moderate stage of CKD

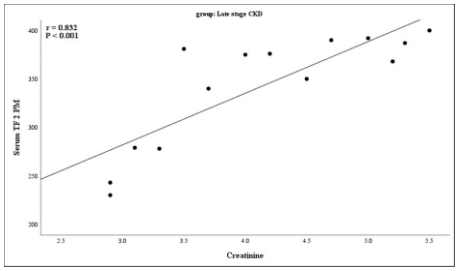


(Fig 7) :Negative correlation between serum TFF2 and eGFR in moderate stage of CKD

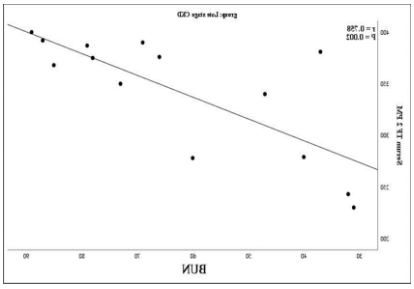


(Fig 8) :Negative correlation between serum TFF2 and creatinine clearance in moderate stage of CKD

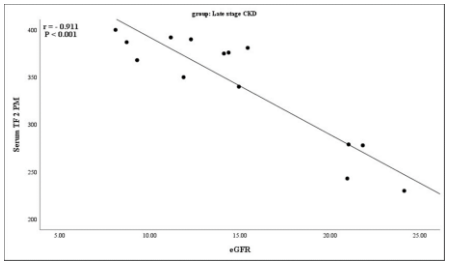
c- Correlation between different parameters and serum and urinary TFF2 in severe stage CKD:
In serum TFF2: there was significant positive strong correlation between serum TFF2 with both creatinine and BUN (r=0.832, p=<0.001), (r=0.758, p=0.002) respectively **(fig 9), (fig 10)**. Also, there was significant positive moderate correlation between serum TFF2 with both platelets and P/C ratio (r=0.533, p=0.049), (r=0.555, p=0.040) respectively. There was significant negative strong correlation between serum TFF2 with eGFR (r= -0.911, p=<0.001), creatinine clearance (r= -0.788, p=0.001) and Hb (r= -0.781, p=0.001) **(fig 11), (fig 12).** In urine TFF2: there was significant positive strong correlation between urine TFF2 with both eGFR and creatinine clearance (r=0.814, p=<0.001), (r=0.872, p=<0.001) respectively **(fig13), (fig 14)**.
There was significant positive moderate correlation between urine TFF2 and Hb (r= 0.663, p=0.010) and negative moderate correlation between urine TFF2 and creatinine (r= -0.662, p=0.010) .



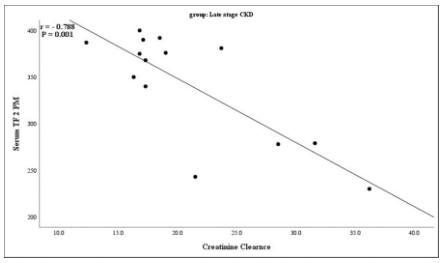
(Fig 9): Positive correlation between serum TFF2 and creatinine in severe stage CKD



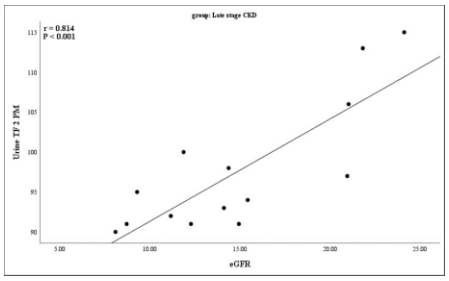
(Fig 10): Positive correlation between serum TFF2 and BUN in severe stage CKD



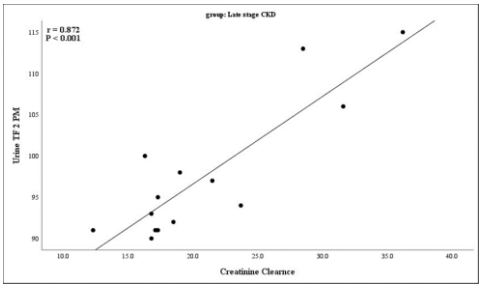
(Fig 11): Negative correlation between serum TFF2 and eGFR in severe stage CKD



(Fig 12): Negative correlation between serum TFF2 and creatinine clearance in severe stage CKD



(Fig13): Positive correlation between urine TFF2 and eGFR in severe stage CKD



(Fig 14): Positive correlation between urineTFF2 and creatinine clearance in severe stage CKD.

Discussion

(CKD) one of the leading cause of death, leading to 1.1 million deaths, worldwide, each year. The role of CKD as a cause of death when renal replacement therapy (RRT) is not available. RRT represents about 3–5% of the world healthcare budget where dialysis is available without restrictions[8]. CKD can progress to kidney failure and known as the end stage renal disease (ESRD)[9]. The patient is known as CKD when they present, for a period equal to or more than three months, glomerular filtration rate (GFR) lower than 60 ml/min/1.73 m^2, or GFR higher than 60 ml/min/1.73 m^2, but injury of the renal structure can be detected by albuminuria, hematuria/pyuria, changes in renal imaging, and histological changes in kidney biopsy. The main causes of CKD include diabetes, chronic glomerulonephritis, chronic pyelonephritis, chronic use of anti-inflammatory medication, autoimmune diseases, congenital malformations, polycystic kidney disease, and prolonged acute renal disease[10]. Trefoil factor family (TFF) peptides are key players in maintaining and repairing the epithelial mucosa.

They have particularly functions in the gastrointestinal tract as regulation of gut homeostasis. Also, they are found in the urinary tract, uterus, eye, respiratory tract, and salivary glands, and have similar functions in mucosal homeostasis and repair[11]. So, in this study, we aimed to validate the presence and the significant of TFF2 in the early detection and its level with the progression of CKD. Serum and urine samples obtained from 40 patients diagnosed CKD divided into three subgroups: Group I (Mild stage of CKD was 13 patients), Group II (Moderate stage of CKD was 13 patients). Group III (severe stage of CKD was 14 patients). They were 25 males and 15 females, their ages ranged from 28 to 71 years. The study also included 20 apparently healthy subjects with matched age and sex as the control group (Group IV). As regard hemoglobin concentration (Hb), HB level in the current study was lowest in patients groups (Moderate and severe group) when compared with control group (p value= <0.001). Also there was significant negative strong correlation between serum TF2 and Hb in severe group (p=0.001). These results were in agreement with Gafter et al., and Narayanan et al. . Regulation of iron metabolism is mediated, mainly by hepcidin and Hepcidin levels are increased in CKD. So iron deficiency anaemia is common with CKD[12,13].

Furthermore, Serum creatinine level in the present study was significant increase in severe group when compared to mild, moderate and control group (p value= <0.001). These results were in agreement with Chaitanyashree et al., and Lee et al.,[14,15]. Chaitanyashree et al., 2019 as majority of creatinine is filtered by the glomerulus and secreted by proximal tubular cells. creatinine is a good marker of a normal functioning kidney and its increase in the serum is indications of kidney impairment. Additionally, blood urea nitrogen level in the present study was significant increase in severe stage when compared to mild, moderate and control group (p value= <0.001). These results were in agreement with Cosola et al., and Di Micco et al.,[16, 17]. Di Micco et al., reported that normal ranges of BUN from 6.1 to 20.2 mg/dL, that corresponds to urea concentrations of 13–43 mg/dL BUN levels are markedly higher in CKD patients, Particularly those with endstage renal disease . But Rodrigues et al., (2020) were disagree with the present results. They explained that by the high accuracy of salivery urea level than blood urea level. Regarding electro-

lytes the present study revealed that there was significant decrease in ionized calcium level in severe group when compared to mild, moderate and control group (P value= <0.001). These results were in agreement with Okamoto et al.,[18] As in the early stages of CKD parathyroid hormone (PTH) secretion is stimulated to keep serum calcium and phosphate levels within normal range. However as CKD progresses from early to advanced stages, this mechanisms destroy leading to hypocalcemia[18]. Concerning the special markers of the present study serum and urine terfoil factor 2. The mean±SD of serum trefoil factor 2 (S. TFF2) was 15 pg/ml in the mild group, 70 pg/ml in moderate group, 371.5 pg/ml in severe group and 2 pg/ml in control group. There was statistically significant increase in serum.TFF2 level in severe group when compared to mild, moderate and control group (p value= <0.001). Also, there was statistically significant increase in moderate group when compared to mild and control group (P-value= <0.001).

In addition there was statistically significant increase in mild when compared to control group (p value= <0.001). The mean ±SD of urine trefoil factor 2 (U. TFF2) was 460 pg/ml in mild group, 245 pg/ml in moderate group, 94.5 pg/ml in severe group and 2 pg/ml in control group. There was statistically significant decrease in urine.TFF2 level in severe group when compared to mild, moderate and control group (p value= <0.001). Also, there was statistically significant decrease in moderate group when compared to mild and control group (p value=<0.001). But there was significant increase in mild group when compared to control group (p value= <0.001).

These results confirm the upregulation of serum and urine TF2 in the injured kidney and indicate epithelial destruction[11]. These results were in agreement with Lebherz E et al.,[19] who found that TFF2 serum concentrations were significantly higher in mid and later CKD stages as compared to healthy controls (p= <0.001). Furthermore, TFF2 serum levels in later CKD stages differed significantly from early stages (p=<0.001). Urine TFF2 levels were significantly higher in early and mid CKD stages as compared to later stages. The contrary rising of TFF2 in serum and urine could indicate changes in kidney function and offer potential to examine CKD course and treatment progression[19]. Another study Yamanari et al.,[20] who studied activities of TFF2 and found that TFF2 concentrations were significantly higher in mild or moderate CKD stages than in severe CKD stages unlike serum TFF2 and this elevation in the urine . These results were in agreement with the results of the present study.

Also, Galura et al., reported that trefoil factor 2 expression is increased on kidney disease, and explained that by TFF2 play a role in cellular restitution, TFF2 increases Aquaporins 3 (AQP3) expression on the migrating cells which mediate water influx to the cell which is essential in the formation of the lamellipodium[21]. In this study there was significant positive strong correlation between serum TFF2 with both serum creatinine and BUN. While there was signficant negative strong correlation between urine TFF2 with both eGFR and Cr Clearance. These results were in agreement with Lebherz et al.,[19] who explain this by higher TFF2 urine levels during early kidney diseases and that TFF2 normal serum levels is facilitated by increased fractional excretion of TFF2. However, as kidney function further decreases, the compensatory increase of TFF2 excretion is exhausted, which in turn leads to a successive increase of serum TFF2 levels. Another studies Stürmer et al., and Yamanari et al.,[20,22] were in agreement with the present results. They explain that by the genetic co-regulation of TFF1 and TFF2. In Yamanari et al., study TFF1 secretion is increased obviously by elevated urinary levels in early group than moderate and late group. Also, there was significant negative strong correlation between urine TFF1 with both eGFR and Cr Clearance[20]. This genetic co-regulation of TFF1 and TFF2 reflect the role of TFF2 in the present results.

Conclusion

Serum TFF2 concentrations increased progressively in severe stages than mild and moderated stage. Urine TFF2 levels were significantly higher in mild and moderate CKD stages as compared to severe stages. Moreover, TFF2 concentration pattern in urine and serum play a role in chronic kidney disease. A signifi-cant positive strong correlation between serum TFF2 with both serum creatinine and blood urea nitrogen. And significant negative strong correlation between urine TFF2 with both eGFR and Cr Clearance. Further studies with

larger sample size in different population may confirm these results.

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