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Transforming Growth Factor- Beta1 in Relation to Glomerular Filtration Rate in Healthy Elderly Egyptians

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ABSTRACT

Background: Owing to high burdens of chronic kidney disease (CKD) worldwide, research targeting early detection of impaired kidney function merits attention. Transforming growth factor-β1 levels (TGF- β1) regulates renal fibrosis and rises in different kidney diseases.

Objectives: The present study aimed at evaluating the serum level of TGF- β1 in relation to estimated glomerular filtration rate (eGFR) in elderly healthy individuals.

Methods: This cross-sectional study included 55 individuals, aged above 60 years. Serum level of TGF- β1, microalbuminuria and eGFR were estimated. Systolic and diastolic blood pressure were measured.

Results: There was a significant negative correlation between GFR and TGF-β1 values (r value= -0.731, P = 0.001). Twenty-four hours urinary proteins and TGF-β1 levels showed a positive significant correlation (r-value = 0.713, p<0.001). There was no statistically significant difference in TGF- β1 values between males and females (P = 0.832). TGF-B showed good sensitivity: 90.7%; poor specificity: 41.7% at a cut-off of 41.5 with AUC: 0.85; p value < 0.001 to be an indicator for reduced e GFR.

Conclusions: TGF- β1 levels could be a potential marker for early detection of renal diseases in elderly individuals.

Keywords: Chronic kidney disease, Transforming growth factor, fibrosis, elderly, glomerular filtration rate

INTRODUCTION

Chronic kidney disease (CKD) is frequent in the older people, presenting mainly with decline in estimated GFR rather than proteinuria [(1)]. One of the attributable factors to occurrence of CKD among the elderly people is the mounting prevalence of traditional risks like diabetes mellitus, [(2)] hypertension and arteriosclerosis disorder [(3)] . Moreover, the range of the estimated glomerular filtration rate (eGFR) for CKD has been broadened by new definitions [(4)].

Aging itself seems to be associated with a higher incidence of nephrosclerosis and recurrent tissue inflammation [(5)]. Better understanding of the morphological, physiological, and functional changes in aging kidneys draws up the attention to the geriatric nephrology care [(6)].

For high burden of CKD associated with rising morbidities, early detection, and management of potential factors of developing CKD are mandated. The end-tributary for CKD regardless the causes is increased intraglomerular hypertension produced by hormones and cytokines milieu as Angiotensin II resulting for arteriolar vasoconstriction, whereas transforming growth factor- β1 (TGF-β1) causes fibrosis [(7,8)]

TGF-β1 is a multifaceted protein family. Its production is adjusted by gene polymorphism of TGF-beta gene. TGF-β1 plays a role in controlling cell proliferation & differentiation, and particularly it modulates fibroblast action thus stimulating fibrogenesis [(9)]. Moreover, it is potentially associated with the fibrogenic response that disturbs the layers of glomerular filtration membrane barrier and causes functional loss with proteinuria [(10)]

TGF-β1 has significant growth inhibitory actions in a variety of cell types including renal cells in normal elderly population [(11)], and earlier research has related TGF-β1 to cellular senescence and ageing [(12)].

Accordingly, the present study aims at evaluating the serum level of Transforming growth factor-β1 in relation to eGFR as a early predictor for CKD in elderly apparently healthy individuals

SUBJECTS AND METHODS

Study setting and sampling:

This cross-sectional study was conducted on 55 Egyptian apparently healthy individuals aged over 60 years. A convenient sample of patients was recruited from outpatient clinic of nephrology at Cairo University. While patients with hypertension, diabetes mellitus, liver diseases or chronic kidney diseases were ruled out from the study.

Methodology

All patients underwent a thoroughgoing medical history, and clinical examination.

Laboratory investigations

All laboratory workup was performed within one hour after the collection of samples. Complete blood

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count (CBC) was estimated using cell counter (Cell Dyn, Abbott,USA). Serum, creatinine , blood urea nitrogen (BUN) and liver enzymes were estimated by using chemistry analyzer (Dimension , Siemens), Also HbA_1C, urine analysis, and 24 hr urinary protein were evaluated. Measurement of GFR using Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) [13]:

$$GFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if African American]}$$

Measurement of serum transforming growth factor-β level was done via enzyme-linked immunosorbent assay (ELISA) by using TGF beta-1 Human ELISA Kit [Catalog # BMS249-4. USA].

Ethical consideration

Written informed consent was taken from the participating individuals after explaining the objectives and the methods of the work. Confidentiality was considered on handling the data according to revised Helsinki deceleration of biomedical ethics.

The Cairo University Faculty of Medicine's Research Ethics Committee (REC) had reviewed and approved the study's protocol.

Statistical analysis

The analysis of data using statistical package for social science (SPSS) version 24 had been done. Qualitative data were described as frequencies and percentages while quantitative data were presented in the form of means and standard deviations.

Normality of data was assessed using Kolmogorov-Smirnov test. Pearson correlation tested the association between numerical dependent and independent variables. Independent sample T test examined the statistical difference between 2 groups. Linear regression analysis was done to test the effect of the independent variables on the occurrence of dependent variables.

RESULTS

Baseline characteristics

The study included 31/55 (56.4%) males. The mean age at assessment was 65.67 ± 3.75. It ranged from 61-76 years. The sociodemographic and laboratory data were tabulated in table (1).

Table (1): The demographic and laboratory data of the participants

Variable	Value	
Age (Years)	**Mean ± SD** **Range**	65.67 ± 3.75 61 – 76
Gender **Male** **Female**	 **N(%)** **N(%)**	 31 (56.4) 24 (43.6)
Creatinine	**Mean ± SD**	0.96 ± 0.22
eGFR	**Mean ± SD**	74.33 ± 6.02
BUN	**Mean ± SD**	17.44 ± 4.3
TGF-β1	**Mean ± SD**	68.84 ± 3.86
HbA1C	**Mean ± SD**	4.95 ± 0.39
Systolic Blood pressure	**Mean ± SD** **Range**	111.58 ± 9.63 (90 – 128)
Diastolic Blood pressure	**Mean ± SD** **Range**	67.35 ± 6.42 (55 – 80)
24- h urinary proteins	**Mean ± SD**	0.125 ± 0.012

eGFR: Estimated glomerular filtration rate,
BUN: Blood urea nitrogen, **TGF-B1:** Transforming growth factor, **HbA1C:** Glycated hemoglobin

Correlations of TGF-B1 and different variables:

The study revealed that there was no statistically significant difference between males and females concerning TGF-β1 values (p=0.832). Mean value in case of males was 68 ± 31.2 ng/dl while in females it was 69.9 ± 35.6 ng/dl.

There was a good positive correlation between creatinine levels and TGF-β1 values (correlation coefficient= 0.614). This correlation was statistically significant (p=<0.001) as demonstrated in figure (1); however, BUN didn't correlate with TGF-β1 values (correlation coefficient= 0.047, p=0.732). Twenty four-hour urinary proteins and TGF-β1 levels showed a positive significant correlation (r= 0.713, p<0.001). No correlation between TGF-β1 values and age of the participants was detected (r = 0.093, p=0.498). Notably, there was a strong negative correlation between GFR and TGF-β1 values (correlation coefficient= -0.731). This correlation was statistically significant (p=<0.001). as shown in figures (2& 3)

Regarding the blood pressure, there was a good positive correlation between SBP and TGF-β1 levels (r= 0.748). This correlation was statistically significant (p<0.001). Also, diastolic blood pressure was proportionately correlated with TGF-B1 (r=0.715, p<0.001) figures (4A, B)

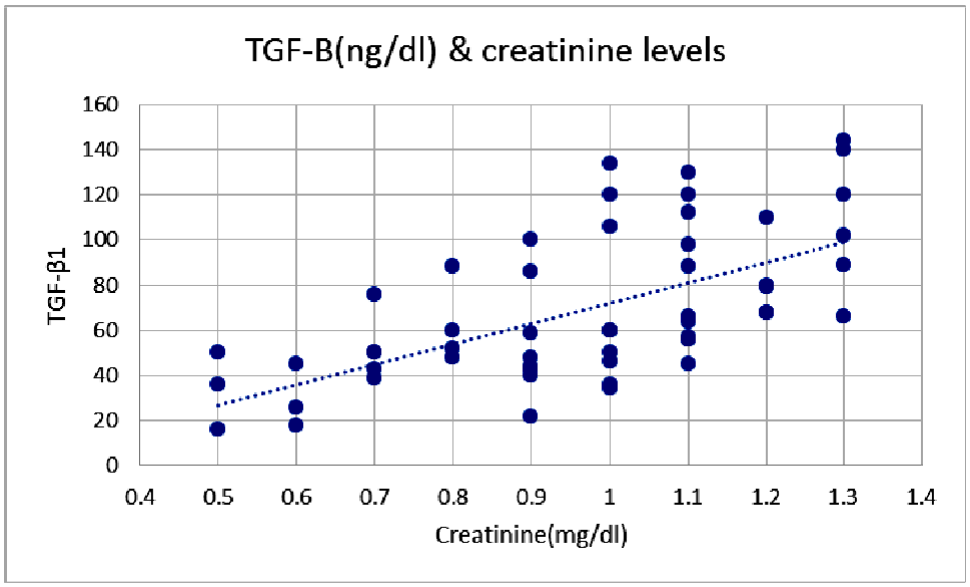


Figure (1): Correlation between TGF-β1 and creatinine among participants

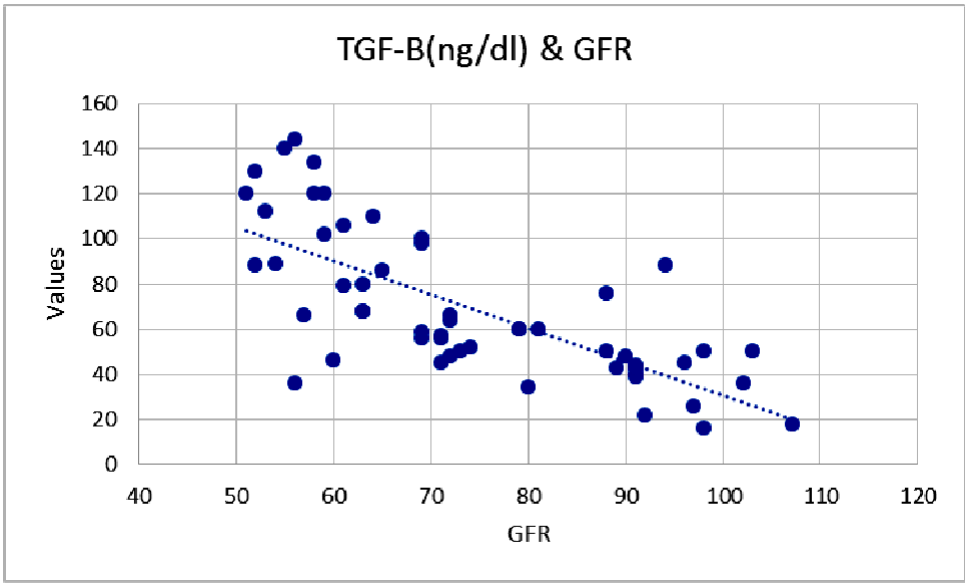


Figure (2): Correlation between TGF-β1 and GFR among participants

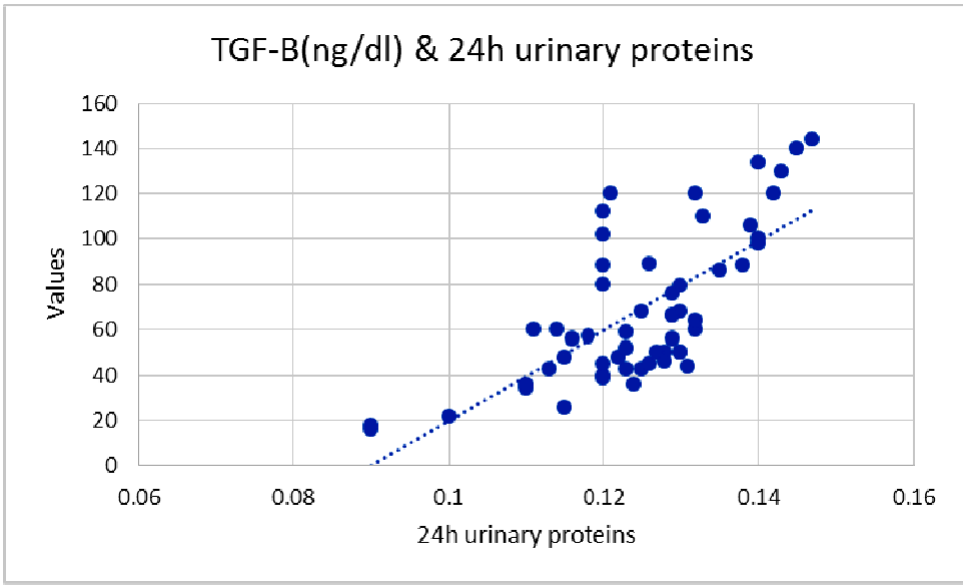
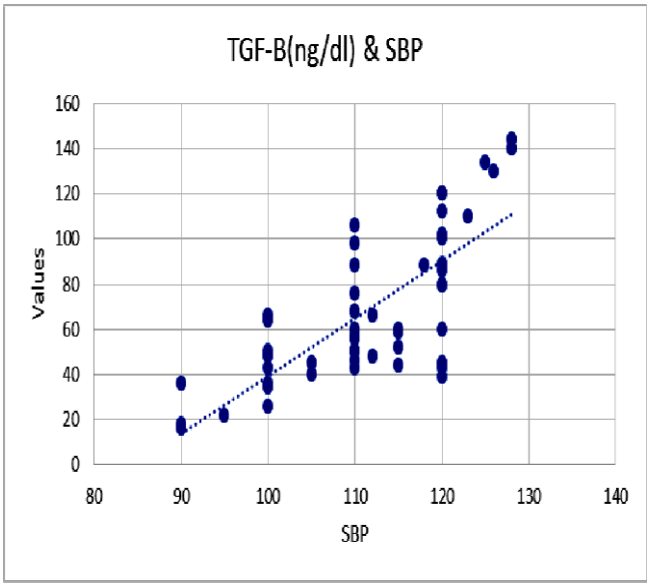


Figure (3): Correlation between TGF-β1 and 24-hr urinary protein among Participants

(A) (B)



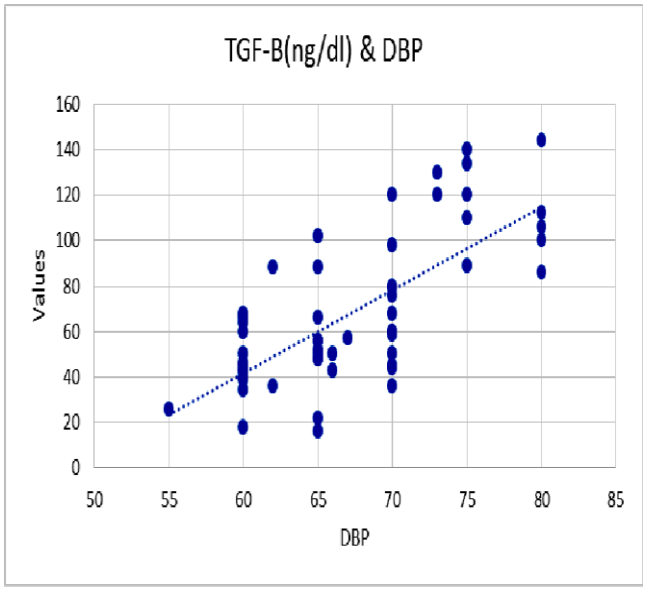


Figure (4) A: correlation between TGF-β1 and systolic blood pressure (SBP) among participants.
B: Correlation between TGF-β1 and diastolic blood pressure (DBP) among participants

Multivariate Linear Regression analysis

After adjusting for other factors, linear regression analysis was done and yielded that both eGFR, SBP and 24-h urinary proteins were independent variables for increased level of TGF- β1 as demonstrated in table (2)

Table (2): Linear regression analysis to detect independent variables

	Unstandardized Coefficients		T	Sig.	95% Confidence Interval for B	
	B	Std. error			Lower Bound	Upper Bound
(Constant)	-38.031	46.276	-.822	.415	-131.027	54.965
Creatinine	1.196	18.256	.066	.948	-35.492	37.884
eGFR	-.883	.266	-3.320	.002	-1.418	-.349
Systolic BP	32.549	8.359	3.894	.000	15.752	49.347
Diastolic BP	15.862	8.051	1.970	.054	-.317	32.041
24- h urinary proteins	949.361	221.174	4.292	.000	504.895	1393.827

Diagnostic role of serum TFG-B to predict reduced eGFR

ROC curve analysis was performed to evaluate the discriminate validity of TGF-B and 24-h urinary proteins as predictors of decreased eGFR. Area under curve (AUC) of urinary proteins was (0.7), at a criterion of 0.1205, with sensitivity 72.1% and specificity 58.3% with p value < 0.001; 95% CI: 0.537 – 0.866. while TGF-B showed good metrics [AUC: 0.85; p value < 0.001; 95% CI: 0.737– 0.973; Cutoff:41.5; sensitivity: 90.7%; specificity: 41.7%] as shown in figure (5)

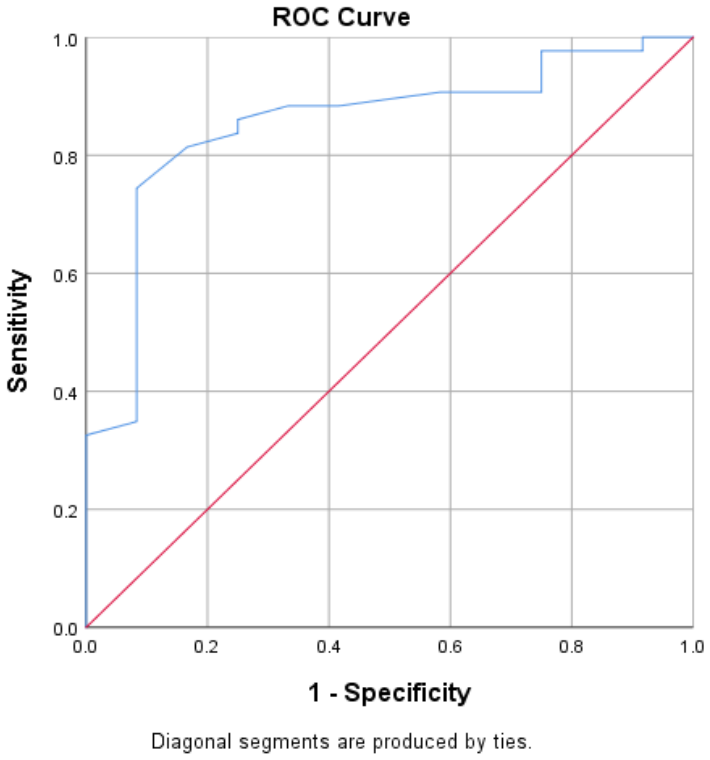


Figure (5): Roc curve analysis to determine the predictors of low eGFR.

DISCUSSION

Early optimal detection of kidney affection is never-ending target for its high financial and social burden as health problem particularly in old individuals [6]. The current study tried to investigate the impact of TGF-B1 as marker for renal impairment in apparently healthy elderly individuals with normal creatinine.

The study found that TGF-B1 is highly correlated to reduced eGFR and microproteinuria despite normal kidney function.

TGF-beta1 is a pleiotropic protein, that takes part in normal biophysiological processes and involves various chronic diseases [14]. Notably CKD prevail in elderly, due to reduced estimated GFR rather than albuminuria. Aging per se seems to associate with decline of renal cells function and fibrotic kidneys [15]. In addition, aging causes malfunctioning protein homeostasis. Strikingly, TGF-β signaling has been related to aging-associated disorders, including Alzheimer's disease, and cardiovascular disease by alteration in TGF-B signaling through dysregulated crosstalk at the cellular level, causing cytostasis and cell aging [16]. Inhibition of renin-angiotensin-aldosterone (RAAS) system might suppress TGF-β1 production [17], yet evidence suggests that the RAS may become more dysregulated by aging thus elevating Angio II with increasing TGF- β1 expression [18].

Given the fact that early endothelial and renal injury is associated with microproteinuria among apparently healthy individuals [19]. We investigated microproteinuria in relation to TGF-β1and we noticed a significant correlation between 24-h urinary proteins and TGF-β1 levels which is consistent with previous studies that have found elevated TGF-β1 levels in glomerulonephritis and diabetic nephropathy as TGF- β1 increases glomerular permeability to albumin while decreasing proximal tubular reabsorption, enhancing protein excretion [20, 21]

Notably, gender, age, hormonal state and lifestyle factors as obesity and smoking seem to influence TGF-1 levels in part [22]. Our study revealed no statistically significant difference in TGF-1 values between males and females and no correlation between age and TFG-B1. This may be explained by the age homogeneity of studied population. Parallel to our results Frydecka D et al., who reported that men and women of cognitive disorders had comparable serum TGF-β1 levels and raised TGF-B1 was irrespective to age [23]. A meta-analysis was carried out on 1,006 healthy European subjects and concluded no relation between plasma TGF-1 levels and age [24] . In contrary, Lin Y and Co observed that in males; TGF-β1 levels reached a trend toward significant difference, that serum TGF-β1 levels decreased with age [22]. These inconsistent findings could be attributed to the genetic milieu of the study participants, and variable sample preparation processes.

Based on the hypothesis that level of genetically determined TGF-1 protein may influence the control of blood pressure and the emergence of hypertension in both animal models and humans. TGF- β1 appears to induce vascular smooth muscle cell hypertrophy and glomerular sclerosis that can accentuate hypertension [25]. It has been proved that elevated serum TGF- β1 levels could predict the development of hypertension in normotensives [26]

Both systolic and diastolic pressure were correlated with TGF-B1 . In consistence with our results a study done among end stage kidney disease patients where serum TGF- β1 levels were proportionately associated with mean arterial pressure, systolic, and diastolic pressure [27]. So TGF-B is considered as known mediator for renal disorder, it modulates multiple risk factors including hypertension. However, an earlier study had found no connection between serum TGF- β1 values and mean blood pressure [28]

Here in , there was a good positive correlation between TGF-β1 values and creatinine levels, yet no relation with BUN. Along with our finding is Shukla et a**l** who studied that TGF-β1 had significant positive correlates with serum creatinine and urinary ACR [29]

In the term of assessing the validity, on applying ROC curve, we found that both creatinine, TGF-B and 24-h urinary proteins can be indicators for renal impairment in healthy population; TGF-B has poor specificity but significant sensitivity in prediction of chronic kidney disease. So, we can rule out the suspected cases of kidney disease.

In clinical settings, prevention of renal aging may be a suitable approach to combat progression of age-related renal diseases. Such, drugs that block RAS can reduce serum levels of TGF-β1 and protect the renal function

This study is one of the early introductory research projects that measure concentrations of serum TGF- β1 in relation to glomerular filtration rate in healthy elderly individuals.

The shortcomings of the current study are the following: sample size was small. Other drivers leading to glomerular injury that could erroneously increase serum TGF-β1 have not been studied. Lack of renal biopsies for histo-pathological examination to illustrate age-related findings was one of the limitations of the study. Our cross-sectional study cannot demonstrate a causal effect of serum TGF-1 on renal impairment. So longitudinal studies will be needed to determine if these findings indicate that levels of circulating TGF-1 contribute to kidney disease development or speculate other unstudied intersecting factors.

CONCLUSION

The study results indicate a significant negative correlation between GFR and TGF-β1 values in elderly healthy individuals. So that measuring serum TGF- β1 may be a proper diagnostic method for detection of early renal affection among old individual with normal kidney function tests.

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