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Research Article

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Impact of Fibrosis Related to TGF-B1and TNFR-1 Growth Factors in Renal Failure Patients

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Abstract

Renal fibrosis (RF) is the sign of last common pathway of Chronic renal Disease, which is independent of the underlying etiology. Renal fibrosis is described by advanced tissue scarring such as glomerulosclerosis, tubulointerstitial fibrosis, and loss renal parenchyma, including tubular atrophy, loss podocyte and capillary. The current study was performed to identify the effect of TGF-B1and TNFR-1 growth factors in Renal Failure (RF) patients focusing on it is role on the renal fibrosis . This study comprises 68 patients diagnosed with kidney failure clinically and serologically (38 males and 30 females) and their age varies from (17 to 80) years

,also 20 healthy individuals as a healthy control group. SerumTGF-B1and TNFR-1 concentrations were measured using a commercially available ELISA kit. The results showed TGF-B1and TNFR-1 have significantly increased (232.3±14.46 , 2267± 104.78µg/ml) respectively when compared with the control (68.9± 11.58 and 299± 11.48pg/ml) respectively ($p<0.05$) .On other hand, the result showed TGF-B1and TNFR-1 level not influenced by the age of patients ($p >0.05$). However, TGF-B1and TNFR-1growth factors elevated gradually with advance the severity of the RF disease and the highest value showed in end stage of renal failure disease.

Keywords: Renal fibrosis, TGF-B1, TNFR1, Growth factors , Renal failure

Introduction

Fibrosis is a complicated tissue condition characterized by an improper and excessive deposition of extracellular matrix (ECM) components , that can affect different organs, like lung, kidney , liver and skin. Fibrosis can be thought of as a semi-permanent state of scar tissue during which the healing process does not resolve. Long-term fibroblasts activation in the affected organs results in massive fibrous extracellular matrix (ECM) deposition and excessive fibroblast/myofibroblast proliferation, thus contrast with normal wound healing during which feedback mechanisms counterbalance the initial activation of fibroblast into myofibroblasts (1). Kidney fibrosis represents the common final pathway of nearly all chronic and progressive

nephropathies. During chronic damage, such as that seen in CKD, fibrotic matrix deposition continues unchecked , eventually distorting organ architecture, limiting blood flow, and interfering with organ function. Fibrosis impairs tissue healing and, as a result, leads to renal failure(2).

Transforming Growth Factor-β-1 (TGF-β1) is a pro-fibrogenic cytokine ,and it is a pleiotropic growth factor that has been linked to a variety of activities, many of which are diametrically opposed, including as proliferation, differentiation, fibrosis, and apoptosis. TGF-β1 is likely playing a critical role in the pathogenesis of chronic renal disease. TGF-1 primarily leads to glomerular filtration barrier modification, fibrosis, and sclerosis, which lower the filtration surface and eventually induce glomerular collapse. TGF-1 promotes the accumulation of monocytes and the stimulation of fibroblasts via boosting expression monocyte Chemo- attractantprotein-1 (MCP-1/CCL2) . Chemokines are important in infiltrating cells' motility and adherence to an inflammatory lesion in renal disorders (3) .Over the last two decades TGF-1 has been identified as a critical mediator in the genesis of CKD and as important in promoting the creation of ECM proteins, which results in structural and functional alterations in the kidney, culminating in unfavorable outcomes (4).TGF- 1 dysregulation is thought to be one of the key pathogenic pathways in the course of CKD since TGF- 1 may enhance ECM synthesis and change ECM component breakdown. According to vitro studies, TGF-1 induces ECM production, myofibroblast differentiation, or the transformation of mesangial cells, interstitial fibroblasts, and tubular epithelial cells into matrix-producing myofibroblasts. Tumor necrosis factor receptor1 (TNFR1) belongs to the TNF receptor superfamily, a group of type I single transmembrane glycoproteins. When TNFα binding to TNFR1 that activates signaling pathways and control inflammatory condation and immune responses (5) . In human ,TNFR1 gene located in chromosome 12p13.31. TNFR1 is expressed on practically all cell types. . TNFR1 is mostly found in glomerular and tubular endothelial cells of the kidney. TNF binding to TNFR1 is well established to trigger two disfferent signaling pathways that affect inflammation and fibrosis.(6).

Soluble receptors (sTNFRs) in plasma have been demonstrated to be implicated in inflammatory and stress response pathways that induce renal fibrosis by preventing TNF- from binding to its target receptor. (7).The Joslin Kidney Study results in humans indicated that increasing levels of circulating TNFRs are extremely good indicators of the development of chronic renal disease stage 3 or ESRD (5). Many pathophysiological events leading to renal fibrosis have been reported. This shows that TNF-α contributes, in part, to alterations in interstitial volume, myofibroblast differentiation, and NF-kB activation in the kidney during ureteral obstruction. These alterations appear to be mediated by the TNFR1 gene product, with TNFR1 effects predominating. Furthermore, it appears that the angiotensin II and TNF-α systems interact with one another, contributing to total renal fibrosis (8).In renal inflammation, increasing evidence has showed that the TNF-α/TNFR1 signaling pathway plays a essential role in the pathogenesis of kidney diseases. Additionally, the interaction between TNF and TNFR1 has been demonstrated to drive a variety of bioactivities, including cell proliferation, migration, and apoptosis; induction of vasculitis and angiogenesis; generation of inflammatory cytokines; and enhancement of fibrogenic responses (9).

Materials and Methods

Study subjects : The study conducted l in laboratories of Al Zahraa Teaching Hospital , the total of 68 patients with renal failure (30 females and 38males) have joined in this study .Also, the study included 20 (10males and 10 females) healthy volunteers as control group, who had no pathological state at the time of the study. All individuals in the control group were matched to patients in gender and age groups. Blood samples and their sera were taken from hospitalized renal failure patients. The patients divided into three age groups, (25<), (26 - 55)years , and elderly (56>) for statistical analysis to realize the effect of age on TGF-B1and TNFR-1 in RF patients. The study protocol in agreement with ethics of AlZahraa Hospital.

Stages of Renal Failure : The National Kidney Foundation’s as part of its Kidney Disease

Outcomes Quality Initiative (NKF KDOQI) clinical practice guidelines, proposed kidney disease stages classification. This system is calculated of the Estimated Glomerular Filtration Rate (eGFR) based on severity determined by renal function levels. The MDRD equation was used to calculate eGFR and that proposed by Levey et al. (1999) (10)as following :

MDRD eGFR = 186 × [serum creatinine (mg/dL)]−1.154 × (age)−0.203 × (0.742 if female)

According to GFR value and MDRD equation, the RF patients classified in to five stages. The prevalence of patients in this study according to the RF staging showed as Stage 1 (5 %), Stage 2 (7 %), Stage 3A (19 %), Stage 3B (22 %), Stage 4 (19 %), and Stage 5 (dialysis patients) (28 %).

Materials

Blood samples (3 ml) allowed to clot in the gel tube for limited minutes at the room temperatur, after that located in centrifuge at (1500 rpm for 5 min) to get serum. The serum was divided in eppendorf tubes, then stored frozen at -70 °C for ELISA assay procedure, must avoid thawed, refreezing.

Determination of TGF-B1andTNFR-1 concentrations

Serum TGF-B1and TNFR-1 concentrations were measured using a commercially available ELISA kit. ELISA assay were done according to (Elabscience) the instructions of manufacture company .

Statistical analysis

The statistics were interpreted into a computerized structure . Data is presented as means ± Standard deviation (SD). SPSS version 25 and Microsoft Excel 2010 Computer software were used for conducted Statistical analysis. Data were normally distributed, an unpaired t-test was used to compare the difference between patients with renal failure and control group . An estimate was considered statistically significant if its P value was less than 0.05.

Results

Data in table (1) indicated significant elevations of TGF-B1and TNFR-1 levels (232.3±14.46 , 2267± 104.78µg/ml) respectively when compared with the control (68.9± 11.58 and 299± 11.48pg/ml) respectively (p<0.05) . Table (2) showed the distribution of TGF-B1 and TNFR1 levels in RF blood serum according to age groups. The result showed no significant difference in the concentration of serum TGF-B1 for RF patients (176.98 for age -25< , 236.02 for age 26-55 ,and 246.79 pg/ml for age > 55 years among three age groups)with p- value(>0.05). Also, the findings found that non- significant variance in the level of serum TNFR1 among the age groups of RF patients) 2004.7 , 2277.7 , 1872.9 pg/ml) with p-value (>0.05) .Data in table 3 revealed significant increase in mean concentration of TGF-B1 with advance the stages of disease (94.71 for Stage1, 106.53 for Stage2 , 170.17 for Stage3A , 212.54 for Stage3B , 232.23 for Stage4 , 284.93 pg/ml for Stage5(dialysis patients)) with p-value (<0.05).

On other sides, the level of TNFR1 growth factors showed progressively increase in the five stages of RF disease (1021.2 for S1, 1671.4 for S2, 2139.8 for S3A , 2653.5 for S3B , 2694.6 for S4 , 2792.2 pg/ml for S5) with p-value (<0.05)

Table (1) :The concentration of TGF-B and TNFR1 in serum of renal failure patients.

Immunological parameters	Subjects understudy				P- value
	Patients	No.	Control group	No.	
TGF-B(pg/ml)					
Mean ± SD	232.3±110.13	68	78.9± 47.74	20	<0.05*
Males	247.5±26.29	38	93.20±40.31	10	<0.05*
Females	214.10±23.83	30	64.10±15.98	10	<0.05*
P-value	0.10**				
TNFR1(pg/ml)					
Mean± SD	2267± 784.1	68	299± 47.3	20	<0.05*
Male s	2213±162.77	38	198±13.46	10	<0.05*

Females	60.127±2328	30	.26 20±174	10	<0.05*
P-value	0.68**				

***=Significant at (p<0.05) ; **= non- Significant at (p>0.05)**

Table (2): Distribution of TGF-B1 and TNFR1 levels in renal failure patients according toage groups.

Age group (years)	**Patients No.**	**TGF-B1**(pg/ml) **Mean**	**TNFR(**pg/ml) **Mean**
25<	6	176.98	2004.7
26-55	28	236.02	2277.7
> 55	34	246.79	1872.9
L.S.D	-----	**85.63**	**647.1**
P-value	-----	**0.35****	**0.25****

LSD ;least significant difference ; *= Significant (p<0.05) ;**= no Significant(p>0.05) .

Table (3): The concentration of serum of TGF-B1 and TNFR1 according to stage of renalfailure disease.

Stage of RF Disease	**GFR ml/min/1.73 m^2**	**Patients No.**	**TGF-B1 mean pg/ml**	**TNFR1 mean pg/ml**
Stage 1	90>	5	94.71	**1021.2**
Stage2	60–89	6	106.53	**1671.4**
Stage3A	45–59	10	170.17	**2139.8**
Stage3B	30–44	12	212.54	**2653.5**
Stage4	15–29	15	232.23	**2694.6**
Stage5	<15	20	284.93	**2792.2**
p-value	----------		**<0.05***	**<0.05***

***= Significant difference at (p<0.05)**

Discussion

The result detected statistical evolution in the mean of concentration for both TGF-B and TNFR1 growth factors in RF patients ,this result coincide with previous study [5; 11]. Consistently, they also found a remarkable increase in TGF-β levels implied that its encoded gene TGFB1 might be unregulated in RF patients. Gohda, and his colleagues (12) found the concentrations of TNFR1 was strongly associated with risk for early renal decline .Renal fibrosis develops when the extracellular matrix progressively replaces the normal tissue architecture (13) .The cells involved exhibit enormous plasticity or phenotypic variability. Renal fibrosis, like wound healing, is thought to begin as a protective reaction to injury. If an injurious condition is persistent, which seem like to be the case in most advanced renal diorders, pathological fibrosis results in glomerulosclerosis, tubular atrophy and dilation, tubulointerstitial fibrosis and rarefaction of the glomerular, as well as peritubular capillaries (14). There is now substantial evidence that inflammation plays a significant role in the beginning and progression of renal fibrosis. In the event of acute kidney injury (AKI), temporary renal ischemia may induce comparable responses to those seen in RF, such as enhanced cytokine release, inflammatory cell infiltration, epithelial to mesenchymal transition (EMT), and fibroblast activation (15).

TGF-β is the major cause that drives fibrosis in majorty, if not all forms of chronic renal diseases

. It is a fibrogenic cytokine that is released in an inactive (latent) form that has to be processed before it may be physiologically active. TGF-B1 increases the buildup of extracellular matrix by raising the expression of extracellular matrix

genes and decreasing the synthesis of proteins that break down extracellular matrix (16).

Tumor necrosis factor receptor 1 (TNFR1) is single Tran's membrane glycoproteins and referred to as markers of the TNF pathway (17). In the human kidney, receptor expression is tightly controlled.Regarding kidneys, TNFR1 is mostly found in endothelial cells of the glomerulus and tubule. When TNF binds to this receptor, it activates common and separate signaling pathways, resulting in cellular outputs that may cause tissue damage on the one hand but also produce protective and beneficial responses on the other. The previous study showed thepatients with the highest levels of TNFR in their serum had more severe renal interstitial fibrosis than those with the lowest values (18).

Aaccording the result in table 3, the concentration of TGF-B1and TNFR1 growth factors progressively increase with of the stage of RF disease, and reaches their highest levels in stage 5 also called (end stage renal disease) when patients undergo to dialysis treatment. Other previous results confirm with our results [19;20]. ,whereas these studies reported that higher TGFB1 and TNFR1 levels in patients serum affected by last stage kidney failure during dialysis therapy for a long time and as well as predialysis stages . TGF-B1 is a key stimulus in the events that contribute to chronic progressive renal disease, having been implicated in the control of cell proliferation, hypertrophy, apoptosis, and fibrogenesis. August, and Suthanthiran, (16) hypothesized that heightened TGF-B1 concentration is a mechanism for the progressed of end stage kidney disease and the hyper level is not necessarily the cause of renal disease; it is, rather, an important cofactor that results in enhanced progression the stage of kidney failure. In hemodialysis patients, Gohda in 2017(5). discovered that a relatively high level of circulating TNFR was highly related with the probability of mortality. According to Carlsson and his colleagues [21]TNFR is closely linked to renal failure stage and progression risk. They also showed that participants with a GFR of <60 ml/min per 1.73 m2 had a higher level of TNFR than those with a GFR of ≥60 ml/min per 1.73 m2.

Conclusion : Our results concluded that high TGF-B1and TNFR1 serum concentration correlated with pathogenesis and progression of CKD disease in related to renal fibrosis .Binding of TNFα to TNFR1 activate distinct signaling pathways that may stimulate tissue damage or prompt defensive responses and predict the prognosis of hemodialysis patients.

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