Tumor Necrosis Factor Superfamily Impact on Cellular and Humoral Immunity in Hemodialysis Patients

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ABSTRACT

Introduction: The immune system is commonly affected in end-stage kidney disease (ESKD) patients on regular hemodialysis (HD). Few studies have investigated the cellular and humoral immunity in such patients. **Objective:** This study aimed to evaluate the impact of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), TNF-like weak inducer of apoptosis (TWEAK), and osteoprotegerin (OPG) on immunoglobulins (IgM and IgG), and CD 4+ T cells in HD patients. **Methods:** This observational study included 64 ESKD patients on HD, and 32 healthy controls. Following history taking, clinical examination, and routine laboratory investigations, serum levels of TRAIL, TWEAK, OPG, IgM, IgG, and CD4+ cells were measured using ELISA. **Results:** The mean serum levels of TRAIL (ng/ml), TWEAK (pg/ml) and OPG (ng/ml) (0.5±0.2), (390±152), and (0.74±0.07) in HD patients were significantly higher than in healthy control (0.09±0.0.36), (130.4±10.1), and (0.37±0.106), p< 0.001 (HS). HD patients showed significantly higher mean serum levels of IgM (mg/ml) (4.2 ± 1.4), and lower levels of IgG (mg/ml) (2.8 ± 1.3), and CD4+ T cells (ng/ml) (6.85 ± 2.05) than healthy control (1.34 ± 0.3), (12.4 ± 3.7), and (15.6 ± 6.3), p< 0.001 (HS). Using multiple regression analysis, dialysis vintage was a significant negative predictor for serum IgM and CD4+ levels, whereas transferrin saturation test (TSAT) was a significant negative predictor for IgG serum levels (p<0.05) (S). **Conclusion:** Serum levels of TRAIL, TWEAK, and OPG are increased in HD patients, but they have no significant association with IgM, IgG, or CD4+ T cells.

Keywords: TRAIL; TWEAK; OPG; Immunity; HD.

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INTRODUCTION

End-stage kidney disease (ESKD) patients on regular hemodialysis (HD) usually suffer from immune system dysfunction, and chronic inflammation [1]. High-flux HD significantly decreases the inflammatory response and immune dysfunction [2].

Cell-mediated immune response significantly improves one or two days after regular HD sessions, with an increase in CD4+ count, but no effect on CD8+ count [3]. Moreover, the antibody titers after vaccinations are lower in HD patients, compared to healthy subjects [4].

The tumor necrosis factor (TNF) superfamily ligands are a class of cytokines, that through their binding to receptors, they induce inflammation and cell differentiation [5]. TNF-superfamily members, such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), and TNF-like weak inducer of apoptosis (TWEAK) can induce cell proliferation, inflammation, and apoptosis,

and in HD patients, high levels of soluble TWEAK are correlated to inflammatory markers; C-reactive protein (CRP), and interleukin-6 (IL-6) and are associated with increased mortality [6].

TRAIL, a pro-apoptotic protein is released by activated leucocytes and has a role in inflammation, and circulating TRAIL is inversely correlated to cardiovascular disease, diabetes mellitus, and neurological diseases [7,8]. TRAIL administration helps protect against atherosclerosis, and diabetes mellitus through regulating both the innate and adaptive immune systems [9].

The osteoprotegerin (OPG), a member of the TNF receptor superfamily that inhibits the TRAIL-related apoptosis, is released by immune cells, vascular smooth muscle cells, osteoblasts, and endothelial cells [10]. OPG has a potential role in osteoporosis treatment, as it prevents bone remodeling, and modulates TRAIL effects, whereas its blood levels may normally fluctuate with age, gender, and blood group [11, 12].

TWEAK plays a role in inflammatory and autoimmune diseases. Soluble TWEAK is expressed by the immune system, natural killer cells, monocytes, and dendritic cells, and promotes the innate immunity to swift to adaptive immunity, and is represented also by endothelial and smooth muscle cells [13].

TRAIL plays an important role in immune cell interactions and has cytotoxic effects mediated by neutrophils, cytotoxic T lymphocytes, and natural killer cells [14]. Activation of peripheral T cells is regulated by OPG, and dysregulation of this system can lead to autoimmune disease [11].

CD4+ T lymphocytes carry out multiple immune functions, ranging from activation of the cells of the innate immune system, B-lymphocytes, cytotoxic T cells, as well as non-immune cells, and also play a critical role in the suppression of immune reaction. Flow cytometry is the gold standard technique in calculating the CD4+ count, however new techniques are currently available to measure the CD 4+ T lymphocytes, with an easier and less expensive technique using ELISA, especially in HIV-infected people [15,16].

Immunoglobulins (Ig) or antibodies are glycoproteins produced by plasma cells. B cells differentiate into plasma cells after exposure to immunogens, and play a role in humoral immune responses against bacteria, viruses, and cellular antigens [17]. ELISA is usually used for assaying the IgG and IgM antibodies [18].

This study aimed to investigate the impact of the TNF-Superfamily members (TRAIL, OPG, and TWEAK) on the cellular and humoral immunity in ESKD patients on regular HD.

PATIENTS AND METHODS

Ethical Considerations

This observational study protocol was approved by the Institutional Research Board (IRB), and Ethical Committee at the Faculty of Medicine, Zagazig University, Egypt, under the No. ZU-IRB#7029/4-7-2021, and a written consent was obtained from all participants, according to Helsinki's Declaration.

Sixty-four patients with ESRD on regular HD were included in the study, in addition to 32 age- and sex- matched healthy control subjects. This work was conducted at the Nephrology and Dialysis Unit, Internal Medicine, and Clinical Pathology Departments, Faculty of Medicine, Zagazig University, Egypt, during the period from June 2022 to November 2022.

Sample Size

Assuming the mean TWEAK was 893.5±480 vs 598.8±350 ng/ml among control vs HD group [19], at 80% power and 95% confidence interval (CI), the estimated sample was 64 HD patients vs 32 control. Open Epi.

Inclusion and Exclusion Criteria

ESKD patients on regular HD aged >18 years, 3 times/week, each session 3-4 hours, using bicarbonate-buffered dialysate, and duration of dialysis more than 6 months were included. Patients underwent hemodialysis via arteriovenous fistula, temporary dialysis catheters, or tunneled permanent dialysis catheters. Otherwise, patients aged <18 years, pregnant females, HD less than 6 months, or those with an active infection were excluded from the study.

Methods

All participants were subjected to history taking, clinical examination, and laboratory investigations including serum creatinine, blood urea nitrogen (BUN), complete blood picture (CBC), serum iron and ferritin, transferrin saturation, total iron binding capacity (TIBC), transferrin saturation test (TSAT), serum calcium, phosphorous, intact parathormone (iPTH), albumin, lipid profile, and C-reactive protein (CRP). For HD patients, dialysis details including dialysis vintage (duration), Kt/V, and medications were reviewed.

TNF superfamily (TRAIL, TWEAK & OPG) and CD4+ T lymphocytes Assays

Blood samples were drawn from the 64 HD patients from the vascular access, before the mid-week HD session, and before heparin administration. Serum levels of TRAIL, TWEAK, OPG, and CD4+ T lymphocytes were also measured in 32 age- and sex-matched healthy control subjects. ELISA Kits: Wuxi Donglin Sci & Tech Development Co., Ltd. Method: Sandwich. 1- Human TNF Related Apoptosis Inducing Ligand (TRAIL): Catalog No: DL-TRAIL-Hu. 2- Human TNF Ligand Superfamily, Member 12 (TNFSF12) (TWEAK): Catalog No: DL-TNFSF12-Hu. 3- Human Osteoprotegerin (OPG): Catalog No: DL-OPG-Hu. 4- Human Cluster of Differentiation 4 (CD4+): Catalog No: DL-CD4+-Hu. Assay Summary: Microtiter plate wells were pre-coated with specific antibodies. Standards or samples were added with a biotin-conjugated specific antibodies. Detailed steps were done as per manufacturer's instructions for each kit.

Statistical Analysis

It was conducted using SPSS (version 21, Chicago, IL, USA). Numerical values were expressed as mean ± standard deviation (SD) or median, and range as appropriate, and categorical variables were expressed as number (%). Comparison of groups was performed using the non-parametric Kruskal-Wallis (for continuous variables) and Chi-Square (for categorical variables) tests. Pearson's correlation coefficient was used for correlation analysis of continuous and normally distributed data. Otherwise the non-parametric Spearman's correlation coefficient was used. Multiple regression analysis was used as appropriate. Statistical significance was defined as a P-value < .05, and a P-value < .001 is highly significant.

RESULTS:

The mean age of the studied 64 HD patients was (50.2 ± 13.4) years, (30 males) (46.9%) & (34 females) (53.1%), dialysis vintage (duration) was (9.67 ± 4.23) years, and Kt/V (1.42 ± 0.17). Of them, 89% used calcium supplements, 57.8% alfacalcidol, 85.9% erythropoietin stimulating agents (ESA), and 12.5% iron supplements.

Table (1) shows the comparison of demographic data, laboratory investigations, and serum levels of TRAIL, TWEAK, OPG, IgG, IgM, and CD4+ T cells between HD patients and healthy control. There was a significantly higher serum creatinine, BUN, ferritin, TIBC, phosphate, intact

iPTH, cholesterol, and CRP and a significantly lower serum iron, TSAT, hemoglobin and platelets ($p<0.05$) (S).

The mean serum levels of TRAIL (ng/ml), TWEAK (pg/ml) and OPG (ng/ml) (0.5±0.2), (390±152), and (0.74±0.07) in HD patients were significantly higher than healthy control (0.09±0.0.36), (130.4±10.1), and (0.37±0.106), $p< 0.001$ (HS). HD patients showed significantly higher mean serum levels of IgM (mg/ml) (4.2 ± 1.4), and lower levels of IgG (mg/ml) (2.8 ± 1.3), and CD4+ T cells (ng/ml) (6.85 ± 2.05) than healthy control (1.34 ± 0.3), (12.4 ± 3.7), and (15.6 ± 6.3), $p< 0.001$ (HS) (Table 1).

Table (1): Comparison of demographic data, and laboratory investigations between HD patients, and healthy control.

Variables Mean±SD	**HD Patients (n=64)**	**Healthy Control (n=32)**	**Test of signif.**	**P-value**
Age (years)	50.2 ± 13.4	49.1±14.6	-0.36	0.7
Sex No. (%) - Male - Female	30 (46.9%) 34 (53.1%)	14 (43.7%) 18 (56.3%)	0.08	0.77
Body Mass Index (BMI) (Kg/m^2)	23.6 ± 3.1	24.1±2.4	0.8	0.4
S. Creatinine (mg/dL)	9.74 ± 2.38	0.83±0.42	-14.5	<0.001**
Bl. Urea Nitrogen (BUN) (mg/dL)	62 ± 17.2	11.1±5.4	-18.3	<0.001**
Bl. Hemoglobin (g/dL)	11.3 ± 2.42	13.2±2.6	3.5	0.007*
S. Ferritin (ng/ml) Median (min, max)	76.5 (3, 1034)	36.7 (25, 301)	U= -184	<0.001**
S. Iron (ug/dL) Median (min, max)	62.5 (10, 263)	82.3 (71.1, 180)	U= 76	0.005*
Total iron binding capacity (TIBC) (ug/dL)	350 ± 159	290±37.9	-2.1	0.038*
Transferrin saturation test (TSAT) (%) Median (min, max)	24.2 (1.85, 235)	30.1 (26.2, 36)	U= 21	0.09
White blood cells (WBCs) ($*10^3/mm^3$)	7.6 ± 3.4	8.6±2.9	1.4	0.16
Platelets ($*10^3/mm^3$)	229 ± 91.7	298±87.6	3.5	0.007*
S. Phosphate (mg/dL)	4.8 ± 1.6	3.2±0.9	-5.2	<0.001**
S. Calcium (mg/dL)	8.8 ± 0.9	8.9±1.8	0.36	0.72
S. Intact PTH (pg/ml) Median (min, max)	328 (6.3, 1691)	32.5 (11.2, 53)	U= 264	<0.001**
S. Albumin (g/dL)	3.9 ± 0.48	4.1±0.9	1.4	0.16
S. Cholesterol (mg/dL)	160 ± 32.5	144±19.1	-2.6	0.012*
S. Triglycerides (mg/dL)	129 ± 28.4	126±14.7	-0.56	0.57
S. C-Reactive Protein (mg/dL) Median (min, max)	10 (1.1, 166)	0.4 (0.3, 0.9)	U= 134	<0.001**
S. TRAIL (ng/ml)	0.5±0.2	0.09±0.0.36	3.4	< 0.001**
S. TWEAK (pg/ml)	390±152	130.4±10.1	4.5	<0.001**
S. OPG (ng/ml)	0.74±0.07	0.37±0.106	2.1	< 0.001**
S. IgG (mg/ml)	2.8 ± 1.3	12.4 ± 3.7	3.9	<0.001**
S. IgM (mg/ml)	4.2 ± 1.4	1.34 ± 0.3	2.9	< 0.001**
S. CD4 (ng/ml)	6.85 ± 2.05	15.6 ± 6.3	- 5.1	< 0.001**

*Level of significance $p < .05$. **Level of significance $p < .001$. S. (serum). Bl. (blood). Independent t-test.

Table (2) shows correlations between serum levels of TRAIL, TWEAK, and OPG with multiple parameters. A significant negative correlation was found between serum iron and serum OPG levels ($p<0.5$) (S), with no other significant correlations between TRAIL, TWEAK, and OPG with other parameters.

Table (2): Correlation of serum levels of TRAIL, TWEAK, and OPG to multiple parameters.

Variables	**Pearson's correlation**	**TRAIL**	**TWEAK**	**OPG**
Age (years)	r	-0.001	0.102	0.071
	p	.994	.421	.58
BMI (kg/m^2)	r	-0.052	0.018	-0.063
	p	.686	.89	.618
Dialysis Vintage (years)	r	-0.003	0.006	-0.01
	p	.978	.960	.939
kt/v	r	-0.032	-0.016	0.117
	p	.801	.899	.356
Bl. Hemoglobin (g/dL)	r	-0.07	0.025	-0.116
	p	.581	.846	.362
S. Ferritin (ng/ml)	r	-0.162	0.04	-0.215
	p	.201	.751	.088
S. Iron (ug/dL)	r	-0.078	0.063	-0.247
	p	.543	.618	.049*
S. Total iron binding capacity (TIBC) (ug/dL)	r	0.098	-0.023	0.099
	p	.441	.855	.438
S. Transferrin saturation test (TSAT) (%)	r	0.012	0.009	-0.229
	p	.926	.943	.069
White blood cells (WBCs) ($^x10^3/mm^3$)	r	0.098	0.001	0.011
	p	.441	.993	.933
Platelets ($^x10^3/mm^3$)	r	0.192	-0.086	0.084
	p	.129	.497	.511
S. Phosphate (mg/dL)	r	0.058	0.058	0.081
	p	.650	.649	.527
S. Calcium (mg/dL)	r	0.161	0.059	-0.136
	p	.203	.644	.283
S. Intact PTH (pg/ml)	r	0.071	0.118	0.013
	p	.575	.354	.917
S. Albumin (g/dL)	r	-0.106	-0.093	0.095
	p	.405	.463	.453
S. Cholesterol(mg/dL)	r	-0.107	0.037	-0.105
	p	.4	.771	.411
S. Triglycerides (mg/dL)	r	0.011	-0.222	0.054
	p	.929	.078	.671
S. C-Reactive protein (mg/dL)	r	-0.003	-0.03	0.009
	p	.983	.813	.944

Pearson Correlation. *Level of significance $p < .05$. S. (serum). Bl. (blood).

Table (3) shows correlations between serum levels of IgG, IgM, and CD4+ T cells with multiple parameters. A significant positive correlation was found between serum phosphorous and both IgG, and IgM, but a negative correlation was found between dialysis vintage and both serum IgM and CD4+ T cells, and between serum TSAT and serum IgG ($p<0.05$) (S).

Table (4) shows multiple regression analysis for predictors of serum levels of IgG, IgM, and CD4+ cells in HD patients. Dialysis vintage was a significant negative predictor for both serum IgM and CD4+ levels, whereas TSAT was a significant negative predictor for IgG serum levels ($p<0.05$) (S).

Table (3): Correlation of serum levels of IgG, IgM, and CD4+ cells to multiple parameters.

Variables	**Pearson's correlation**	**IgG**	**IgM**	**CD4**
Age (years)	r	-0.066	0.014	-0.025
	p	.605	.911	.843
BMI (kg/m^2)	r	-0.122	0.088	-0.030
	p	.337	.488	.814
Dialysis Vintage (years)	r	0.210	-0.308	-0.245
	p	.095	.013*	.04*
kt/v	r	-0.025	-0.121	0.032
	p	.845	.340	.8
Bl. Hemoglobin (g/dL)	r	-0.006	0.094	-0.009
	p	.963	.462	.941
S. Ferritin (ng/ml)	r	-0.156	-0.140	-0.225
	p	.219	.270	.073
S. Iron (ug/dL)	r	-0.221	-0.219	0.148
	p	.079	.082	.243
S. Total iron binding capacity (TIBC) (ug/dL)	r	0.147	-0.008	0.191
	p	.248	.948	.131
S. Transferrin saturation test (TSAT) (%)	r	-0.246	-0.143	0.034
	p	.04*	.260	.791
White blood cells (WBCs) ($^x10^3/mm^3$)	r	0.002	-0.078	0.114
	p	.99	.538	.370
Platelets ($^x10^3/mm^3$)	r	0.021	-0.022	0.159
	p	.866	.865	.209
S. Phosphate (mg/dL)	r	0.243	0.304	-0.085
	p	.043*	.015*	.505
S. Calcium (mg/dL)	r	-0.110	0.040	0.087
	p	.386	.756	.495
S. Intact PTH (pg/ml)	r	0.085	-0.008	-0.086
	p	.502	.948	.497
S. Albumin (g/dL)	r	-0.051	-0.042	0.158
	p	.686	.740	.213
S. Cholesterol(mg/dL)	r	0.025	0.009	-0.137
	p	.847	.943	.282
S. Triglycerides (mg/dL)	r	0.179	0.034	0.224
	p	.158	.789	.076
S. C-Reactive protein (mg/dL)	r	-0.003	-0.030	0.009
	p	.983	.813	.944
S. TRAIL (ng/ml)	r	-0.009	0.081	-0.098
	p	.945	0.524	0.440

S. TWEAK (pg/ml)	r	-0.100	0.009	-0.225
	p	0.430	0.947	0.074
S. OPG (ng/ml)	r	0.137	0.124	0.089
	p	0.282	0.331	0.482

Pearson Correlation. *Level of significance p < .05. S. (serum). Bl. (blood).

Table (4): Multiple regression analysis for predictors of serum levels of IgG, IgM, and CD4+ cells.

Predictor	**Estimate**	**SE**	**t**	**p**
Model Coefficients – IgG (mg/ml)				
Transferrin saturation test (TSAT) %	-0.00885	0.00457	-1.938	0.046*
Serum Phosphorus (mg/dl)	0.05860	0.10197	-0.575	0.568
Model Coefficients – IgM (mg/ml)				
Dialysis vintage (years)	0.0745	0.0261	2.86	0.006*
Serum Phosphorus (mg/dl)	0.1689	0.1093	-1.55	0.128
Model Coefficients - CD4 (ng/ml)				
Dialysis vintage (years)	-0.0868	0.0385	-2.26	0.028*

*Level of significance p < .05. SE (standard error).

DISCUSSION

ESKD patients are immunocompromised and at risk of infection, with increased uremic toxins and cytokines affecting innate and adaptive immunity,[20] and dialysis itself causes inflammation and abnormalities in the immune system [21]. The TNF superfamily and its receptors' pathways regulate cellular differentiation and programmed death, especially in the immune system through co-stimulation or co-inhibition [22].

This observational study included a total of 64 patients on regular HD, 3 sessions/week, and each session 3-4 hours, in addition to 32 healthy control subjects. The serum levels of TRAIL, TWEAK, and OPG were significantly increased in HD patients compared to healthy control. Do Van *et al.*, reported that OPG concentration was higher than normal in CKD, and can predict atherosclerosis in HD patients using low-flux dialyzers [23].

In contrast, Eskandari *et al.* [19] found that serum TWEAK levels were lower in HD patients than in healthy control, and were associated with ongoing inflammation in these patients. On the other hand, Liabeuf *et al.* [24] concluded that serum soluble TRAIL levels were significantly lower in patients with CKD stage 5 and HD than in patients with earlier CKD stages.

HD patients showed higher serum levels of IgM, but lower levels of IgG, and CD4+ T cells compared to healthy control. Yarlagadda *et al.* [25] reported a deficiency of IgM and IgG in CKD patients, with an increase in CRP, which reflects both immunosuppression leading to infection, and immunoactivation leading to inflammation.

Lobo *et al.* [26] found that IgM-anti-leukocyte autoantibodies were increased in dialysis patients, whereas Bassi *et al.* [27] reported lower antibody response to vaccines in HD patients compared to healthy controls.

On the other hand, Pretorius *et al.* [28] found no immediate negative effect for HD on CD4+ T cells, but they increase immediately after the HD session. In another study, HD patients had B-cell lymphopenia, caused by apoptosis, whereas IgG, IgM, and IgA were normal [29].

Serum OPG levels negatively correlated with serum iron in HD patients. Both marked iron excess and severe iron deficiency have negative effects on bones, through various effects on remodeling, osteoblasts, and osteoclasts [30].

Dialysis vintage (duration) was negatively associated with serum levels of IgM, and CD4+ cells. Repeated HD affects the phenotype and proliferation of CD4+ T cells, and the CD4+, and CD8+ cells decrease as HD induces the apoptosis of T lymphocytes [31, 32]. HD induces apoptosis of T lymphocytes, leading to T lymphocytopenia, and as CD4+ T cells are involved in both cell-mediated and humoral immunity, this impairs immunity and increases susceptibility to infection [33].

Serum phosphorous was positively associated with serum levels of IgM, and IgG. Low serum phosphate in HD patients might be a nutritional biomarker to predict increased susceptibility to infection and worse outcome, especially in older age and longer dialysis vintage[34].

Moreover, TSAT was negatively associated with serum IgG levels. TSAT means the ratio of serum iron to TIBC, and reflects iron deficiency, inflammatory, and nutritional conditions [35] .

Using the multivariate analysis, dialysis vintage was a significant negative predictor for serum IgM and CD4+ levels, whereas TSAT was a significant negative predictor for IgG serum levels. Espi *et al.* [36] concluded that CKD-associated immune dysfunctions affect humoral and cellular immunity, and are related to protein-bound uremic retention solutes. Mavrovouniotis *et al.* [37] mentioned that cell-mediated immunity in HD patients is affected by decreased serum albumin, increased iron sucrose intravenous doses, vitamin D deficiency, and ESA supplement.

Although the serum levels of TRAIL, TWEAK, and OPG were increased in HD patients compared to healthy control, they did not show any significant correlation to serum IgM, IgG, or CD4+ T cells. This doesn't cope with the previous studies that showed a regulatory effect for the TNF superfamily on T-cell responses and its interaction with other cell types [38]. An important point to be mentioned here is the necessity for standardization of proposed kits, which is required for accurate estimation of these markers and comparison between the results of different studies must be conducted carefully [39].

Interestingly, the serum levels of IgM were increased, whereas the IgG, and CD4+ T cells were decreased in HD patients compared to healthy control. ESKD patients have increased levels of anti-inflammatory and pro-inflammatory cytokines, related to their poor excretion or increased production. T cell proliferation and function are decreased in HD patients, and their antibody production in response to vaccines was lower than non-CKD subjects [40].

This study is among a very limited number of studies on the immune system in HD patients, with detailed findings. Using ELISA as an alternative to flow cytometry to measure the serum levels of CD4+ cells represents an easy and inexpensive tool that can guide future research. The main limitation in this study is the relatively small number of HD patients that prompts future studies to use a wide scale of hemodialysis patients.

CONCLUSION:

In conclusion, serum levels of TRAIL, TWEAK, and OPG are increased in HD patients, but they have no significant association with IgM, IgG, or CD4+ cells.

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