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Tumor Necrosis Factor-α Gene Polymorphism in Hemodialysis Pediatric Patients: Association with Comorbidity, Functionality and Serum Albumin

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The aim of this study was to evaluate the association between specific alleles genotypes of TNF-α with Index of Coexistent Disease (ICED) score (an index of comorbidity), karnofsky index (a measure of functional status), serum albumin and nutritional marker (body mass index) in 43 ESRD pediatric patients on regular HD. All participants were genotyped for TNF-308 promoter region polymorphism by PCR followed by digestion and gel electrophoresis. The TNF-α high producer genotype (A/A and G/A) had significantly higher comorbidity (ICED scores ≥2) and lower functional scores compared with the TNF-α low producer genotype (G/G). Serum albumin levels were lower in patients with the TNF-α high producer genotype compared with those with the low producer genotype. Also body mass index was lower in the TNF-α high producer genotype compared with the TNF-α low producer genotype ($p<0.05$). On multivariate analysis, the TNF-α high producer genotype was associated with increased significance for a higher ICED score, lower karnofsky index, lower serum albumin and lower body mass index compared with the low TNF-α low producer genotype ($p<0.05$). Single nucleotide polymorphism in the promoter region of TNF-α show a strong association with indices of comorbidity, functionality, biological and nutritional markers in ESRD patients on long-term HD. The TNF-α high producer genotype seemed to be associated with adverse clinical outcome in ESRD patients. Prognostic TNF-α genetic assay provides a more precise approach for identification of high risk ESRD patients and development of accurate individualized treatment strategies.

Key words: TNF-α, gene polymorphism, ICED score, karnofsky index, serum albumin, hemodialysis

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INTRODUCTION

In recent years, a strong association between chronic inflammation and long-term morbidity and mortality in patients with End-stage Renal Disease (ESRD) on hemodialysis (HD) has been shown in several studies (Manchanda *et al*., 2006; Shu *et al*., 2005). Pro-inflammatory cytokines such as tumor necrosis factor-α-(TNF-α) are believed to be key orchestrators of this inflammatory response. These cytokines have been implicated as key factors that link malnutrition, accelerated atherogenesis and excessive morbidity and mortality in ESRD patients on HD (Bologa *et al*., 1998; Stenvinkel *et al*., 2005).

TNF-α is one of the most important cytokines with various immunological functions. It is produced early in the inflammatory process, generating a cascade of other mediators and upregulation of some adhesion molecules and other cytokines such as interferon gamma (IFNγ), interleukin 6(IL-6), IL-8, IL-10 and TNF-α itself (Ruddle, 1992; Tuglular *et al*., 2003).

TNF-α is predominantly produced by monocytes/macrophages and in turn is a strong activator of phagocytic cells (Heidenreich *et al*., 1988; Shu *et al*., 2005). The TNF-α-gene (TNFA) is located on the short arm of chromosome 6, in the class III region, within the major histocompatibility complex (MHC) in a position defined as 250 Kb centromeric to HLA-B locus and about 850 Kb telomeric to HLA-DR locus (Wilson *et al*., 1993).

Single nucleotide polymorphism in TNF gene at position-308, probably have a direct influence on TNF production (Buraczynska *et al*., 2003).

Several studies have shown that there is considerable inter-individual variation in the maximal capacity to produce different cytokines in response to mitogen stimulation *in vitro* (Tambur *et al*., 2001). Furthermore, over all expression and production of cytokines is, in part, genetically determined. Stable allelic variants for TNF-α genes, arising from nucleotide polymorphism within the regulatory region, have been described in recent years. Polymorphism involving the 5-flanking region (promoter) of the TNF-α-gene affecting transcriptional activity and therefore, of functional relevance have been identified (Wilson *et al*., 1993; Shu *et al*., 2005).

The aim of this study was to determine the relative frequency of specific alleles/genotypes of TNF-α-in 43 pediatric patients with ESRD on HD and evaluated the relationship of specific TNF-α-alleles/genotypes to indices of comorbidity, functional status, biological and nutritional markers.

MATERIALS AND METHODS

Subjects: This study was performed on forty three patients (24 males (55.8%) and 19 females (44.2%) with ESRD on maintenance HD. They were recruited from the hemodialysis unit of the Center of Pediatric Nephrology and Transplantation, Children Hospital, Cairo University, from December 2005 to August 2006. Their mean age was (9.95±3.22 years).

Inclusion criteria included children on regular HD for not less than 4 months and had been receiving at least 3 sessions per week. Their ages ranging from 2-16.5 years and had residual renal clearance of urea of less than 1.5 mL/min/35 L of urea distribution volume.

Those patients in acute or chronic care hospitals, with active malignancy, decompensated cardiac, hepatic, or pulmonary disease, serum albumin <2.6 g dL^{-1}, inter-dialytic urea clearance >1.5 mL/min, or a scheduled or recently (<6 months) failed transplant were excluded. An informed written consent was taken from parents of all participants.

All patients were subjected to:

- Full history taking and thorough clinical examination laying stress on demographic, medical and socioeconomic information and these data were completed from chart progress notes, list of current medications and the most recent laboratory data, chest X-ray report, electrocardiogram and hospital discharge summary.

The comorbidities were cataloged using the Index of Co Existing Diseases (ICED), a coding system that classifies the presence and severity of 19 different diseases and 11 physical impairments (Miskulin *et al*., 2001). Disease severity was scored on a scale from 0 to 3, with 0 indicating the absence of disease and increasing values indicating increasing severity of the disease. The highest scores of Index of Disease Severity (IDS) and Index of Physical Impairment (IPI) were combined to create the ICED score, from 0 to 3 (Table 1). Functional status was assessed by means of the Karnofsky Index (KI) (Rettig *et al*., 1997). The KI is an overall indicator of the patient's level of physical functioning and is used frequently in clinical research. KI scores range from 10 (lowest level) to 100 (highest level) (Table 1). Clinical assessment of nutritional status included anthropometric measurements and Body Mass index (BMI).

Table 1: ICED and Karnofsky index scoring system

Level	Status	Proportions of patients (N, %)
ICED		
0	No comorbidity	2(4.7%)
1	Asymptomatic to mildly symptomatic	17(39.5%)
2	Moderate symptoms, require medication for control of disease	21(48.8%)
3	Severe symptoms, inadequate control of disease despite maximal medical therapy	3(7%)
Karnofsky index		
100	Normal	-
90	Normal activity; minor symptoms/signs of disease	10(23.2%)
80	Normal activity with effort	7(16.3%)
70	Cares for self	6(14%)
60	Self-care with minimal help	13(30.2%)
50	Self-care with considerable help	5(11.6%)
40	Disabled needs special care	2(4.7%)
30	Severely disabled	-
20	Very sick; hospitalization needed	-
10	Moribund	-

Laboratory investigations

- Complete blood count
- Pre-and post-dialysis urea for calculation of dialysis efficiency (Kt/V). Equilibrated post-dialysis urea from the rate equation of Daugirdas and Schneditz was used for calculation of Kt/V (Daugirdas *et al*., 1997).
- Serum albumin
- Genotyping of-308 TNF-α-polymorphism.

Blood samples collection: A 3 mL whole blood from all HD patients was collected pre-dialysis on EDTA containing tubes.

Genotyping of-308 TNF-α-polymorphism: DNA was extracted by using spin column Kit (Supplied from Qiagen). Two milligram of genomic DNA was amplified with each of forward primer 5'AAGGAAACA GAC CAC AGA CCTG and reverse primer GGT CTT CTGGGC CAC TGAC. (Supplied by Biosynthesis). PCR thermal cycling conditions were 1 min. Denaturation period at 94°C and 30 cycles of the following 94°C for 1 min, 60°C for 1 min and 72°C for 1 min. This is followed by a 5 min extension at 75°C. NCOI restriction digestion using NCOI restriction enzyme (Sib Enzyme Ltd.) followed by electrophoresis on a 3% agarose gel with ethidium bromide TNF-α-1 gave two bands at 325 bp, 20 bp. and TNF-α-2gaveone band at 345 bp. Since the very light band of 20 bp migrates too fast to be detected on the gel, the TNF-α-1 allele was in practice characterized by only one band at 325 bp. Finally the genotypes were defined as G/G (homozygous) in case of a unique band at 325, as A/A (homozygous) in case of a unique band at 345 bp and as G/A (heterozygous) in case of two bands-one at 325 bp and one at 345 bp. (Wilson *et al*.,1992).

Specifically, high or low producer phenotypes were assigned as follows: TNF-α-position-308: high producer genotypes (A/A and G/A), also referred as (TNF-α-2 allele) and low producer genotype (G/G) also referred as (TNF-α1 allele) (Wilson *et al*., 1997).

Statistical methods: SPSS (Statistical Package for social science) program version 9.00 was used for analysis of data. Data were summarized as mean±SD, range and percentage. One sample student's t-test was used for analysis of difference between two groups and the chi-square test was used for differences in proportions. Univariate and multivariate analysis were performed by general linear models to test the effect of TNF-α genotypes on comorbidity (ICED) score, functional status (karnofsky index), serum albumin and nutritional marker (body mass index). Multivariate analysis was adjusted for age, gender and duration on HD. $p<0.05$ was considered significant.

RESULTS

Proportions of patients according to ICED and karnofsky index scoring system are shown in Table 1.

Demographic characteristics and baseline laboratory data are provided in Table 2.

Figure 1 shows distribution of TNF-α-genotype by phenotypic characteristics (low and high producers). The percentage of TNF-α-high and Low producer genotypes were (58.1%) and (41.9%), respectively.

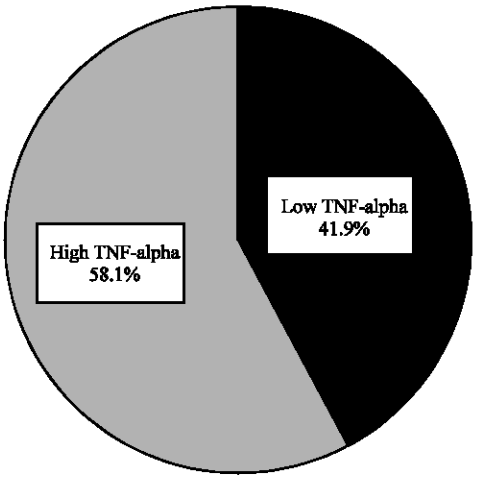

Fig. 1: Distribution of TNF-α-genotype by phenotypic characteristics in HD patients (low and high producers)

Table 3 shows that the TNF-α A allele was more frequent than the TNF-α-G allele in HD patients.

Table 4: shows the relationship between TNF-α-genotypes and different variables. There were statistically significant differences between the TNF-α-high producer genotype and the low produce genotype as regard age duration on HD and adequacy of HD as measured by equilibrated K/t V.

Table 2: Baseline characteristics of HD patients

Age (years)	9.95±3.22
Gender (M/F)	24/19(55.8%-44.2%)
Duration on HD (years)	1.75±1.05 (0.09-4)
Karnofsky index	70 (40-90)
ICED score (%)	2 (1-3)
Body mass index (kg m^{-2})	17.33±3.83
Equilibrated Kt/V	1.49±0.29
Serum albumin (g dL^{-1})	3.90±0.47
Hematocrite (%)	29.03±5.70

Results as mean±SD or number (%) or range as applicable

Table 3: Frequency distribution of TNF-α genotypes and alleles

	TNF-α alleles	
Genotype	low-producer N (%)	high-producer N (%)
A/A	-	5(20(%)
G/A	-	20(80(%)
G/G	18 (100%)	

Table 4: Relationship between TNF-α genotypes and different variables

	TNF-α genotypes		
Parameter	low-producer	high-producer	p-value
No (%)	18(40.9%)	25(59.1%)	
Age (year)	9.98±3.12	9.94±3.36	p<0.001*
Gender (M/F)	6/12(33.3-66.7%)	18/7(72-28%)	
Duration on HD (years)	1.56±0.99	1.91±1.08	p<0.001*
Equilibrated Kt/V	1.5±0.31	1.4±0.26	p<0.001*
ICED Score			
0	2 (11.1%)	-	
1	8 (44.4%)	8 (32%)	
2	7 (38.9%)	15 (60%)	p<0.001*
3	1 (5.6%)	2 (8%)	
Karnofsky index	75±12	66±14	p<0.001*
Serum albumin (g dL^{-1})	3.95±0.45	3.90±0.53	p<0.001*
BMI (Kg m^{-2})	17.54±4.36	17.16±3.12	p<0.001*

One-sample student's t-test was used to compare mean; p<0.05 was considered significant, BMI = Body Mass Index

A higher proportion of patients with the TNF-α high producer genotype had ICED scores of 2 and 3 compared with the TNF-α-low producer genotype n = 15 (60%) and n = 2 (8%) vs n = 7 (38.9%) and n = 1 (5.6%), respectively).

The TNF-α high producer genotype had a lower KI (more functional impairment) compared with those with the respective low producer genotype (66±4 vs 75±12 respectively, p<0.001).

Also, patients with the TNF-α high producer genotype had lower serum albumin compared with those with low producer genotype (3.90±0.53 g dL^{-1} vs 3.95±0.45 g dL^{-1}, respectively, p<0.001).

There was a significant association between TNF-α-genotypes and BMI. Patients with TNF-α high producer genotype had significantly lower BMI compared with those with low producer genotype (17.1±6.12 kg m^{-2} vs 17.54±4.36 kg m^{-2}, respectively, p<0.001).

We found a positive correlation between serum albumin and adequacy of HD, (r = -0.14, p<0.006).

Table 5 shows the relationship between TNF-α genotypes and outcome variables. On both univariate and multivariate analysis, the presence of the TNF-α-high producer genotype was associated with significant higher ICED scores compared with the TNF-α-low producer genotype (B = 0.28, 95% CI, 0.02 to 0.59, p<0.08) after adjustment for age, gender and duration on HD.

Also, univariate analysis revealed a significant relationship between KI and TNF-α-genotypes. On multivariate analysis after adjustment of the above co-variate, having a lower KI (more functional impairment) was higher in patients with the TNF-α-high producer genotype compared with the respective low producer genotype (B = 1.09, 95% CI, 1.04 to 1.26, p<0.05).

Patients with TNF-α-high producer genotype were estimated to have mean serum albumin levels 0.28 g dL^{-1} lower than patients with the TNF-α high producer genotype.

As regards BMI, multivariate analysis showed that, patients with TNF-α high producer were estimated to have lower BMI when compared with those with low producer genotype (B = -8.75, 95% CI, -7.74 to -9.76, p<0.001).

Table 5: Relationship between TNF- α genotypes and outcome variables (ICED Score, Karnofsky index and Serum albumin)

	Univariate analysis			Multivariate analysis		
Outcome	β	95% CI for β	P-value	β	95% CI for β	P-value
High producer (G/A or A/A) vs low producer (G/G)						
Higher ICED score on anordinal scale of 1 to 3	0.42	0.14, 0.71	0.005*	0.28	0.02,0.59	0.08*
Lower Karnofsky index on an ordinal scale of 40 to 90	1.04	1.73, 3.50	0.04*	1.09	1.04, 1.26	0.05*
Serum albumin	-0.4	-0.36,-0.44	0.001*	-0.28	-0.04,-0.57	0.05*
BMI	-9.37	-3.5,-5.39	NS	-8.75	-7.74, -9.76	0.001*

Significant was estimated by using univariate and multivariate general linear models, p<0.05 was considered significant, CI = Confidence Interval, BMI = Body Mass Index, NS = Non Significant

DISCUSSION

End stage renal disease is a final result of various etiologies. Prognostic indicators leading to ESRD in chronic kidney diseases have been studied extensively, of which, genetic factors remain a subject of great concern (Shu *et al*., 2005).

There is considerable evidence for a cytokine orchestrated chronic inflammatory response in patients on long-term HD. Indeed, several studies have shown that plasma TNF-α is elevated among patients on HD (Pereira *et al*., 1994; Balakrishnan *et al*., 2004; Ram *et al*., 2003).

In this study, as regards age, a significant difference between the TNF-α high producer genotype and the low producer genotype was found. Buraczynska *et al.* (2003) found that there is a significant association between TNF-α-genotypes and age of onset of renal failure.

This study showed significant differences as regards HD adequacy and duration on HD. These results are in accordance with the result of Herbelin *et al*. (1990) who found that uremia and duration on hemodialysis influence the circulating TNF-α. Also Ghysen *et al.* (1990) has reported that there is a certain effect of membrane characteristics on TNF-α kinetics during HD

In this study, the TNF-α A allele was more frequent than the TNF-α G allele in these patients. This result is in agreement with the result of Manchanda *et al*. (2006) who found that the AA genotype was more frequent in ESRD patients, but Balakrishnan *et al.* (2004) reported that the G/G genotype was more frequent in HD patients.

The present study demonstrates an important association between single nucleotide polymorphism within the promoter region of TNF-α-gene and indices of co-morbidity and functionality in ESRD patients on long-term HD. The presence of the -308 A allele or the TNF-α high producer genotype (G/A or A/A) was associated with higher comorbidity and lower karnofsky scores than patients with the TNF-α low producer genotype (G/G).

There is a considerable inter-individual variability in TNF-α secretion in response to stimuli. This is determined, at least in part, by polymorphism within the promoter region of this cytokine and may have a significant influence in individual susceptibility to cytokine-induced chronic inflammation and its attendant morbidity (Tambur *et al*., 2001).

The-308A allele has been associated with high promoter activity (Wilson *et al*., 1993) and has been found to correlate with enhanced spontaneous and stimulated TNF-α production both *in vitro* and *in vivo* (Wilson *et al*., 1997) and may contribute to chronic inflammation in HD patients (Gentory *et al*., 2005).

This study showed that on multivariate analysis, TNF-α genotypes retained a strong association with ICED scores and karnofsky index after adjustment for other covariates. These results are in accordance with the results of Balakrishnan *et al.* (2004) who found significantly higher comorbidity (ICED scores ≥2) and lower functional scores (Karnofsky index) in patient with the TNF-α high producer genotype compared with patients with the low producer genotype. TNF-α 308 seemed to be associated with the adverse clinical outcome in ESRD patients (Stenvinkel *et al*., 2005). Comorbidity and functional status are global indices of clinical status and strong predictors of survival and therefore, may be more sensitive phenotypes for the consequences of inflammatory process (Miskulin *et al*., 2001; Rettig *et al*., 1997).

This cytokine has been Incriminated in short- and long-term morbidity experienced by HD patients (Shu *et al*., 2005). Manchanda *et al*. (2006) has concluded that TNF-α-308 had strong association and may thus be a strong predisposition to ESRD in cohort on north Indian population. Further, individuals with G/A genotype may be at higher risk for ESRD. Buraczynska *et al.* (2003) reported that the TNF-2 gene may play a role in chronic renal failure and this role depends on pathophysiological changes in different diseases underlying renal failure. Kimmel *et al*. (1998) found that elevated plasma levels of TNF-α-among HD patients were significantly associated with increased relative risk of death. Cavet *et al.* (1999) observed that higher endotoxin-stimulated cytokine synthesis by peripheral blood mononuclear cells predicted morbidity from cardiovascular events.

Also, Ram *et al*. (2003) found that in hemodialysis patients the high production TNF-α-genotypes had significantly lower cumulative synthetic graft survival at 1 and 2 years compared with patients with the low production genotype and suggested that TNF-α 308 A allele is associated with increased graft thrombosis and failure in HD patients. Tuglular *et al*. (2003) found in a large cohort of patients a significance between the occurrence of IgA nephritis and the TNF-α low producer phenotype. However could not demonstrate significant and independent influences of the various TNF-α genotypes on the progression of the disease to end stage renal failure.

The-308 TNF A polymorphism has been associated with increased risk and severity of infectious diseases (Wilson *et al*., 1995) and infections after renal transplantation (Sahoo *et al*., 2000). Further more, the TNF-2 polymorphism has been associated with increased morbidity and mortality of severe forms of cerebral malaria (Mcguire *et al*., 1994), fulminans purpura

(Nadel *et al*., 1996) and mucocutaneous leishmaniasis (Cabrera *et al*., 1995). Other investigators have shown that the TNF-2 allele is strongly associated with susceptibility to and mortality from, septic shock (Mira *et al*., 1999) and may also be a susceptible factor for systemic lupus erythematosus (SLE) (Rood *et al*., 2000).

In this study, we found that the presence of the-308 A allele was associated with lower serum albumin than patients with the TNF-α low producer genotype(G/G). Proinflammatory cytokines such as TNF-α may suppress appetite (Kirchgessner *et al*., 1997) and induce catabolism (Moldawer *et al*., 1997), leading to a wasting illness that may be indistinguishable from malnutrition. (Bologa *et al*., 1998) found a significant correlation between plasma TNF-α and the degree of hypoalbuminemia and dyslipidemia among HD patients. Also on multivariate analysis we found that patients with the TNF-α high producer were estimated to have mean serum albumin level 0.28 g dL^{-1} lower than patients with the TNF-α low-producer genotype. Agreed with this result was Balakrishnan *et al.* (2004) who reported that mean serum albumin levels was 0.13 g dL^{-1} lower in patients with the TNF-α high producer genotype when compared with patient with TNF-α low producer genotype.

This study showed a significant difference in BMI between patients with TNF-2 allele and patients with TNF-1 allele. However Balakrishnan *et al*. (2004) did not find significant association between TNF-α genotypes and BMI or anthropometric measurements. Stenvinkel *et al*. (2005) stated that polymorphisms in genes related to body composition may be excellent candidates for analysis in the ESRD population, since nutritional parameters are strongly associated with adverse events in these patients.

CONCLUSION

The results from this study indicate that single nucleotide polymorphism in the promoter region of TNF-α show a strong association with indices of comorbidity, functionality, biological and nutritional markers in ESRD patients on long-term HD. The TNF-α high producer genotype seemed to be associated with adverse clinical outcomes in ESRD patients. Prognostic TNF-α genetic assay provides a more precise approach for identification of high risk ESRD patients and development of accurate individualized treatment strategies.

RECOMMENDATIONS

- Prognostic or predictive multigene DNA assays (which allow a simultaneous and rapid assessment of multiple genetic variants) are recommended as chronic inflammation is affected by multiple cytokines in HD patients.
- It would be equally interesting to investigate intra-renal TNF-α expression of different genotypes and measuring the serum or urine TNF-α levels in these patients and correlate them with the genotypes.
- Further studies to demonstrate the influence on clinical outcome in patients with earlier stages of chronic renal disease.

REFERENCES

Balakrishnan, V.S., D. Guo, M. Rao, B.L. Jaber, H. Tighiouart, R.L. Freeman, C. Huang, A.J. King and B.J. Pereira, 2004. Cytokine gene polymorphisms in hemodialysis patients: Association with comorbidity, functionality and serum albumin. Kidney Int., 65: 1449-60.

Bologa, R.M., D.M. Levine and T.S. Parker *et al*., 1998. Interleukin 6 predicts hypoalbuminemia, hypocholesterolemia and mortality in hemodialysis patients. Am. J. Kidney Dis., 32: 107-114.

Buraczynska, K., M. Koziol-Montewka, M. Majdan and A. Ksiazek, 2003. Polymorphisms is tumor necrosis factor-α and myeloperoxidase genes in patients with chronic renal failure on peritoneal dialysis. Mol. Diagn., 7: 175-180.

Cabrera, M., M.A. Shaw and C. Sharples, 1995. Polymorphism in tumor necrosis factor-α associated with mucocutaneous leishmaniasis. J. Exp. Med., 182: 1259-1264.

Cavet, J., P.G. Middleton, M. Segall, H. Noreen and S.M. Davis *et al*., 1999. Recipient tumor necrosis factor-alpha and interlukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood, 94: 3941-3946.

Daugirdas, J., T. Depner and F. Gotch *et al*., 1997. Comparison of methods to predict equilibrated Kt/V in the HEMO Pilot Study. Kidney Int., 52: 1395-1405.

Gentory, G., B. Altun, A.A Kiykim., M. Arici, Y. Erdem, M. Caglarg, U. Yasavul, C. Turgan and S. Caglar, 2005. TNF alpha-308 genotype and rennin-angiogenesis system in hemodialysis patients: An effect on inflammatory cytokine levels? Artif Organs., 29: 174-178.

Ghysen, J., J.F. Deplaen, D.E. Vanypersele and C. Strihou, 1990. The effect of membrane characteristics on tumor necrosis factor kinetics during hemodialysis. Nephrol. Dial. Transplant., 5: 270-274.

Heidenreich, S., M. Weyers, J.H. Gong, H. Sprenger, M. Nain and D. Gemsa, 1988. Potentiation of lymphokine-induced macrophage activation by tumor necrosis factor-alpha. J. Immunol., 140: 1511.

Herbelin, A., A.T. Nguyen and J. Zingraff *et al*., 1990. Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor alpha. Kidney Int., 37: 116-125.

Kimmel, P.L., T.M. Phillips and S.J. Simmens *et al*., 1998. Immunologic function and survival in hemodialysis patients. Kidney Int., pp: 236-244.

Kirchgessner, T.G., K.T. Uysal and S.M. Wiesbrock *et al*., 1997. Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating keptin release from adipocytes. J. Clin. Invest., 100: 2777-2782.

Manchanda, P.K., A. Kumar, A. Kaul and R.D. Mittal, 2006. Correlation between a gene polymorphism of tumor necrosis factor-alpha (G/A) and end-stage renal disease: A pilot study from north India. Clin. Chim. Acta, 370: 152-157.

Mcguire, W., A.V. Hill and C.E. Allsopp *et al*., 1994. Variation of TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature, 371: 508-510.

Mira, J.P., A. Cariou and F. Grall *et al*., 1999. Association of TNF2, a TNF-alpha promoter polymorphism with septic shock susceptibility and mortality: A multicenter study. JAMA, 282: 561-568.

Miskulin, D.C., N.V. Athieites and G. Yan *et al*., 2001. Comorbidity assessment using the Index of Coexistent Diseases in a multicenter clinical trial. Kidney Int., 60: 1498-1510.

Moldawer, L.L. and E.M. Copeland, 1997. Pro-inflammatory cytokines, nutritional support and the cachexia syndrome: Interactions and therapeutic options. Cancer, 79: 1828-1839.

Nadel, S., M.J. Newport, R. Booy and M. Levin, 1996. Variation in the tumor necrosis factor-α promoter region may be associated with death from meningococcal disease. J. Infect. Dis., 174: 878-880.

Pereira, B.J., L. Shapiro and A.J. King *et al*., 1994. Plasma levels of IL-1 beta, TNF alpha and their specific inhibitors in undialyzed chronic renal failure, CAPD and hemodialysis patients. Kidney Int., 45: 890-896.

Ram, S., K. Bass, K. Abreo, R.J. Baier and T.E. Kruger, 2003. Tumor necrosis factor-alpha-308 gene polymorphism associated with synthetic hemodialysis graft failure. J. Invest. Med., 51: 19-26.

Rettig, R., J. Sadler and K. Meyer *et al*., 1997. Assessing health and quality of life outcomes in dialysis: A report on an Institute of Medicine workshop. Am. J. Kidney Dis., 30: 140-155.

Rood, M.J., Van M.V. Krugten and E. Zanelli *et al*., 2000. TNF-308A and HLA-DR3 alleles contribute independently to susceptibility to systemic lupus erythematosus. Arthritis Rheum., 43: 129-138.

Ruddle, N.H., 1992. Tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta). Curr. Opin. Immunol., 4: 327-332.

Sahoo, S., S. Kang, S. Supran and R. Saloman *et al*., 2000. Tumor necrosis factor alpha gene polymorphisms correlate with infections after renal transplantation. Transplantation, 69: 880-884.

Shu, K.H., C.H. Cheng, M.J. Wu, C.H. Chen and W.C. Lee, 2005. Interleukin 1 receptor antagonist and tumor necrosis factor alpha gene polymorphism in patients with end stage renal failure. Ren. Fail, 27: 53-57.

Stenvinkel, P., R. Pecoits-Filho and B. Lindholmz, 2005. Gene polymorphism association studies in dialysis: the nutrition-inflammation axis: Semin Dial; 18: 322-330.

Tambur, A., I. Yaniv and J. Sstein *et al*., 2001. Cytokine gene polymorphism in patients with graft-versus-host disease. Transplant. Proc., 33: 502-503.

Tuglular, S., P. Berthoux and F. Berthoux, 2003. Polymorphisms of the tumor necrosis factor alpha gene at position-308 and TNFd microsatellite in primary IgA nephropathy. Nephrol. Dial. Transplant., 18: 724-731.

Wilson, A.G. and F.S. Di Giovine, 1992. Single base polymorphism in the TNF-gene detectable by Nco1 restriction of PCR products abstract. Hum. Mol. Genet., pp: 1-353.

Wilson, A.G., N. de Vries, F. Pociot, E.S. di Giovine L.B. van der Putte and G.W. Duff, 1993. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA, A1, B8 and DR3 alleles. J. Exp. Med., 177: 557-560.

Wilson, A.G., F.S. di Giovine and G.W. Duff, 1995. Genetics of tumor necrosis factor-alpha in autoimmune, infectious and neoplastic diseases. J. Inflamm., 45: 1-12.

Wilson, A.G., J.A. Symons and T.L. Mcdowell *et al*., 1997. Effects of a polymorphism in the human tumor necrosis factor [alpha] promoter on transcriptional activation. Proc. Natl. Acad. Sci. USA., 94: 15-31.