The Relationship Between Tumor Necrosis Factor-Alpha, Insulin Resistance and Dialysis Adequacy in Hemodialysis Patients

HEMODİYALİZ HASTALARINDA TÜMÖR NEKROZİS FAKTÖR-ALFA, İNSÜLİN DİRENCİ ve DİYALİZ YETERLİLİĞİ ARASINDAKİ İLİŞKİ

Mustafa GÜLLÜLÜ*, Alpaslan ERSOY*, Barbaros ORAL**, Ferah BUDAK**, Mahmut YAVUZ*, Kamil DİLEK*, Mustafa YURTKURAN*

* Dept. of Nephrology, Medical School of Uludağ University,
** Dept. of Immunology, Medical School of Uludağ University, Bursa, TURKEY

Summary

Purpose: It is known that chronic renal failure patients have insulin resistance that can be improved with effective haemodialysis therapy. Many toxins that are removed with haemodialysis therapy are blamed for this resistance. But the tumor necrosis factor-alpha (TNF-a) expression which is known to be strongly correlated with insulin resistance especially in obese patients has not yet been studied in hemodialysis patients. In this study, we aimed to investigate the relation between TNF-a level and insulin resistance with increased dialysis effectiveness.

Materials and Methods: Twenty eight haemodialysis patients 15 male, 13 female were included in the study. Their mean age was 37 ± 15 years. 18 cases with an average Kt/V value of 1.0 with the urea kinetic model were included in the study group and 10 cases with an average Kt/V value of 1.1 were included in the control group. After all cases had intravenous glucose tolerance test (IVGTT), the Kt/V value of the study group was increased to 1.5. No change was done in the control group. After 12 weeks both groups again had an IVGTT and glucose, insulin area under the curves of glucose and insulin and TNF-a levels were compared before and after the study.

Result: The serum glucose and insulin levels before and after the study were statistically compared. In the study group, these parameters were significantly decreased when compared with the control group. Although there was a statistically significant decrement in area under the curves

Received: Oct. 16, 2000

Correspondence: Mustafa GÜLLÜLÜ
Department of Nephrology
Uludag University Medical School
16059, Görükle, Bursa, TURKEY

Özet

Amaç: Kronik renal yetmczlikli hastalarda etkili hemodiyaliz tedavisi ile düzeltilebilen insulin direnci olduğu bilinmektedir. Hemodiyaliz ile uzaklaştınlabilen birçok toksin bu direnç için suçlanmaktadır. Obez hastalarda insulin direnci ile güçlü bir şekilde uyum gösteren tümör nekrozis factor-alfa (TNF-a) ekspresyonu, hemodiyaliz hastalarında henüz incelenmemiştir. Bu çalışmada, diyaliz etkinliğinin artırılması ile insulin direnci ve TNF-a arasındaki ilişkiyi araştırmayı amaçladık.

Materyal ve Metod: Çalışmaya 15'i erkek, 13'ü kadın 28 hemodiyaliz hastası dahil edildi. Ortalama yaşları 37 ± 15 yıl idi. Üre kinetik model ile ortalama Kt/V değerleri 1.0 olan 18 olgu çalışma grubuna dahil edildi. Ortalama Kt/V değeri 1.1 olan 10 olgu ise kontrol grubuna alındı. Tüm olgulara intravenöz glikoz tolerans testi (İVGTT) uygulandıktan sonra, çalışma grubunun Kt/V değeri 1.5'a yükseltildi. Kontrol grubunda değişiklik yapılmadı. Oniki hafta sonra İVGTT her iki grupta da tekrarlandı. Çalışma öncesi ve sonrası serum glikoz ve insulin seviyeleri ve glikoz ve insulin eğrilerinin altında kalan alanlar ve TNF-a seviyeleri karşılaştırıldı.

Bulgular: Çalışma öncesi ve sonrası serum glikoz ve insulin düzeyleri istatistiksel olarak karşılaştırıldı. Çalışma grubunda, bu parametreler kontrol grubuyla karşılaştırıldığında anlamlı azaldı. Onikinci haftanın sonunda çalışma grubunda glikoz (18180 ± 1465'den 12057 ± 602'ye, pO.001) ve insulin (8567 ± 1368'den 5674 ± 902'ye, p<0.05) eğrilerinin altında kalan alanlar istatistiksel olarak anlamlı azalmasına karşın her iki grupta da TNF-a seviyelerindeki değişiklikler farklı değildi (Yüksek Kt/V grubunda 9.18 ± 6.03 pg/mfden 9.91 ± 7.1 pg/ml'ye ve kontrol grubunda 8.68 ± 6.66 pg/ml'den 9.78 ± 8.0 pg/ml'ye, p>0.05).

of glucose (from 18180±1465 to 12057±602, p<0.001) and insulin (from 8567±1368 to 5674±902, p<0.05) in the study group at the end of 12 weeks, the changes in TNF-a levels were not different in both groups (from 9.18±6.03 pg/ml to 9.91±7.1 pg/ml in high Kt/V group and from 8.68±6.66 pg/ml to 9.78±8.0 pg/ml in control group, p>0.05).

Conclusion: As a result, we can say that the increment in hemodialysis effectiveness did not affect TNF-a levels much and upon this point we thought that TNF-a could not be an important factor for insulin resistance in these patients.

Key Words: Insulin resistance, Dialysis adequacy, Tumor necrosis factor-alpha

T Klin J Med Res 2001, 19:120-125

Sonuç: Sonuç olarak, hemodiyaliz etkinliğindeki artışın plazma TNF-a seviyelerini etkilemediğini vc bu noktada TNF-a'nın bu hastalarda insülin direncinde önemli bir faktör olmayabileceğini söyleyebiliriz.

Anahtar Kelimeler: İnsülin rezistansı, Diyaliz yeterliliği, Tümör nekrozis faktör-alfa

T Klin Araştırma 2001, 19:120-125

Tumor necrosis factor-alpha (TNF-a) is closely associated with acute and chronic inflammatory processes in hemodialysis patients. However, the mechanisms concerning cytokine production by monocytes during hemodialysis are still conflicting (1). In addition, TNF-a is potentially an important mediator of protein wasting in chronically uremic patients (2).

The role of TNF-a, which is a cytokine produced by immune cells during skeletal muscle damage, has repeatedly been investigated in insulin resistance in recent years (3,4). A strong positive correlation has been observed between TNF-a mRNA expression levels in fat tissue and the level of hyperinsulinemia, an indirect measure of insulin resistance (5). Moreover, the insulin sensitivity in obese rats was improved by neutralizing TNF-a (6). Insulin resistance, as evidenced by reduced peripheral sensitivity to the hypoglycemic action of insulin, is common in patients with uremia on dialysis (7). The etiology of insulin abnormalities in CRF is probably multifactorial but not clearly understood (8). It has been reported that insulin resistance can be improved with effective hemodialysis therapy (9,10). We did not find any data on whether TNF-a play a role in the etiology of insulin resistance in hemodialysis patients in English literature. Therefore we aimed to study the relationship between TNF-a level and the improvement of insulin resistance with increasing dialysis effectiveness.

Materials and Methods

28 outpatients with end-stage renal disease who were on hemodialysis treatment were included in the study. All of them had 4 hours of dialysis treatment three times a week. All hemodialysis treatments were performed using acetate dialysate and cellulose triacetate dialysers. Their medications did not change throughout the study, including erythropoietin. None had diabetes mellitus, obesity and any disease known to be associated with elevated TNF-a values.

Kt/V values of all cases were calculated by ureakinetic modelling [dialyzer urea clearance (K), duration of dialysis (t), and volume of distribution of urea (V)]. Mathematically, this relationship has been designated as a dose of dialysis = Kt/V (11). During 12 weeks we tried to keep Kt/V almost equal to 1.0 in all patients. After 12 weeks 18 (10 M, 8 F) cases who had mean Kt/V value of 1.0 ± 0.02 were selected as the study group. 10 cases (5 M, 5F) with the mean Kt/V value of 1.1 ± 0.02 were selected as control group. The characteristics of both groups were showed in Table 1. Among groups, there was no difference in terms of age and the duration of dialysis (p>0.05).

Intravenous glucose tolerance test (IVGTT) was performed to all patients after an overnight fast. The Kt/V values of the patients in the study group were elevated to 1.5 ± 0.02 by increasing the pump rate and the dialysis duration. The Kt/V values in the control group were tried to be kept con-

Table 1. Characteristics of both groups

	STUDY GROUP	CONTROL GROUP
Sex, M/F	10/8	15/5
Age, year	36 ± 10	37 ± 15
The dialysis duration, month	76 ±28	89 ±44
Body mass index, kg/m²	22.2 ± 4.5	23 ± 5.2
Primary Diseases		
Glomerulonephritis	4	2
Pyelonephritis	-	1
Acute Tubular Necrosis	1	-
Amyloidosis	1	-
Alport Syndrome	2	-
Hypertension	3	-
Unknown	7	7
Protein catabolism rate	1.2 ±0.05	1.2 ±0.07
Kt/V		
Pre-study	1.0 ±0.02	1.1 ±0.02
Post-study	1.5 ±0.02	1.1 ±0.02
PTH, pg/ml		
Pre-study	588 ± 133	424 ± 104
Post-study	567±125	464± 130
Calcium, mg/dl		
Pre-study	10± 1.5	9.2 ± 1.2
Post-study	10± 1.2	8.3 ± 1.0
ionised calcium, mg/dl		
Pre-study	0.68 ±0.41	0.68 ± 0.41
Post-study	0.57 ±0.15	0.64 ± 0.08
Phosphorus, mg/dl		
Pre-study	7.2 ± 1.5	5.8 ± 1.2
Post-study	5.8 ± 1.1	6.0 ± 1.2

stant (1.1 ± 0.03). After 12 weeks, IVGTT was repeated in both of the groups. Glucose and insulin values were determined from all samples during IVGTT. Total areas under the curves of glucose and insulin values between 0 and 120 minutes were calculated by using the trapezoidal integration. In addition to the changes in all of these parameters calculated in both of the groups, the values were also compared with each other.

Plasma samples were collected from above mentioned study groups and stored at -20° C until the assay performed. Human TNF-a concentrations in plasma were measured by a sandwich enzyme immunoassay (Quantikine human TNF-a immunoassay kit; R & D Systems, Minneapolis, USA) according to the manufacturer's instructions. TNF-a levels were determined by reference to a standart curve. The minimum detectable concentration of TNF-a was 4.4 pg/ml.

Statistical analyses were performed by using student's t-test, and nonparametric Mann-Whitney and Wilcoxon signed rank tests.

Results

After 12 weeks, while the fasting glucose levels of study group decreased from 91.8 ± 4.8 mg/dl to 73.5 ± 2.4 mg/dl ($p<0.01$), those of control group did not significantly change (from 84 ± 5.8 mg/dl to 87.2 ±3.3 mg/dl, $p>0.05$). The changes in the fasting insulin levels of both groups were not significant (from 38.0 ± 4.8 U/L to 39.5 ±3.3 U/L in high Kt/V group and from 39.5 ± 6.2 U/L to 35.7 ± 3.1 U/L in control group, $p>0.05$). Stimulated glucose and insulin levels in the study group markedly decreased during IVGTT compared to the pre-study values. But those of control group did not change (Figure 1).

After the study, the changes that had occured in serum glucose and insulin levels of the study and control groups were compared with each other. The decrement amounts in glucose values of study group during IVGTT were significantly different from the increments or decrements in the control group ($p<0.01$ in the second minute, $p<0.05$ in the other minutes). When the decrement amounts in insulin values of the study group except baseline and 120th minute values (1 ± 4 and 26.8 ± 10.5, respectively) were compared with the decrement amounts in the baseline and 90th minutes of the control group and the increment amount in 120th minute (27 ± 13.5),

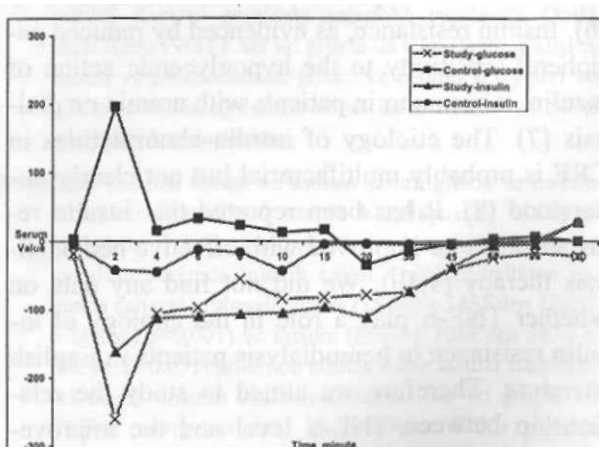

Figure 1. The changes in serum glucose and insulin levels of study and control groups during IVGTT.

Table 2. TNF-a levels and total areas under glucose and insulin curves of both groups.

	High Kt/V Group		Control Group	
	Pre-Study	Post-Study	Pre-Study	Post-Study
TNF-a pg/ml	9.18 ±6.03	9.91± 7.1a	8.68 ± 6.66	9.78 ±8.0a
Total area under insulin curve	8567±1368	5674 ± 902b	5510 ±734	5515 ± 657a
Total area under glucose curve	18180±1465	12057 ± 602c	16457 ± 1647	15791 ± 1437a

a $p > 0.05$, compared to prestudy values
b $p < 0.05$, compared to prestudy values
c $p < 0.001$, compared to prestudy values

there was only a significant difference between the second and 45th minutes ($p<0.05$).

After the study, the total area under the curves of glucose and insulin of the study group were significantly decreased when compared with the prestudy values. We determined a significant difference between the changes in areas under the glucose and insulin curves of both groups after the study (-6123±1181 vs -666±1280, $p<0.01$ and -3212 ± 1158 vs 4.5 ± 616, $p<0.05$, respectively). The changes in TNF-a levels were not different in both groups (Table 2).

Among groups, there was no difference in terms of parathyroid hormone, serum calcium, phosphorus and albumin levels measured before and after the study ($p>0.05$) (Table 1).

Discussion

Glucose intolerance, characterised by moderately elevated fasting glucose and increased insulin concentrations is frequently observed in patients with chronic renal failure. It is already well known, that tissue sensitivity to insulin, metabolic clearence of insulin and glucose uptake are all decreased in these patients (12). Finally, it is also known, that the above described abnormalities of glucose metabolism are partially corrected by dialysis therapy, suggesting that dialysable factors are responsible (13). The factors that interfere with the biological effects of insulin in uremic sera can be eliminated with dialysis (10). But, this improvement can never reach to the levels in normal healthy subjects. Urea kinetic modelling demonstrates the dialysis efficacy best.

In our study we observed that the hemodialysis therapy which elevated the KT/V values by urea kinetic modelling increased the insulin sensitivity. We suggested that there was a correlation between the increment in dialysis efficacy and insulin sensitivity due to the increase in metabolized glucose and decrease in insulin concentrations.

The role of TNF-a in the pathogenesis of insulin resistance in non-uraemic obese patients has been proven. The precise mechanism of action of TNF-a on insulin resistance and how TNF-a interferes with insulin signalling is still unclear. Treatment of cultured murine adipocytes with TNF-a was shown to induce serine phosphorylation of insulin receptor substrate 1 (IRS-1) and convert IRS-1 into an inhibitor of the insulin receptor tyrosine kinase activity in vitro. The cells that had endogenous IRS-1 were very sensitive to this effect of TNF-a (14,15). Recently, other molecular mechanisms associated with TNF-a and insulin action were reported (3). In our study, the increments in TNF-a levels of both groups were not statistically significant. There was no difference between the changes of TNF-a levels of groups. Because of the baseline glucose levels of our patients were in normal ranges the results could be affected. The increased dialysis efficacy in patients with impaired glucose tolerance and increased insulin resistance could resulted in more marked improvement in insulin resistance and a significant change in TNF-a levels.

Cytokine production (including TNF-a) is stimulated during the hemodialysis procedure in patients with chronic renal failure. Immunoreactive TNF-a levels are measured in the plasma from long-term hemodialysis patients. Ghysen et al. (16) found that TNF-a, although not very prominent, was higher, in dialysed patients than in uremic ones, serum TNF-a was shown to be more than 15 pg/ml in 23 cases among 52 dialysed patients. In our cases, TNF-a levels were high in two patients of the study group and in one patient of the control group before the study. After the study, all of them decreased under 15 pg/ml. TNF-a could be at least partly implicated in the clinical manifestations observed in these patients (17). The same observations were obtained in another study (18). In long-term hemodialysed patients, they found at the beginning of the dialysis increased plasma TNF-a levels and enhanced monocyte capacity to produce TNF-a spontaneously ex vivo. In addition during dialysis with cellulose acetate or polysulphone membranes, plasma TNF-a levels and the spontaneous and lipopolysaccaride-induced production of TNF-a by monocytes remained at predialysis levels while there was a significant increase in these parameters when cuprophane membranes were used (18). In conrast, it has been reported that hemodialysis either with cuprophan or P M M A dialysers had no influence on basal cytokine release during a 24-hour period following dialysis (19). Similarly, Mandalfo et al (20) did not detect post-dialysis increase in the intracellular content of TNF-a with high-flux benzly-cellulose, acetate-cellulose and low-flux polysulfone membranes.

As a result, the increment in hemodialysis effectiveness did not affect TNF-a levels. However, we came to the conclusion that TNF-a did not change concomittantly with the increase in dialysis intensity does by no means prove that it does not play a role in the pathogenesis of insulin resistance. It is clear to be the requirement for the more detailed studies in this object.

REFERENCES

1. Lin YF, Chang DM, Shaio MF et al. Cytokine production during hemodialysis: effects of dialytic membrane and complement activation. Am J Nephrol 1996; 16 (4): 293-9.
2. Guarnieri G, Toigo G, Fiotti N et al. Mechanisms of malnutrition in uremia. Kidney Int Suppl 1997; 62: S41-S44.
3. del Aguila LF, Claffey KP, Kirwan JP. TNF-alpha impairs insulin signaling and insulin stimulation of glucose uptake in C2 C12 muscle cells. Am J Physiol 1999; 276 (5 Pt 1): E 849-55.
4. Hotamisligil GS. Mechanisms of TNF-alpha-induced insulin resistance. Exp Clin Endocrinol Diabetes 1999;' 107 (2): 111-2.
5. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995; 95 (5): 2409-15.
6. Hotamisligil GS, Budavari A, Murray D, Spiegelman B M. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994; 94 (4): 1543-9.
7. DeFronzo RA, Alvcstrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. J Clin Invest 1981; 67 (2): 563-8.
8. Mak RHK, DeFronzo RA. Glucose and insulin metabolism in uremia. Nephron 1992; 61: 377-82.
9. Foss MC, Gouveia LM, Mayses-Neto M, Paccola GM, Piccinato CE. Effect of hemodialysis on peripheral glucose metabolism of patients with chronic renal failure. Nephron 1996; 73 (1): 48-53.
10. Dzurik R, Hupkova V, Cemacek P. The isolation of on inhibitor of glucose utilization from the serum of uremic subjects. Clin Chim Acta 1983; 46: 77.
11. Ahmad S. Dose of hemodialysis. Manual of clinical dialysis. Science Press, 1999:44-52.
12. Smith D, DeFronzo RA. Insulin resistance in uremia mediated by postbinding defects. Kidney Int 1982; 22(1): 54-62.
13. DeFronzo RA. Pathogenesis of glucose intolerance in uremia. Metabolism. 1978; 27(12 Suppl 2): 1866-80.
H. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996; 271 (5249): 665-8.
15. Kenety H, Feinstein R, Papa MZ, Hemi R, Karasik A. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. J Biol Chem 1995; 270 (40): 23780-4.
16. Ghysen J, De Plaen JF, Van Ypersele de Strihou C. The effect of membrane characteristics on tumour necrosis factor kinetics during haemodialysis. Nephrol Dial Transplant 1990; 5: 270-4.

17. Herbclin A, Nguyen AT, Zingraff J, Urena P, Descamps-Latscha B. Influence of uremia and hemodialysis on circulating interlcukin-1 and tumor necrosis factor a. Kidney Int 1990; 37: 116-25.

18. Chollet-Martin S, Stamatakis G, Bailly S, Mery Jp, Gougerot-Pocidalo M A. Induction of tumour necrosis factor-alpha during haemodialysis. Influence of the membrane type. Clin Exp Immunol 1991; 83(2): 329-32.

19. Schaefer R M, Paczek L, Heidland A. Cytokine production by monocytes during hemodialysis. Nephrol Dial Transplant 1991; 6(Suppl.2): 14-7).

20. Mandalfo S, Tetta C, David S, Gervasio R, Ognibene D, Wratten M L, Tessore E, Imbasciati E. In vitro and in vivo biocompatibility of substituted cellulose and synthetic membranes. Int J Artif Organs 1997; 20(11): 603-9.