Effect of Dialysis Duration on the Immunochemical Changes

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Abstract

Objective: To study the effect of dialysis on the immunochemical changes of some interleukins and other biochemical parameters.

Design setting: A total of 25 patients with end stage renal disease (mal and female) underwent dialysis with different duration were included in this study. Their age ranged from (22-75).The patients were divided according to the duration, the first group who received dialysis less than one year and the second group who received dialysis more than one year Healthy control group were sex, ethnic, matched and semimatched in age with patients group were selected.

Results: Using ELISA technique, serum IL-1α and IL-8 were estimated for dialyzed patients when divided in to two groups (less than one year and more than one year). Also Serum FT3 and albumin were also measured for the above two groups compared with healthy controls. All the above parameters showed different pattern.

Conclusion: Hemodialysis duration was influenced serum levels of interleukins and other biochemical parameters.

Key words: End stage renal disease, Dialysis, Interleukins.

Introduction

Hemodialysis is the most common method used to treat advanced and permanent kidney failure. Chronic inflammation is a common feature of end stage renal disease; failure refers to temporary or permanent damage to the kidneys that result in loss of normal kidney function [1].

Although the uremic state itself may impair lymphocyte, granulocyte & monocytes/macrophage function, defects in immunity can occur as a direct consequence of therapy. About 35_ 65% of ESRD patients receiving hemodialysis (HD) show signs of inflammation, whereas the prevalence in pre-dialysis patients may be somewhat lower. Dialysis has been associated with acute changes in the complement activation, granulocyte markers, macrophage function, T-cell activation and the release of various proinflammatory cytokines. Actually, recent data suggest that levels of the proinflammatory cytokines in HD patients are eight-fold to 10-fold higher than in healthy controls. Chemokines play a central role in inflammatory processes by regulating leukocyte migration into sites of tissue damage.[2]

IL-1α is a polypeptide hormone produced by activated macrophages that mediates a broad range of biological activities and interacts with surface receptors on numerous cell types. It is clear that IL-1α is an early proinflammatory cytokine, which cause a rapid up-regulation of other cytokines, chemokines and inflammatory factors, as well as oxidative stress and inflammation.[3,4]

IL-8 is mitogenic and chemotactic for endothelial cells, it is released by several cell type: Monocytes, Macrophages, T-cells, and within neoplasm. [5,6]

It is clear that not only the quantity but also the quality of T-cell activation is influenced by chronic renal failure. In dialysis patients there is not only reduction of T-helper cell activation, but also preponderance of the Th1 cytokine pattern. T-cell activation follows Th2 pattern in hemodialysis patients.[7,8]

Thus patients with end-stage renal disease (ESRD) have an impaired immune response with a dysregulated Th1/Th2. [9,10]

Impaired leukocyte function in renal failure is due to an accessory cell defect, and that T-cell of these patients have normal function capacities when they get the costimulatory signals required.[11]

The Biochemical changes:

Albumin is a protein, found in animal sources and in plant sources, it provides the body with the protein needed to both maintain growth and repair tissues. It can also help with fluid removal during the dialysis treatment. If the albumin level is good, fluid will move more easily from swollen tissues into the blood, where it can then be removed by the dialyzer. [12,13]

The notion that end-stage renal disease (ESRD) affects thyroid function has solid ground and ESRD is now formally listed as an established cause of chronic

Non-thyroidal illness .A variety of alterations in thyroid hormone levels and/or metabolism has been described in patients with ESRD and low plasma

triiodothyronine (T3) has been consistently found to be the most common disturbance in thyroid function in this population. Dialysis patients frequently display low T3 levels as an effect of impaired extra-thyroidal T4 to T3 conversion, or as a phenomenon secondary to peritoneal loss of thyroid binding globulin [14,15].

The recent observation that biomarkers of inflammation are consistently associated with low T3 levels in haemodialysis (HD)patients is a stimulating new finding which may have implications also for dialyzed patients [16,17].

Because risk factors for inflammation in PD patients do not coincide with those in HD patients, we thought that it is important to confirm the inflammation-T3 link in PD patients and explore the association between low T3 and hard outcomes (death) in this population[18,19].

Materials and Methods:

Subjects

A total of 25 patients with ESRD (female and male) underwent dialysis with different duration were included in this study .Their age ranged from 22-75 with a mean age of 45 ± 3.

The patients were divided according to the duration, the first group that received dialysis less than six months and the second group those who received dialysis more than six months. The blood samples were collected pre and post dialysis.

Healthy control group were sex, ethnic, matched and semi matched in age with patients group were selected.

Kits:

1- IL-1α ELISA kit, Beckman coulter, France.
2- IL-8 ELISA kit, Beckman coulter, France.
3- ALb Kit.
4- FT3 Kit.

Statistical analysis:

Data are presented as mean SD, median and inter-quartile range or as percent frequency and comparison between groups were made by t-test, Mann–Whitney test or chi-squared test, as appropriate.

Results:

All parameters were estimated in dialyzed patients those whom underwent dialysis for less than one year with those for more than one year to identify the effect of long term dialysis compared with healthy control.

Using ELISA technique, serum IL-1α level was estimated for the two groups of dialyzed patients compared with healthy control. The results for the first group which is less than one year reveled that there is significant increase in pre-dialyzed patients (mean=33.06±7.86) as compared with healthy control (mean= 14.31±4.065) with P-value = 0.0001.

The same result was observed when the comparison has been done between post-dialysis (mean=37.35±7.91) and healthy control (mean =14.31± 4.065) which is significant with P-value = 0.0001 .

For the same group (less than one year on dialysis) we compare pre-with post-dialysis, there was increase in the concentration but statically it was not significant, in pre-dialysis the result of IL-1α was (mean=33.06±7.86) while in post-dialysis was (mean=37.35±7.91), with P-value = 0.162 .

Concerning the long term dialysis, more than one year on dialysis the result of IL1α was highly significant in pre-dialysis (mean=44.94± 11.47) as compared with healthy control (mean=14.31±4.065) with P-value= 0.0001.

Higher result was obtained when we compare post-dialysis (mean= 49.97±7.058) with healthy control (mean=14.31±4.065) with P-value= 0.00001.

Interestingly there was increase in the concentration of IL-1α in pre- (mean=44.94±11.47) and post-dialysis (mean= 49.97±7.058) but statistically not significant with P-value = 0.256.

All these results are shown in Table (1) and Fig (1).

Using ELISA technique, serum IL-8 level was estimated in dialyzed patients with less than one year duration compared with healthy control group. The result of IL-8 in pre-dialysis showed highly significant increase level in patients (mean= 101.25±53.88) compared with healthy control (mean =8.95±5.702) with P-value = 0.0001

When we compare serum IL-8 concentration in post- dialysis which is less than one year with healthy control, showed significant increase in patients (mean=75.06±21.63), while in healthy control (mean=8.95±5.702), with P-value = 0.00001.

This study observed that there is decrease in IL-8 concentration between pre- (mean=101.25±53.88) and post-dialysis (mean=75.06 ±21.63) but statistically there is no significance P-value = 0.109.

Using the same technique the level of IL-8 in patients with more than one year on dialysis showed that there is significant increase in IL-8 concentration in pre-dialysis (mean=68.08±24.96) while in healthy control (mean= 8.95±5.702) with P-value = 0.0001.

The same result was obtained when we compare post-dialysis (mean=69.77±19.03) with healthy control (mean=8.95±5.702) with P-value = 0.0001.

Unfortunately there no statistically changes in the level of IL-8 in pre-dialysis (mean=68.08± 24.96) while in post-dialysis (mean=69.77±19.03) with P-value = 0.86.

The results of IL-8 are represented in Table (1) and Fig (2).

Now we study the effect of long term dialysis on the level Albumin, this study revealed that there is increase in the level of serum Alb in dialyzed patients for less than one year in pre–dialysis(mean= 4.73±2.23) as compared with healthy control (mean= 4.46±0.606)but statistically not with P-value = 0.66.

No significant correlation between the level of albumin in post-dialysis and healthy control (mean= 4.04± 1.72, mean= 4.46± 0.606) correspond, P-value = 0.32.

Furthermore the same results when we compared the albumin level in post-dialysis (mean=4.04± 1.72) with pre-dialysis (mean= 4.73± 2.23), P-value = 0.368. Concerning the duration on dialysis, there is significant decrease in the level of albumin in pre-dialysis patients (mean=2.72±1.19) as compared with healthy control (mean= 4.46±0.606) with P-value =0.001.

On the other hand there was no significance decrease (P-value = 0.06) in the level of albumin in post –dialysis (mean= 3.38± 1.57) as compared with healthy control (mean= 4.46± 0.606).

In addition, this study showed no significant correlation between post-and pre-dialysis. Mean = 3.38± 1.57, mean = 2.72± 1.19 correspond, with P-

value = 0.302. The results which represent albumin levels are shown in Table (1) and Fig (3).

The highest degree of significancy were recorded in the comparison of free T3 level, for the first group (less than one year), comparing the level of free T3 I pre-dialysis (mean = 1.63± 0.377) and healthy control (mean = 2.99± 0.97) there is significant decrease (P-value= 0.00001), while there was lower significancy when we compared albumin level in post- dialysis (mean= 2.03± 0.64) with healthy control (mean= 2.99± 0.97) with P-value = 0.0001.

It should be mentioned that the comparison of pre-and post-dialysis revealed that there is increase in the level of FT3 in post-dialysis (mean=2.03± 0.64), but statistically not significant with P-value = 0.06.

In long term on dialysis (more than one year on dialysis) we observed that there is highly significant decrease in the level of FT3 in pre-dialysis (mean=1.41±0.186) than in healthy control (mean=2.99±0.97) with P-value = 0.000001.

Less significant decrease has been showed when we compare the level of FT3 in post-dialysis (mean=1.71±0.48) with healthy control (mean= 2.99±0.97) with P-value = 0.0001.

There is increase in the level of FT3 in post-dialysis (mean=1.71±0.48) as compared with pre-dialysis (mean=1.41±0.186) but statically not significant, with P-value = 0.088.

All the results of FT3 concentration are in Table (1) and Fig (4).

At the end if we compared IL-1α, I L-8,Alb,and FT3 between each other before and after dialysis. This study revealed that there is inverse relationship between serum Alb level and serum IL-1α before dialysis (r=-0.377) .The relation is highly significant with P-value=0.00001.

The same result obtained when we compared IL-1α with the level of albumin in post-dialyzed patients (r= -0.22958) with P-value= 0.00001.

There is significant positive correlation between IL-1α and FT3 in pre-dialyzed patients (r=0.63), with P-value=0.00001.

Interestingly this study revealed that there is significant inverse correlation when we compare IL-1α and FT3 in post-dialyzed patients (r= -0.4589), with P-value=0.00001.

The comparison between the level of Alb and IL-8 before dialysis showed direct relationship(r=0.5), with P-value =0.0001. While the same comparison but after the dialysis revealed that there is an inverse relationship(r= - 0.0773), P-value=0.00001.

There is significant direct correlation between IL-8 and the level of FT3 in pre-dialysis(r= 0.379), with P-value=0.0001. While there is highly significant inverse correlation between them in pre-dialyzed patient(r = -0.0852) with P-value= 0.00001.

It is worthy to mentioned that there is significant positive correlation between Alb level and serum FT3 before and after dialysis, (r=0.63, r= 0.6) respectively. With P-value=0.001 for both of them.

All the results are shown in Table (2)

Discussion:

The results of our study demonstrate that the serum concentrations of IL-1α are increased during dialysis but the levels of IL-8 are decreased over the course of measurement [(2)].

During dialysis, patient's blood leukocytes come into contact with several exogenous challenges including the surface material of the dialyzer materials, and substances (solutes and microbial products) from the dialysis batch. These interactions can trigger the synthesis of proinflammatory cytokines such as IL-1, thus during dialysis, a new gene expression for cytokines occurs, and the intracellular content of this IL is elevated.

IL-1α as the pre-eminent cytokine in dialysis, promoting the stimulus, signal transduction, gene transcription into m RNA, m RNA translation in to cytokine protein, post-translational processing from precursors to mature forms and extracellular release represent the various intracellular passage.

Each of these steps is induced by specific signals and they have to be differentiated, as some stimuli only start the process and produce primed monocytes with m RNA not translated into a specific cytokine. Once IL-1 production has started, it stimulates its own gene expression and synthesis with an amplification loop effect [(20)].

Our data revealed that hemodialysis duration influenced serum level of IL-8 since they were higher in short term hemodialysis patients than medium or long term, this result not agreed with [(20)] which the serum concentration of IL-8 is decreased during Hemodialysis while IL-1 remained unchanged. The rate of IL-8 gene transcription was correlated with the level of IL-8 expression, suggesting that a constitutive level of IL-8 gene transcription was a major contributing factor in differential IL-8 expression. The removal of IL-8 from the circulation by continuous hemo-filtration but a reduction of plasma IL-8 levels is contradictory, thus the cytokine profile is perturbed in uremia and during dialysis, and this should be considered as an inflammatory status.

There are a wide variety of reasons an albumin level may become low. May include: 1)-Inadequate nutrition ,Lack of appetite possibly from poor dialysis (a low Kt/V), an illness, a side effect of medications, or feeling depressed can all lead to a poor intake of protein-rich foods. Not knowing what foods to eat or not having protein-foods available can also create a poor albumin level.2)-Protein loss with some types of kidney disease, protein may be lost in the urine (proteinuria), with peritoneal dialysis, some protein crosses the peritoneal membrane and exits the body in the effluent dialysate (the solution drained from the peritoneal cavity). This loss increases in a person with peritonitis, an infection of the peritoneum, liver disease (protein is synthesized in the liver) or blood loss can also cause the albumin level to drop. 3) -

Inflammation: albumin levels decrease when an inflammation is present. Examples of a sudden inflammation include an access infection, an infected foot, gum disease, a urinary tract infection, a myocardial infarction (heart attack), or recent surgery.4)-Chronic inflammation, such as present with arthritis or cancer, also can cause the albumin to drop.

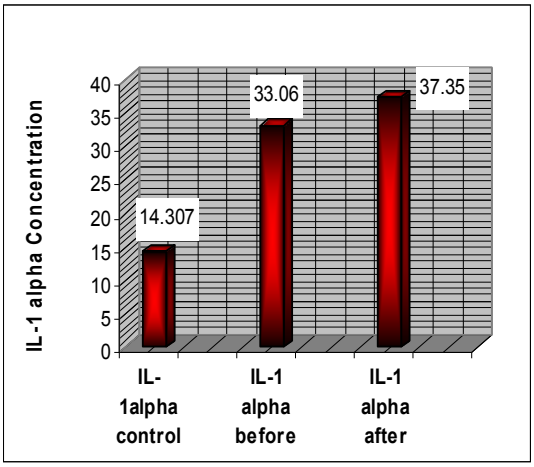
This study shows that thyroid function, as characterized on the basis of the plasma concentration of the active form of the thyroid hormone (fT3), is associated with markers of inflammation and endothelial activation in stable patients with ESRD. This association may entail a causal link because we also found that fT3 is suppressed during inflammatory processes that are triggered by intercurrent infections and that it reverts to normal as inflammation resolves. Intensive studies revealed that renal insufficiency affects thyroid function in multiple ways, including altered peripheral hormone metabolism, disturbed binding to proteins, reduction in tissue thyroid hormone content, and iodine accumulation in thyroid glands .Furthermore, uremic patients have a variety of non-renal, non-thyroidal disorders that affect thyroid hormone metabolism, such as diabetes, infections, and malnutrition, and they are often treated by drugs that interfere with thyroid function .In ESRD, both plasma fT3 and T3 are often reduced, and this alteration is attributed to impaired extra-thyroidal T4 to T3 conversion, whereas T4 and fT4 are much less frequently depressed in these patients .The different behavior of fT4 and fT3 in ESRD may depend on the fact that the depression of T3 is much greater than that of T4 and/or that, T3 being less tightly bound to thyroid-binding globulin than T4, alterations in thyroid hormone binding in ESRD are more apt to disturb the interpretation of T4 assays than those of T3.

Table (1): Shows the characteristic values of all parameters in dialyzed patients for the duration of less and more than one year with healthy control.

Parameters	Less than one year		More than one year		Healthy Control
Conc. of IL-1 α (pg/ml)					14.31 ± 4.06
	Pre-dialysis	**Post-dialysis**	**Pre-dialysis**	**Post-dialysis**	
Mean ± SD	33.06 ± 7.86	37.35 ± 7.91	44.94 ± 11.47	49.97 ± 7.05	
P-value	0.0001	0.0001	0.0001	0.00001	
P-value for pre and post dialysis groups	0.162		0.256		
Conc. of IL-8 (pg/ml)					8.95 ± 5.702
	Pre-dialysis	**Post-dialysis**	**Pre-dialysis**	**Post-dialysis**	
Mean ± SD	101.25±53.88	75.06±21.63	68.08 ± 24.96	69.77 ± 19.03	
P-value	0.0001	0.00001	0.0001	0.0001	
P-value for pre and post dialysis groups	0.109		0.86		
Conc. of Albumin (mg/dl)					4.46 ± 0.606
	Pre-dialysis	**Post-dialysis**	**Pre-dialysis**	**Post-dialysis**	
Mean ± SD	4.73 ± 2.23	4.04 ± 1.72	2.72 ± 1.19	3.38 ± 1.57	
P-value	0.66	0.38	0.001		
P-value for pre and post dialysis groups	0.368		0.302		
Conc. of FT3 (nmol/L)					2.99 ± 0.79
	Pre-dialysis	**Post-dialysis**	**Pre-dialysis**	**Post-dialysis**	
Mean ± SD	1.63 ± 0.377	2.03 ± 0.64	1.41 ± 0.186	1.71 ± 0.48	
P-value	0.00001	0.0001	0.000001	0.0001	
P-value for pre and post dialysis groups	0.06			0.088	

Table (2): Shows the correlation and predictive value for patients before and after dialysis for all the parameters.

Parameters	IL-1 alpha pre-dialysis		IL-8 pre-dialysis		Alb. pre-dialysis, FT3 pre-dialysis	
Albumin pre-dialysis	Correlation coefficient (r)	-0.377	Correlation coefficient (r)	0.5043		
	P-value	0.00001	P-value	0.0001		
FT3 pre-dialysis	Correlation coefficient (r)	0.630	Correlation coefficient (r)	0.3791		
	P-value	0.00001	P-value	0.0001		
Alb. pre-dialysis, FT3 pre-dialysis					R	0.63
					P	0.001
	IL-1 alpha post-dialysis		IL-8 post-dialysis		Alb. post-dialysis, FT3 post-dialysis	
Albumin post-dialysis	Correlation coefficient (r)	-0.22958	Correlation coefficient (r)	-0.0773		
	P-value	0.00001	P-value	0.0000		
FT3 post-dialysis	Correlation coefficient (r)	-0.45894	Correlation coefficient (r)	-0.0852		
	P-value	0.00001	P-value	0.00001		
Alb. post-dialysis, FT3 post-dialysis					r	0.6
					p	0.001



Fig(1): Shows IL-1 alpha level for patients (Duration less than one year)

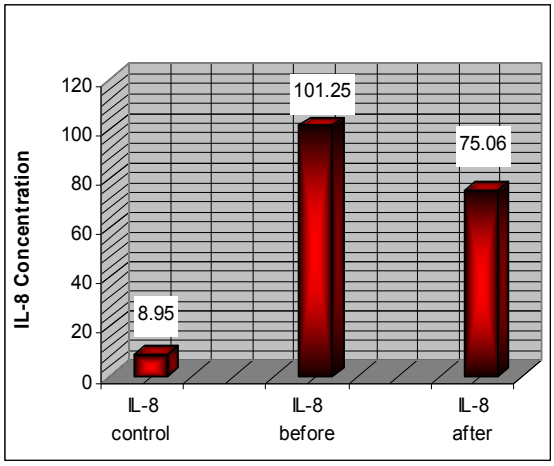


Fig. (2): Shows IL-8 level for patients on dialysis (Duration less than one year)

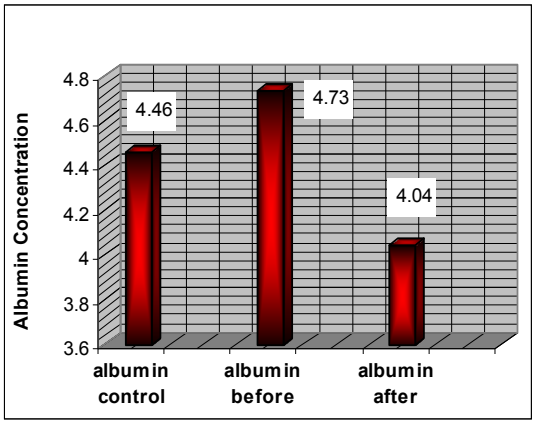


Fig (3): Shows Albumin level for patients on (duration less than one year).

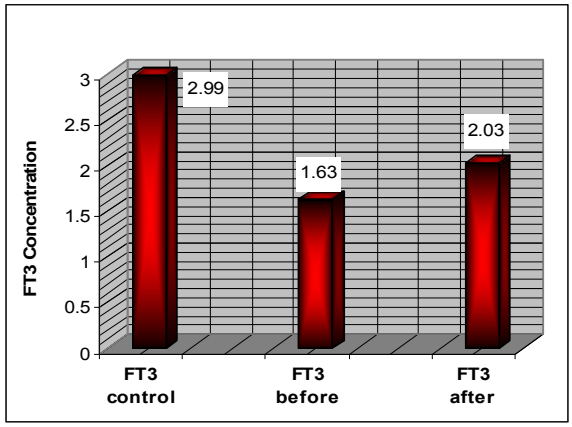
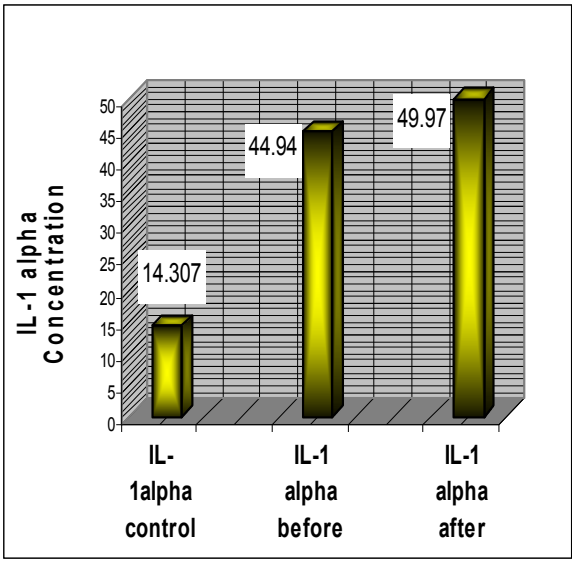


Fig.(4): Shows FT3 level for patients on dialysis (duration less than one year)



Fig(1): Shows IL-1 alpha level for patients on (Duration more than one year).

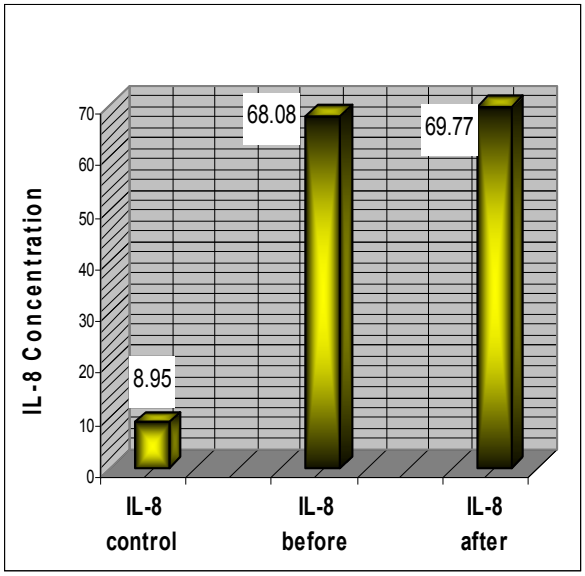


Fig. (2): Shows IL-8 level for patients on dialysis (Duration more than one year)

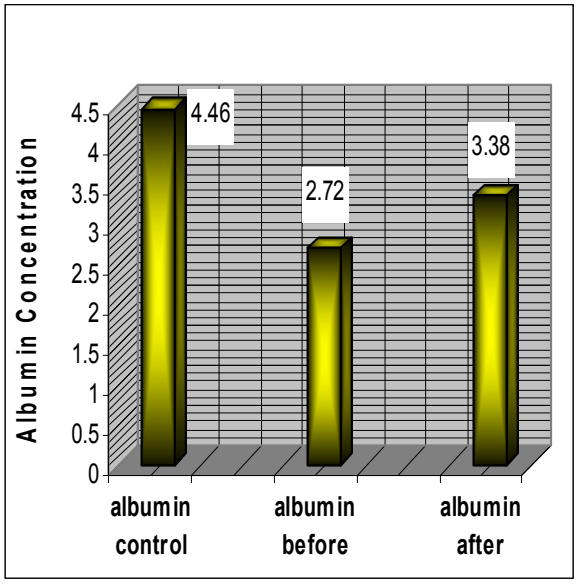


Fig (3): Shows Albumin level for patients on (Duration more than one year).

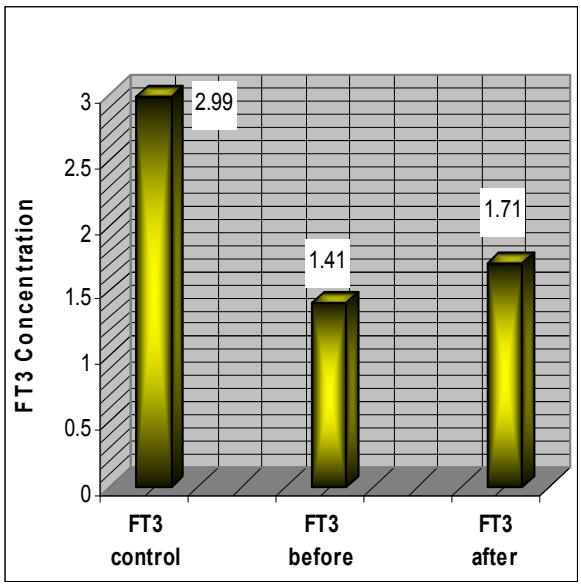


Fig. (4): Shows FT3 level for patients on dialysis (Duration more than one year)

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