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The Effect of Age Onleptin Hormone and Some Biochemical Parameters in Patients Pre-Dialysis

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Abstract: ***To evaluate impact the difference in stages ofage and related incidence of hemodialysis patients.Two hundred and fifty patients undergoing hemodialysis were collected from general hospital in Baghdad city /Iraq. The samples with renal failure before hemodialysis were divided into (138) male,(112)female. The sera were separated from samples to physiological investigation. We found that renal failure was more predominant among the patients ages group ranging from (51-70) years old. The results shows A significant increase in the levels of urea, creatinine, in younger patients (≤ 30 years) when compared with older patients (>70 years). Furthermore a significant decrease in serum levels of total protein in patients in older patients (>70 years) compared with younger group (≤ 30 years). While there are no significant differences in sera levels of albumin among aging group.While this study revealed a significant increase in the level of cholesterol when compared the aging group (>70 years) patients with younger group (≤ 30 years). This study also revealed significant increase in the level of fasting blood glucose (FBS) in the aging group (51-70)year when compared with aging group (31-50) year only. In additionleptin, CRP,Hb and PCV levels a significant increase in the age group (>70) years when compared with (≤ 30 years) group.***

Keywords: Hemodialysis, Age, Creatinine and urea profiles, Hemoglobin

1. Introduction

Chronic kidney disease (CKD) is described by persisting loss of renal function and/or kidney damage[1].It develops slowly, people with CKD are often not diagnosed or diagnosed late when CKD is at an advanced stage[2], hence leading to end-stage renal disease (ESRD) that refers to the final stage of CKD occurs when the kidneys have deteriorated to the point that they are no longer capable of sustaining life[3].The termination result of renal failure is usually death unless the blood is filtered by some other means[4]. Planned dialysis should start when glomerular filtration rate(GFR) is between (10-20) ml/minper 1.73 m^2 (normal range 80-120) [5]. Hemodialysis (HD) is a medical method that uses a special machine to filter waste products from the blood and to restore normal constituents to it when the kidneys are unable to do so[6].Some decline in renal function is a normal part of the aging process[7].Age is considered a risk factor for death in the renal replacement therapy population[8].According available data of age related differences and effect in the clinical status of HD patients are limited[9].

2. Materials and Methods

Two hundred and fifty patients(138 males and 112 females), on maintenance HD were included in this study that was carried out from April 2016 to March 2017. The patients were collected from four different major HD centers in public hospitals in Baghdad governorate in Iraq. Data collected from all the patients by interview was used for filling in a questionnaire, which designated for matching what the study needs.Laboratory investigations of serum HD patients pre dialysis consist of creatinineurea, total protein, albumin, cholesterol, FBS, blood hemoglobin, packed cell volume(PCV), leptin hormone and CRP. Blood sampling was collected before dialysis sessions.Leptin hormone and CRP were measured by using leptin and CRP intact ELISA kit. Serum creatinine urea, total protein, albumin, cholesterol and FBS were measured by standard laboratory techniques using diagnostic kits.Blood samples were collected using tubes containing ethylene diamine tetra acetic acid (EDTA) for hematological disorder were measured in different centers by automated analyzer (Abbott auto analyzer) for hematologypatients were treated by HD program 3-4 hours, two- three times a week. All patients with demonstrated ESRD (GFR<15 mL/min) were recruited based on the following inclusion criteria :-(Age above 18 years old.,Hemodialyzed schedule of a minimum three months with regular HD sessions for two- three per week at least and three hours for each session, No longer hospitalized, Absence of enteral or parenteral feeding,Participant with no acute illness such as acute myocardial infraction, pneumonia, or septicemia).Statistical Analysis: 20 (SPSS 20) was used for data analysis.

3. Results and Discussion

In the current study, the mean age of the HD patients was 52.6±12.5 years (range 18-83 years), this study indicated that the smallest proportion of patients was in the age group > 70 years which was limited on 14 (5.6%) of patients and 14 (5.6%) of patients in the age group ≤ 30. several reasons may explain the current study result, recent studies revealed that very old patients with CKD the chances of dying were greater than the chances of undergoing dialysis [10].Whereas, the proportion of cancer as the cause of early deaths decreased with patient age. Infection was responsible for as many as 25% or slightly less of early deaths in all age groups. Another attributable factor is that the most of the elderly patients refuse long-term RRT due to the financial, social and psychological burden on them and more so, on their families[11]. In this study, the largest proportion of patients was in the age group 51-70 years and then followed by the age group up to 31-50 years.A study done by [12]who

indicated that there is raise in number of young adults who are diagnosed with ESRD on hemodialysis and the causes are hypertension with diabetes. That ESRD occurs in Baghdad, at 31-50 years old, (38.4%) proportion of patients that may be due to certain risk factors in the environment in Iraq, which should be identified and prevented**.**

Table 1: Distribution of samples study according to age groups

Age groups (year)	No.	Percentage (%)
≤ 30	14	5.6
31-50	96	38.4
51-70	126	50.4
>70	14	5.6
Chi-Square	---	10.337 **
** (P<0.01)		

3.1 Association between age and renal function test

In the current study, increase levels of serum creatinine and urea in all patients age groups in table (2) as an expected result from an impairment of renal function either due to the decrease in the number of functioning nephrons, which would reduce the glomerular filtration rate (GFR), reduction of GFR or obstruction that interferes with urinary excretion,which causes major decreases in renal excretion of water and solutes [13].Other causes which make increase in urea and creatinine level in serum of HD patients is blocking the flow of urine [14]. leading to high concentration of creatinine in the serum, and it's concentration inversely related to the speed of glomerular filtration, that any slight decrease in the glomerular filtration leads to an increase in the concentration of creatinine in the blood[15],from our results found there were differences among the ages groups and it is statistically (P<0.05) significance. In our study, the highest level of urea and creatinine were in the patient's age group of ≤ 30 years, while the mean values of serum urea and creatinine level in the age group >70 years were observed to be less than other groups. This finding might be partly due to bad nutritional status and less physical activity in elderly patients than younger as suggested by [16]. Study by [17] supported this result , who observed that there was a decrease in creatinine levels in the older patients. In another study by [18] who showed that a significant lower predialysis serum creatinine in patients older than 50 years old which suggests insidious malnutrition in these patients might become more aggravated with their lower dialysis efficacy.

Table 2: Effect of age groups in renal function test

Age group (year)	Mean ± SE	
	Urea 10-45 mg/dL	Creat. 0.6-1.2 mg/dL
≤ 30	157.42 ± 18.37	7.68 ± 0.46
31-50	133.19 ± 5.05	7.42 ± 0.31
51-70	124.26 ± 3.50	6.65 ± 0.21
>70	119.71 ± 7.30	5.96 ± 0.53
LSD value	24.785 *	1.369 *
* (P<0.05), NS: Non-significant.		

3.2 Association between age and some biochemical parameters

Albumin and total protein levels

A serum albumin level of 3.0–3.4 g/dL was associated with significantly greater risk of death compared with the reference level of (4.0–4.4 g/dL). This study demonstrated that serum albumin level decreased in patients who suffered from CRF and treatment with hemodialysis, This hypoproteinemia may result from protein restriction, anorexia, protein loss during dialysis especially peritoneal, protein-energy malnutrition and chronic inflammation [19]. Also, hemodilution contribute to the hypoalbuminemia of patients on dialysis[20].Table (3) demonstrates the non-significant (p >0.05) difference in the concentration of serum albumin in all patients age groups. The result of serum albumin concentration of this study is matched with the results of[21].The serum albumin level in this result was influenced not by age, and close to study done by[22] in patients divided into two age group (≤40 and >40)years demonstrated from results non-significant between two groups of age. This study is in disagreement with those conducted by [23] who demonstrated that serum albumin level significant (p <0.05) decreased in the young patient when compared with old patients. This discrepancy, which was presumably caused by studying subjects with different age groups and/or different ethnicities [24], the study was done by [23] their patients were divided by age using 60 years as a cutoff point. Also, inflammation is considered the principal cause of a decrease in the serum albumin [25], it was more common in elderly than in younger patients[26].From the results of the table (3) The result shows that the mean value of total protein of patients in above 70 years group significant (p< 0.05) decrease when compared with ≤ 30 years group. This study is in disagreement with those conducted by [22]. That is may result from restricted protein intake[27] or metabolic acidosis which increase whole body protein degradation[28].The decrease in the serum levels of albumin and total protein may be attributed to the following reason Changes in the structure of basement membrane of glomeruli which consequent lead to the leakage of albumin and some low molecular weight proteins. Proteinuria is considered as a marker of renal disease progression [29]. Serum protein is affected by capillary permeability, drugs, impaired liver function, nutritional status and inflammation and a host of other factors [30].

Table 3: Effect of age groups in biochemical parameters

Age group (year)	Mean ± SE			
	Total protein 6- 8.3	Albumin 3.2-5.0	Cholesterol 140-200	FBS
≤ 30	6.79 ± 0.11	3.59 ± 0.11	144.78 ± 15.71	117.85 ± 11.23
31-50	6.34 ± 0.07	3.69 ± 0.05	167.14 ± 5.19	105.71± 5.74
51-70	6.47 ± 0.16	3.82 ± 0.17	173.68 ± 5.67	136.12 ± 5.59
>70	6.03 ± 0.14	3.52 ± 0.06	196.00 ± 20.54	115.64 ± 5.23
LSD value	0.468 *	0.339 NS	31.99 *	28.461 *
* (P<0.05), NS: Non-significant.				

Cholesterol level and Serum sugar level

The cholesterol levels were lower than the reference range of < 200 mg/dl for all age groups. Interestingly, the results from this table show total cholesterol levels are normal or even subnormal in hemodialysis patients and this in line

with [31]. Previous data on the effect of age on serum lipids levels gave mixed results. [32]who revealed that age correlated negatively with total cholesterol. Conversely, studies prove a significant increase in total cholesterol with age [33]. The changes in the results of previous studies on the possible effect of age and gender in serum lipids levels could be due to studying subjects with different age groups and/or different ethnicities, These results variations can partly be explained by the age range of the subjects in each study [24]. The current results of our study revealed age correlated positively with total cholesterol table (3-14). we observed a significant ($p < 0.05$) increase in the levels of cholesterol in patients group with above 70 years when compared with patients age group ≤ 30 years, and the explanation may be attributed to either a highly significant increase in FFA. The increase in the latter component leads to increased formation of acetyl-CoA with a subsequent increase in cholesterol synthesis[34].

Table (3-14) shows the levels of serum sugar in pre-dialysis patients with CRF. The results have shown that there was a significant increase in the serum levels of sugar in age group (51-70).These findings were confirmed by[17] and [21]. In another study by [35] who observed from his result that serum blood glucose levels were significantly ($p < 0.05$) increased in older patients. Hyperfiltration caused by insulin resistance, increased sympathetic activity and activated renin-angiotensin system [36]. Several factors, including uremic toxins, may increase insulin resistance in ESRD, leading to a blunted ability to suppress hepatic gluconeogenesis and regulate peripheral glucose utilization. Hemodialysis further alters insulin secretion, clearance, and resistance as the result of periodic improvement in uremia, acidosis, and phosphate handling [37].

3.3 Association between age and concentrations of leptin, CRP, Hb and PCV

Serum leptin

The results showed a significant increase in mean serum leptin levels in >70 (years) compared with ≤ 30 (years) patients Table (4) this agreed with a previous suggestion [38], predicted that the rise in leptin production with age. These findings are in a close agreement with another result reported by Beberashvili et al., who have also determined increased leptin levels in HD patients. They suggested that Hyperleptinemia observed in their studies might be the result of decreased renal clearance and consequent leptin retention in HD patients [39].In the study prepared by [40] they have shown in human subjects, reported that leptin is unchanged during age, furthermore another study showed a negative correlation between age and serum Leptin[41]. The differences in the results may be a consequence of many factors as both young and old subjects had similar percent body fat, different statistical analysis, or the studies was made in adult healthy subjects and possibly all participants from the same gender, or the fact that often the age leptin relationship was not the main focus of most of the studies. [42]observed that there is an increasing level of serum leptin hormone as aging is associated with increased adiposity in humans and animals. It is well established that age affects body composition, such as reduced muscle strength and increased fat depots[43]. It has been suggested that these phenomena might be related at least in part to changes in serum leptin levels. Our findings show that persons with young age have lower leptin levels, while old age has higher leptin levels. This finding in agreement with the data obtained by[44].The cause of increasing body fat with age is probably multifactorial. It has been postulated that changes in body composition with age could partially be the result of insensitivity to the action of the Ob gene product leptin[45]. Increased leptin levels were found to be associated with increased IL-6 and CRP [45],[46]. The mechanisms linking leptin and CRP are not clear. Adipose tissue is the source of circulating leptin[47].CRP is synthesized by the liver, largely under the regulation of the proinflammatory cytokines, primarily IL-6 [48].

Table 4: Effect of age groups in Leptin and CRP

Age group (year)	Mean ± SE			
	Leptin	CRP	Hb 11-16 g/dl	PCV 37-50/ul
≤ 30	10.00 ± 4.99	35.36 ± 7.84	7.98 ± 0.29	24.07 ± 0.87
31-50	25.37 ± 3.16	35.55 ± 2.73	9.23 ± 0.18	27.96 ± 0.55
51-70	25.11 ± 2.35	44.75 ± 2.33	9.02 ± 0.14	27.12 ± 0.45
>70	27.37 ± 6.81	51.73 ± 6.51	9.33 ± 0.37	28.70 ± 1.63
LSD value	13.878 *	12.518 *	0.811 *	2.596 *
*** (P<0.05)**				

Serum CRP level

In the same table, the mean of CRP serum level shows a significant variation among age groups of patients. We see that the mean value of CRP was high level 51.73 ± 6.51 (Mg/ml) in >70 age group and low level 35.36 ± 7.84 (Mg/ml) in ≤ 30 age.CRP is a very sensitive marker of systemic inflammation and can be increased with any kind of infection/inflammation [49]. The high blood CRP a marker of inflammation is demonstrated as a powerful predictor of the mortality in HD patients [50]. Although inflammatory marker levels are clearly increased in HD patients, this may be related to the exposure of blood to bioincompatible dialysis membranes [51]. Other causes, including dietary and behavioral factors, cardiovascular diseases[52],and high serum leptin levels are also associated with an increase in CRP levels[50]. Results of the current study showed a rise in levels of C reactive protein among dialysis patients. In study by[53]refer that the activation of inflammatory cytokines in hemodialysis patients is associated with a deficiency of kidney function and range of other factors induced infections in dialysis patients such as permanent contact or repeated with membranes of dialysis machine period of dialysis [54], CRP in the present study was significantly more prevalent in patients (>70 years) this agreed with a previous suggestion by [55] who predicted that the rise in CRP levels increases with age. Elevated CRP, which mainly reflected the presence of infection/ inflammation and was more common in elderly than in younger patients[26]. The current results are in agreement with the observation of[56] who stated age associated with the increase in inflammatory markers in CRF patients. Moreover, the present findings are in agreement with those of Pecoits-Filhoet al they mentioned significantcorrelation of CRP and IL-6 with ages [57].In another study by[58] who mentioned elevated CRP levels were positively associated with age. Aging is a multifactorial process involving

morphological and biochemical changes in single cell and in the whole organism[59]. Previous studies [60] have shown that aging is associated with significant alterations in the expression patterns of cell surface cytokine receptors. Aging seemed to be associated with an increase in systemic oxidative stress, which is one of the leading causes of increasing inflammatory markers in CRF patients[61].On the other hand, the current results are in inconsistency with the observation of [62] who reported there is no relationship between a high level of C-reactive protein and age. In addition, study done by [63] who demonstrated that CRP concentrations are not influenced by age in Caucasian populations. A possible explanation for the discrepancy between our study and previously mentioned studies is the population recruited, sample size, and design. In addition, this context particularly in using CRP and relation with aging requires further studies.

Hb and PCV concentration

Anemia was defined according to the World Health Organization criteria as a hemoglobin level < 13.0 g/dLin men and < 12.0 g/dL in women[64], anemia first appears when the GFR falls below 40 ml/minute and is present in most patients with ESRD because, in renal failure, erythropoietin production usually is insufficient to stimulate adequate red blood cell production by the bone marrow[65].Erythropoietin is the hormone which is the major humoral regulator of red cell production(RBC). The present study shows a significant difference in the age wise distribution of Hb and PCV concentration in patients group (table 3- 18) The results reveal a significant difference between the hemoglobin level in age group ≤ 30 years and other groups. These results in line with previous studies done by[66],[67]. These finding could explain as that ESRD patients on HD with HCV infection have higher Hb and HCT levels compared with HCV-negative patients [68], inflammation and aging are suggested to raise EPO levels, whereas diabetes lowers EPO levels[69]. Deficiency of the EPO is the main cause of the progressive decline in Hb concentration, PCV, and RBC, which occur frequently in patients with CRF. Erythropoietin stimulates terminal differentiation of committed erythroid progenitors in the bone marrow[70].The prevalence of metabolic syndrome was significantly high in ages 50-59 and 60- 69 years in hemodialysis patients when compared with other age groups [71].According to a study by [72]. Prevalence of iron deficiency anemia was found more in elderly age group. In this study, contradictory results are found probably because of more young patients developing CKD in Iraqi population.

4. Conclusion

Based on the results of this study, we found that renal failure was more predominant among the patients ages group ranging from (51-70) years old. The results shows a significant increase in the levels of urea, creatinine, in younger patients (≤ 30 years) when compared with older patients (>70 years). The results also revealed a significant decrease in the level of uric acid and total protein in older patients when compared with the younger patients group.

It was found that those aging group (>70 years) were significantly more associated with increased serum cholesterol, leptin, CRP, Hb and PCV levels when compared with (≤ 30 years) group.

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