Abnormal Serum Concentrations of Creatinine, Phosphorous, Triglycerides and Total Cholesterol in Males with End-Stage Renal Disease: A Comparative Study

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Abstract:

This study investigated the concentration abnormalities of serum creatinine (SCr), serum phosphorous (SP), serum triglycerides (STg) and serum total Cholesterol (STc) in males suffering from End-StageRenal Disease (ESRD). The serum concentration of each biochemical was determined in 30 males with ESRD (patient group) and in 30 normal males (control group). Except for STc, the mean concentration for all the biochemicals in the patient group was higher than the mean of the equivalent biochemical in the control group. However, the difference was only significant in case of SCr pair and SP pair ($P < 0.01$). Two significant relationships ($P < 0.05$) were found in patient group between two pairs: SCr-SP and STg-STc. These two relationships were not recorded in the control group. Similarly, the only significant relationship between age and SCr ($P < 0.05$) found in the control group was not recorded in the patient group. These limited differences in relationships were accompanied by limited differences in similarity ratio according to the results of cluster analysis. The findings of this study indicate that ESRD has a diverse effect on serum concentration of the studied biochemicals, with more prominent impact on SCr and SP. According to the results of statistical analysis, SP can be used in combination with SCr for early identification of ESRD.

Keywords: ESRD; creatinine; phosphorous; triglycerides; total cholesterol; t-test; correlation.

IINTRODUCTION

A patient diagnosed to have Chronic kidney disease (CKD) when glomerular filtration rate (GFR) decreases to < 60 mL/min/1.73m^2 or when kidney damage lasts for at least 3 months [1-3]. This decrease of GFR leads to accumulation of electrolytes, toxins [4], urea, creatinine and other chemicals [5] in blood, and if the accumulation of these components remains

untreated the patient condition will deteriorate and death becomes more probable. According to the level of GFR, CKD is divided into five stages. In the fifth stage, known as end-stage renal disease (ESRD), GFR declines to less than 15 mL/min per 1.73 m^2 [6] resulting into numerous complications, such as bone disorders [7], cognitive impairment [8], anemia [9], depression [10], inflammation and malnutrition [11]. Furthermore, as the CKD patient advances to ESRD, there will be a decrease in the quality of life, an increase of health-care cost [12] and a rise of death risk [13-15]. ESRD patients should either undergo kidney transplantation or dialysis to remove wastes from blood. There are two types of dialysis: hemodialysis and peritoneal dialysis [6]. Due to the total failure of kidney to remove wastes from blood and hence accumulation of wastes in bloods, it is expected that the serum level of some biochemicals will be altered in ESRD patients compared with healthy people. Some studies have reported that ESRD is accompanied with abnormal serum level of several biochemicals, including, but not limited to, creatinine [5, 16], phosphorous [17, 18] and lipids [19, 20]. Lipid abnormalities include elevated level of triglycerides (hypertriglyceridemia) and low level of total cholesterol (hypocholesterolemia). Information about serum concentration of these biochemicals, and other ones, could help in early identification of ESRD, and in gaining more knowledge and more understanding of the health state of the patient. The current study aims to provide some information about concentrations of serum creatinine (SCr), serum phosphorous (SP), serum triglycerides (STg) and serum total cholesterol (STc) in ESRD male patients receiving hemodialysis at Gharyan's Educational Hospital, Libya, an Arabic country located in North of Africa. The study approach included comparing concentration values of each biochemical in sera of ESRD male patients (patient group) with regard to their reference intervals. Also, the obtained data of each biochemical in the patient group was statistically compared with similar data of healthy males free from ESRD (control group). Although that the effect of ESRD on the concentration of SCr has been investigated in a similar previous study [21], creatinine was also included in the current study, due to the fact that its level usually elevates as CKD patient advances to ESRD. Therefore, creatinine was included in the current study to investigate its relationships with the studied biochemicals, and when any biochemical found to be related to creatinine it could be used for early identification of ESRD in combination with creatinine.

EXPERIMENTAL

Sampling and analysis were carried out at Gharyan's Educational Hospital, Libya.

Subjects and sampling

Sixty males were subjected to blood analysis to determine SCr, SP, STg and STc concentrations. Half of these subjects (30 males) representing patient group and were suffering from ESRD and receiving hemodialysis at Gharyan's Educational Hospital, while the other half (30 males) were normal males representing control group. All normal males were from Gharyan city, Libya. Females were not included in this study to eliminate the effect of gender on the concentration of studied biochemicals. Two mL of the venous blood was collected from each fasting subject before the dialysis session, then the collected sample was transferred to a plain tube and left at room temperature until a clot formed. Subsequently, each sample was centrifuged at 4000 rpm for 10 minutes. After obtaining the serum of each sample, it was analyzed twice for its content of creatinine, phosphorous, triglycerides and total cholesterol.

Methods

The source of all reagents used to determine the concentration of SCr, SP, STg and STc was Spinreact (Spinreact, Girona, Spain). The analysis of each sample was done directly after obtaining its serum. Measurement of SCr, SP, STg and STc concentrations was done photometrically, at 505, 340, 505 and 505 nm, respectively. The analysis was accomplished by Selectra Prom chemistry analyzer (ELITech Group, Puteaux, France). All analysis steps were carried out according to the instructions supplied with the kits from Spinreact (Girona, Spain). Determination of SCr concentration was based on the reaction of creatinine with alkaline sodium picrate. The reaction formed a red complex (creatinine picrate) with an intensity proportional to the concentration of SCr [22]. In case of SP, the analysis was based on the reaction between the phosphate ion and ammonium molybdate in acidic medium. A yellow color was produced due to the formation of phoshomolybedate complex, with color intensity proportional to the concentration of SP [23]. As for STc concentration, it was measured through three reactions: Cholesterol esters in Serum was hydrolyzed by cholesterol esterase (CHE) resulting into free cholesterol. The produced free cholesterol then oxidized by cholesterol oxidase (CHOD) to form Cholest-4-en-3-one with simultaneous production of

hydrogen peroxide, which then coupled with 4-aminphenazone (4-AP) and phenol in the presence of peroxidase (POD) yielding quinonimine, a red compound, with an intensity that proportional to STc concentration [24]. Concentration of STg was determined through series of reactions as follows: The triglycerides were hydrolyzed with lipoprotein lipase (LPL) liberating glycerol and free fatty acids. The produced glycerol was converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and Adenosine-tri-phosphate (ATP). G3P was then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Finally, hydrogen peroxide reacted with 4-aminophenazone and p-chlorophenol in presence of POD producing quinone, a red colored dye with an intensity proportional to STg concentration [25].

Statistical analysis

Minitab 17 (Minitab Inc., State College, Pennsylvania, US) and Microsoft Excel 2013 (Microsoft Corp., Seattle, WA, USA) were used to carry out statistical analyses and to represent the data graphically. In addition to running some descriptive statistics and presenting the biochemicals' data graphically for comparison, three statistical tests were conducted. These three tests were: t-test, correlation test (r) and cluster analysis. In order to investigate the difference between the biochemical means (patient group versus control group), t-test was employed at a significance level (α) = 0.01. Correlation test was applied at α = 0.05 to examine the correlation between age and the data of each biochemical. To measure the similarity between thee biochemical, cluster analysis was applied to the data.

Ethical consideration

Permission to carry out the experimental part was obtained from chemistry department, Gharyan's faculty of science and from the head of Gharyan's Educational Hospital. All subjects were informed of the nature of the study and they agreed to participate in it.

RESULTS AND DISCUSSION

Except for STc, all concentration means of the studied biochemicals were higher than their reference intervals. In case of SCr, all concentrations values exceeded the higher limit of its reference interval (0.7-1.4 mg/dL). As for SP there was only one reading below the lower

limit of the reference interval (2.5-5 mg/dL), and about %66.7 of the readings exceeded its higher limit, while nearly 30% of them were within the reference interval. Only 37% of the STg concentration values were higher than the higher limit of its reference interval (40-160 mg/dL), while 63% of the concentration values were within the reference interval. In case of STc, 80% of its concentration values were lower than the lower limit of the reference interval (200-239 mg/dL), 10% of the readings were higher than the higher limit of the reference interval, and 10% of the readings within the reference interval. It is clear that all patients suffer from high level of SCr, and most of these patients suffer from high level of SP and low level of STc. Only nearly 1/3 folds of the patient suffer from high level of STg. The standard deviation (SD) and the coefficient of variance (CV%) of all the studied biochemicals, shown in Table 1, were found to be higher in patient group, reflecting that concentration values of SCr, SP, STg and STc are more dispersed in patient group than in control group. The highest CV% value in patient group was for STg, suggesting that ESRD has more effect on data dispersion in case of STg compared to the other biochemicals in patient group. The CV% for the other biochemicals in patient group was nearly the same, indicating that, except for STg, the concentration values of each biochemical were equally dispersed around their means.

By comparing the differences in CV% between each pair (the same biochemical in both groups) it is clear that the highest difference in CV% was between SCr pair. This indicates that ESRD has more effect on data dispersion in case of SCr pair compared to the other pairs.

		Mean	Minimum	Maximum	SD	CV%
Age	Patient	41	26	83	13	32
(years)	Control	30	21	51	7	23.2
SCr	Patient	10.33	5.25	15.65	2.64	25.5
(mg/dL)	Control	0.93	0.51	1.2	0.14	14.7
SP	Patient	5.44	2.45	7.90	1.32	24.3
(mg/dL)	Control	3.60	2.50	5.05	0.61	16.81
STg	Patient	162	61	405	83	51
(mg/dL)	Control	123	43	278	55	44
STc	Patient	170	109	274	44	25.7
(mg/dL)	Control	172	123	252	34	19.7

Table 1. Some descriptive statistics for age & the biochemicals of patient and control groups

Table 1 and Figures 1-4 show that the concentration values of each biochemical are more spread in patient group compared to its equivalent in the control group.

Figures 1-4 show that, except for STc, all biochemical means in patient group were higher than that in control group. However, according to the results of t-test, the difference between the means of each two equivalent biochmicals was significant in the case of SCr and SP ($P < 0.01$), and were not significant in the case of STc and STg ($P > 0.01$). Our finding of the high level of SCr is in agreement with the results of previous studies [26-28]. The Elevated level of SCr is used as an indication of kidney failure, however; since it has been demonstrated that age, sex, muscle mass [29] and diet [30] affect SCr, thus one should be careful when interpreting results of SCr. In Our study we minimized the role of those effects that contribute to altering the SCr. This was achieved by not including female patients in the study, and also by comparing SCr of the patient group with a control group consisted of healthy males relatively close in age.

The elevation of SP is in agreement with previous studies [26, 31]. This elevation has been reported to be associated with increasing mortality in patients with ESRD [32-34] because of it is association with many diseases such as cardiovascular disease [35, 36] and secondary hyperparathyroidism [34]. Since SP and serum calcium were found correlated with raising mortality rate in ESRD patients [37], thus more studies are needed to explore the interaction between SP and serum calcium in ESRD patients receiving dialysis at Gharyan's Educational Hospital. In ESRD patients, common lipid abnormalities include hypertriglyceridemia (STg>150 mg/dL [38]) and hypocholesterolemia (STc<160 mg/dL [39]). In the current study, 40% of the patient group exhibited hypertriglyceridemia, while 47% exhibited hypocholesterolemia. Patients on hemodialysis (as in this study) usually have high level of triglycerides and normal or low level of cholesterol, while patients on peritoneal dialysis tend to have high level of triglycerides and high level of total cholesterol [40]. It has been reported that the elevation of triglycerides results from abnormalities of enzymes involved in lipoprotein metabolism [41] while hypocholesterolemia is associated with inflammation and malnutrition [42]. These two lipid disorders, hypertriglyceridemia and hypocholesterolemia, contribute in increasing health risks and mortality rate in ESRD patients on hemodialysis.

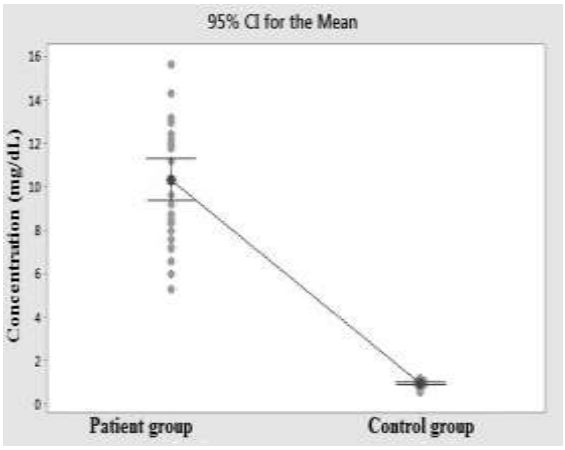

Figure 1. Interval plot of SCr concentration for patient and control groups with a diagonal line connecting the means

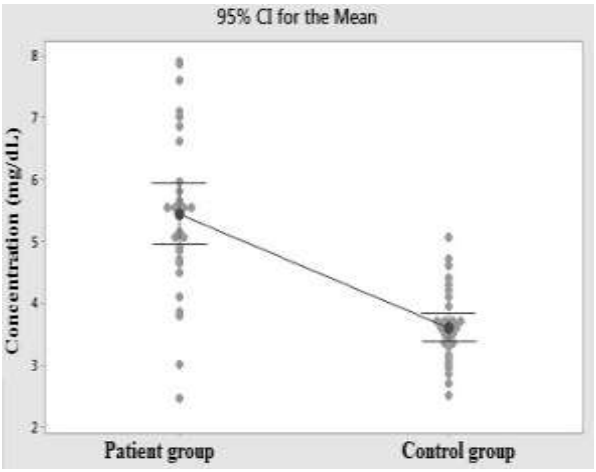

Figure 2. Interval plot of SP concentration for patient and control groups with a diagonal line connecting the means

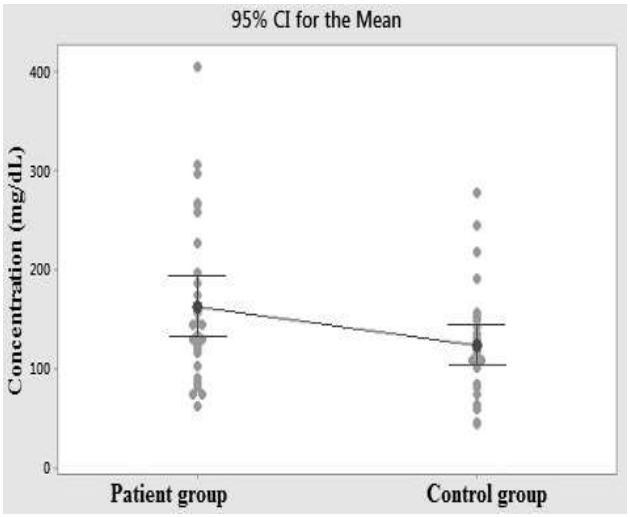

Figure 3. Interval plot of STg concentration for patient and control groups with a diagonal line connecting the means

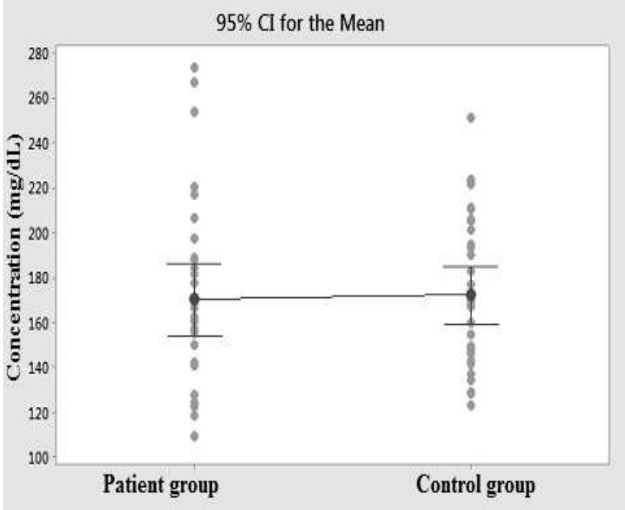

Figure 4. Interval plot of STc concentration for patient and control groups with a diagonal line connecting the means

As for the correlation test, few significant relationships were observed, and these relationships were observed in one group but not in the other. Table 2 shows that there was two significant positive relationships in patient group. One of these two relationships was between the SCr and SP concentrations ($r = 0.58$, $P < 0.05$), and the other one was between STg and STc concentrations ($r = 0.38$, $P < 0.05$), a weaker relationship compared to the SCr-SP relationship.

		Age		SCr		SP		STg	
		r	P	r	P	r	P	r	P
SCr	Patient group	-0.27	0.15						
	Control group	0.43	0.02						
SP	Patient group	-0.18	0.33	0.58	0.001				
	Control group	-0.09	0.65	0.08	0.69				
STg	Patient group	0.06	0.74	-0.08	0.67	-0.11	0.57		
	Control group	0.32	0.09	-0.02	0.92	-0.16	0.39		
STc	Patient group	0.11	0.57	-0.15	0.44	-0.18	0.33	0.38	0.04
	Control group	0.12	0.52	-0.05	0.80	-0.08	0.69	0.35	0.06

Table 2. Correlation coefficients (r) and P-values between age and the studied parameters

In control group there was only one significant positive relationships, which was not recorded in patient group. This relationship was observed between age and SCr concentrations ($r = 0.43$, $P < 0.05$). These limited differences in relationships were accompanied by limited differences in similarity ratio of the biochemical concentrations. The similarity ratios were estimated by cluster analysis for the four biochemicals in each group, and the results are presented in Figure 5 and Figure 6. The two figures show that the concentration values of the studied biochemicals were divided into two clusters, each cluster contained the same pair in both groups (patient and control groups). These clusters were: SCr-SP cluster and STc-STG cluster. The similarity ratio between SCr-SP in patient group (79%) was higher than that in the control group (54%), while the similarity ratio between STc-STg was nearly the same in both groups; 69% for the patient group and 67% for the control group.

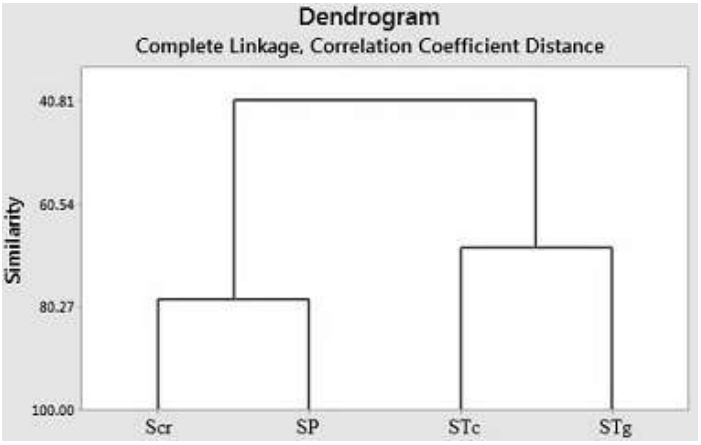

Figure 5. Dendrogram of biochemicals for patient group.

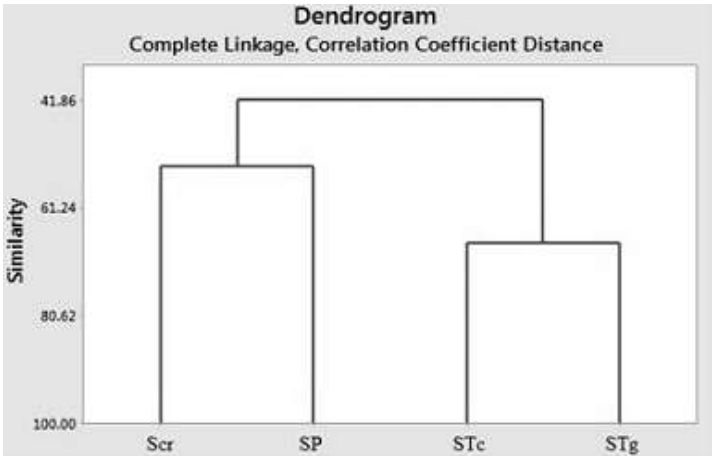

Figure 6. Dendrogram of biochemical parameters for control group.

The high similarity ratio between the SCr-SP pair in the patient group supports the previously mentioned significant relationship between this pair which was noticed in the correlation test. This association between SCr and SP indicates that both biochemicals can be used together for early identification of ESRD. The findings of this study reflect the role of ESRD on altering the serum concentration of the studied biochemicals compared with their reference intervals and with healthy people, and also reflect ESRD effect on the relationships between the serum concentrations of these biochemicals. Patients included in this study should be treated for the elevation of SCr and SP.

CONCLUSION

ESRD has a diverse effect on the serum concentration of the studied biochemicals, with more prominent impact on SCr and SP concentrations. The elevation of SP level relative to the reference interval could be used, combined with SCr, for early identification of ESRD in Libyan males. Further investigation of these biochemicals in ESRD female patients, and of other biochemical in both sexes, will provide more information by following the same approach applied in this study.

LIMITATIONS OF THE STUDY

The current study has some limitations which are: (1) we haven't taken into consideration the diet type which could have altered the serum concentration of SP in the subjects included in this study; (2) although the number of subjects was adequate for statistical analysis, increasing their number would have made the results more reliable; (3) considering serum cholesterol, we have measured only STc in each subject; it could have been more appropriate if we measured serum concentrations of high density lipoprotein (HDL-C) and low density lipoprotein cholesterol (LDL-C) in each subject, that would have provided clearer information about cholesterol profile.

Acknowledgement

We thank the staff at Gharyan's Educational Hospital, Libya, where sampling and analysis were carried out. We also thank Mr. Abdulmotlip Mohammed for his assistance with the statistical analysis.

Conflict of interest

None to declare.

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