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Distribution of plasma proteins in patients with chronic renal disease and hepatic insufficiency

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Abstract: Chronic renal disease (CRD) is a serious condition associated with mortality, and decreased quality of life. Moreover, patients of CRD are often suffering from hepatic insufficiency (HI). The plasma protein profile could be used as a predictor, as well as a diagnostic marker of preoperative liver and renal failure. The present study was carried out to evaluate the fluctuation in the plasma protein profile in patients with chronic renal failure and hepatic insufficiency. The blood samples were collected from 68 pre-dialysis patients with CRD and HI with their prior consent from a local hospital matched with the normal samples. Our results showed a significant rise in the levels of urea and creatinine, total bilirubin and glutamate pyruvate transaminase and alkaline phosphatase in patients with CRD and HI than normal individuals. Since studies indicated that albumin and fibrinogen are insufficient for diagnosis and staging, therefore the complete plasma protein profile was designed to clarify physiological relationships and emphasize pathological conditions through pattern recognition. SDS-PAGE of plasma proteins was carried out to evaluate the effect of CRD and HI on individual proteins present in plasma. Characteristic band patterns were obtained in patients that fully describe the state of the disease. Reduced or low concentrations of albumin, pre-albumin, haptoglobin, transferrin and alpha-1-acidglycoprotein were noted through the gel electrophoresis. The decrease of haptoglobin (100 KD) and the increase of alpha-1-antitrypsin (54 KD) in CRD and HI were a characteristic change, respectively. Our results suggest that pre albumin and haptoglobin are useful markers of CRD and HI.

Keywords: Plasma proteins, alpha-1-antitrypsin, haptoglobin, chronic renal disease, hepatic insufficiency
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INTRODUCTION

The chronic renal disease (CRD) is defined as a deterioration of kidney function that results in the retention of nitrogenous waste products. It is increasingly prevalent in individuals with diabetes or hypertension and postoperative patients[1-8]. Substantial reduction of functioning nephrons from any origin is followed by a relentless progression to chronic renal failure. Glomerular hypertension and hyper filtration are the major contributors to this progressive nephron loss[9]. Once glomerular damage reaches a certain threshold value, the progression of renal disease becomes consistent and irreversible[10].

One of the common mechanisms that leads to the renal failure through tubulointerstitium injury is massive proteinuria. Accumulating evidence suggested the critical effects of filtered macromolecules on tubular cells, including lysosomal rupture, energy depletion and tubular injury that are directly induced by specific components such as blood complement components[10].

Renal failure develops in approximately 55% of all patients referred to a hospital with acute liver failure. The renal failure may be secondary to the liver failure itself (and is termed as hepatorenal syndrome) or the renal failure may be a secondary insult that directly affects both liver and kidney[11-13]. Patients with liver cirrhosis are susceptible to pre-renal renal failure because of gastrointestinal bleeding, diuretics and paracentisis[14].

Since the CRD is characterized by oxidant stress, it results in increased reactive oxygen species production by neutrophils. Thus plasma concentrations of methylglyoxal are increased, contributing to oxidant stress associated with renal failure[15]. Blood plasma is exposed to this severe oxidative stress more than intracellular fluid [16]. Among the plasma proteins, albumin is the major target of oxidant stress in CRD[17] and low serum albumin level is a strong independent predictor of total and cardiovascular mortality in haemodialysis patients[18]. In addition, the blood urea and creatinine concentrations are routinely assayed as indirect markers of glomerular filtration rate and have been reported to be highly correlated with the progressing CRD and HI[19-24].

Since liver is a detoxifying centre of the body and if the detoxifying ability of liver gets altered and is accompanied by chronic renal failure, there will be a change in the plasma protein profile. The derangements of protein metabolism in chronic renal failure or in liver disease were studied separately through out the world but less work has been done (on the combined study) on CRD and HI.

The aim of this study was to evaluate the changes in plasma protein profile in patients having CRD and HI. The electrophoretic pattern of plasma protein profile in patients will give us the complete picture of the diseased state when compared with normal individual plasma protein profile. To our best knowledge, this type of study has not been done previously that correlates the combined effect of altered kidney and liver function on plasma proteins.

From this study long term survival and quality of life of patients with renal and hepatic insufficiency will be improved as the factors responsible for the development of disease and the complications associated with the CRD and HI will be diagnosed earlier before any cellular damage can occur or any irreversible change can occur.

MATERIALS AND METHODS

Collection of samples

The blood samples were collected from 68 pre-dialysis patients with chronic renal disease and hepatic insufficiency with their prior consent from local Hospital in Karachi. The blood samples were also collected from 50 age and sex matched healthy individuals with their prior consent from the general population. The plasma was prepared and then stored at -20°C until further processed.

Estimation of total protein and separation of proteins by SDS PAGE

Total Protein was estimated by Lowry's Method[25]. The proteins were separated on SDS-polyacrylamide gel on the basis of their molecular weight as described by Laemmli[26].

Determination of total and direct bilirubin, creatinine, urea, glutamate pyruvate transaminase and alkaline phosphatase

Determination of total and direct bilirubin was carried out by colorimetric method[27]. Estimation of plasma creatinine was carried out by colorimetric method[28], whereas, urea was determined in plasma by enzyme kinetic method (UV method)[29]. Estimation of glutamic pyruvic transaminase (GPT) and alkaline phosphatase were carried out by UV method[30] and colorimetric method[31] respectively.

RESULTS

The levels of the measured plasma parameters are shown in table 1 and figures 2-5. It was observed that the mean value of plasma proteins in patients was (7.99±1.82 mg/dl) as compared to the normal individuals (8.53±0.99 mg/dl). The mean value of total bilirubin (6.47±10.57 mg/dl) in patients with CRD and HI was significantly ($p<0.001$) higher than the normal individuals (0.60±0.38 mg/dl at 95% C.I). The mean values of direct (4.8±8.69 mg/dl) and indirect bilirubin (1.67±2.10 mg/dl) were also found to be significantly higher in patients than the mean values in normal individuals, i.e. 0.15±0.203 mg/dl and 0.47±0.28 mg/dl respectively (Figure 1). Likewise, the level of creatinine (3.51±2.83 mg/dl) in patients was also found to be significantly higher ($p<0.001$) than normal individuals (0.79±0.24 mg/dl at 95% C.I.). The mean value of urea (114.44 ± 62.12 mg/dl) in patients was found to be increased significantly ($p<0.001$) than the normal individuals (23.82±7.68 mg/dl at 95% C.I.) as shown in figure 1.

Table 1: Comparison of parameters in normal individuals and patients with chronic renal disease and hepatic insufficiency.

No	Parameters	Normal	Patients
1	Urea mg/dl	23.82±7.68(50)	114.44±62.12(68)*
2	Creatinine mg/dl	0.79±0.24(50)	3.51 ± 2.83(68)*
3	Total bilirubin mg/dl	0.60±0.38(50)	6.47 ± 10.57(51)*
4	Direct bilirubin mg/dl	0.15±0.203(50)	4.82 ± 8.69(51)*
5	Indirect bilirubin mg/dl	0.47 ± 0.28(50)	1.67 ± 2.10(51)*
6	GPT U/l	21.22 ± 8.01(50)	293.94±63.19(68)*
7	ALP U/l	82.62±23.52(50)	184.64±19.13(51)*
8	Protein gm/dl	8.53 ± 0.99(49)	7.99 ± 1.82(67)

Values are mean±SD (n) number of individuals is given in parenthesis. The normal individuals were compared with the patients having chronic renal disease and hepatic insufficiency, the significance of difference is indicated by p-values calculated by independent t-test. *p<0.001

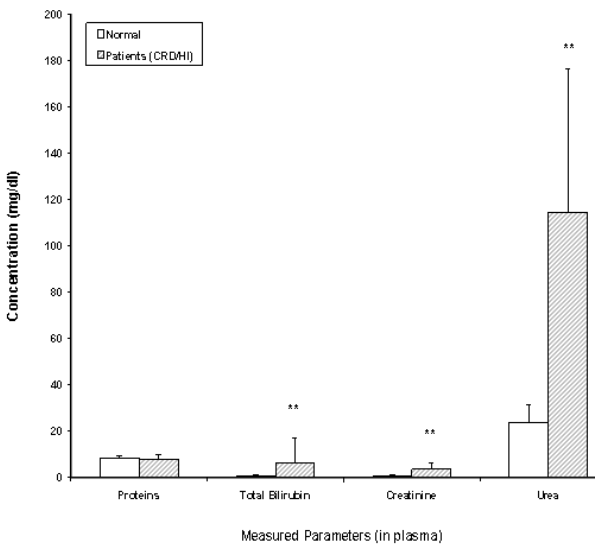

Figure 1: Total plasma proteins, total bilirubin, creatinine and urea in patients with CRD and HI. Each value represents mean±SD (n=given in table 1). **p<0.001

Both the liver enzymes i.e., alkaline phospatase (184.64±19.13 U/l) and GPT (293.94±63.19 U/l) were measured as an index of HI. Figure 2 show that the activity of both the enzymes increases significantly ($p<0.001$) in patients of CRD and HI.

SDS-PAGE of plasma proteins (Figure 3, Table 2) was carried out to see the effect of hepatic and renal insufficiency on individual proteins present in plasma. Molecular weights of proteins were

calculated by graph plotted between log molecular weight and relative mobility. As a result characteristic band patterns were obtained which comprehensively described the diseased state. Reduced or low concentration of albumin, pre-albumin, haptoglobin, transferrin and alpha-1-acid glycoprotein were seen through out the electrophoretic gel analysis.

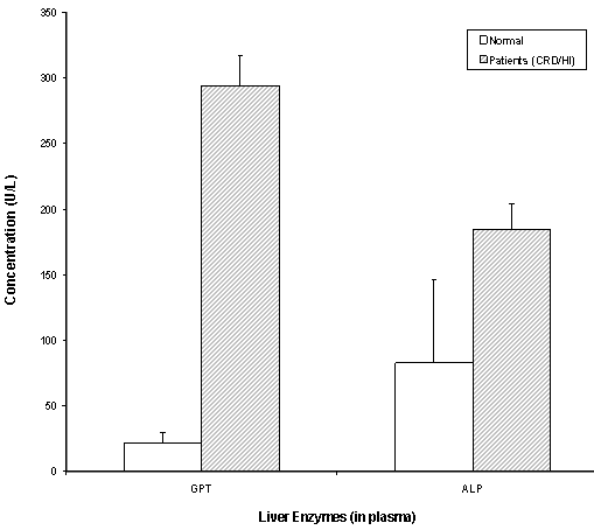

Figure 2: Total plasma GPT and ALP activity in patients of CRD and HI. **p<0.001

The plasma proteins profile obtained showed reduced patterns of proteins in CRD and HI patients as compared to healthy normal individuals. The pattern shows reduced levels of proteins having molecular weight 340 KD, 190 KD, 170 KD, 160 KD, 150 KD, 100 KD, 66 KD, 64 KD, 56 KD, 40 KD and 21 KD when compared with normal plasma protein pattern.

The difference of each protein level amongst disease groups clarified that the decrease of haptoglobin (100 KD) and the increase of alpha-1-antitrypsin (54 KD) in CRD and HI were a characteristic change respectively.

DISCUSSION

Chronic renal disease (CRD) is an irreversible deterioration in renal function. The resulting impairment of excretory, metabolic and endocrine functions of the kidney leads to the development of the clinical syndrome. This catabolic state is associated with alterations of protein and amino acid metabolism, as it is characterized by negative nitrogen balance, impaired growth and reduced body mass[32,33]. The prevalence of hepatic dysfunction is higher in patients with chronic renal disease as compared to the general population[34-36].

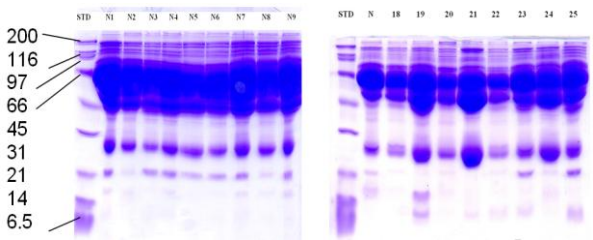

Figure 3: Comparative electrophoretic pattern of plasma proteins separated on SDS-PAGE. Standard proteins (STD) used were myosin (200 KD), β-galactosidase (116.25 KD), phosphorylase b (97.4 KD), bovine serum albumin (66.2 KD), ovalbumin (45 KD), carbonic anhydrous (31 KD), Trypsin inhibitor (21.5 KD), Lysozyme (14.4 KD) and Aprotinin (6.5 KD). A) N=Normal individuals (N1-N9), B) 18-25= Patients with Chronic Renal Disease (CRD) and Hepatic Insufficiency (HI).

Table 2: Comparative electrophoretic pattern of plasma proteins separated on SDS-PAGE in normal individuals and patients with chronic renal disease and hepatic insufficiency.

No	N1#		18#		19#		20#		21#		22#		23#		24#		25#	
1	340	102	340	38	340	65	340	72	340	87	340	38	340	66	340	38	340	72
2	190	119	190	46	190	46	190	46	190	46	190	46	190	75	190	46	190	46
3	170	202	170	56	170	56	170	32	170	56	170	56	170	82	170	56	170	32
4	160	138	160	60	160	60	160	60	160	89	160	60	160	102	160	60	160	60
5	158	140	158	185	158	133	158	99	158	170	158	185	158	64	158	185	158	99
6	150	360	150	89	150	89	150	89	150	89	150	89	150	112	150	89	150	89
7	132	228	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	100	260	100	66	100	66	100	96	100	66	100	66	100	108	100	66	100	96
9	76	303	76	285	76	285	76	285	76	285	76	285	76	185	76	285	76	285
10	69	241	69	301	69	301	69	301	69	301	69	301	69	196	69	301	69	301
11	66	350	66	330	66	330	66	330	66	330	66	330	66	188	66	330	66	330
12	64	207	64	112	64	112	64	112	64	112	64	112	64	210	64	112	64	112
13	56	175	56	85	56	85	56	85	56	85	56	85	56	220	56	85	56	85
14	54	82	54	92	54	59	54	63	54	92	54	92	54	239	54	92	54	63
15	40	96	40	39	40	71	40	109	40	84	40	39	40	142	40	39	40	109
16	28	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	21	85	21	22	21	58	21	92	21	72	21	22	-	-	21	22	21	92
18	-	-	16	-	16	69	16	38	16	130	-	-	-	-	-	-	16	38
19	11	45	11	-	11	-	11	-	11	45	-	-	-	-	-	-	-	-
20	6.5	66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

#MW in KD/band density N1=Normal individual, 18-25= Patients with Chronic Renal Disease (CRD) and Hepatic Insufficiency (HI) as shown in Figure 3.

Underlying severe hepatic insufficiency in patients with CRD may compromise patient survival. Therefore, liver function tests should also be obtained in patients with CRD. Liver function tests routinely combine markers of function (albumin and bilirubin) with markers of liver damage (GPT and ALP). Abnormalities in liver enzyme activities gave useful information about the nature of the liver insult. In our study, we observed significantly elevated levels of markers of liver functions which concurred with previous studies that reported elevated levels of these markers in CRD and HI patients. Increased levels of these enzymes might be due to the liver cell damage such as cirrhosis that is the consequence of chronic injury to the liver which releases the enzymes from the hepatic cytoplasmic compartment[37].

In addition to GPT and ALP, it was also observed that the plasma level of total bilirubin, creatinine, urea in patients with CRD and HI were also markedly increased than the normal individuals. These prognostic markers gave an overall picture of the disease. Due to high urea and creatinine values, the patients with CRD and HI are more susceptible to renal failure and the chances of progression of disease are higher.

In our study we analyzed total plasma proteins of patients with CRD and HI compared with normal individuals. The plasma proteins were further evaluated by separating and analyzing these plasma proteins by SDS-PAGE. Our findings concurred with the earlier studies that have shown[38,39] an alteration in the urine/serum protein pattern in renal failure and in liver cirrhosis. We observed that haptoglobin (100 KD) decreases in the patients compared to that of normal individuals. In contrast, an increase of alpha1-antitrypsin (54 KD) was observed in patients with chronic renal disease and hepatic insufficiency.

In the gels there was a decrease in the density of the bands of molecular weight 340 KD, 190 KD, 170 KD, 160 KD, 150 KD, 100 KD, 66 KD, 64 KD, 56 KD, 40 KD and 21 KD. There was a decrease in the band density of 66 KD and 56 KD proteins which might be albumin and pre-albumin respectively, which were considered to be due to the impairment in liver function and altered activity of functioning nephrons. But in other gels/ samples we have seen normal band density of these proteins which indicates low degree of hepatocellular damage and this was further confirmed by the GPT, ALP and creatinine concentration in the plasma of patients with chronic renal disease and hepatic insufficiency. The protein of molecular weight 40 KD, which might be alpha-1-acid glycoprotein, was present in most of the gels and its concentration is only decreased in severe liver damage of patients**.**

It can be concluded that the decrease in levels of acute phase proteins for example haptoglobin and negative acute phase proteins for example albumin and pre albumin may be the result of decreased synthesis, increased catabolism or a combination of these or they may have been acquired in inflammatory process thus predicting a poor prognosis. The importance of electrophoretic analysis is that it gives a clear picture of plasma proteins in this metabolic syndrome and one can predict the metabolic consequences underlying the disease by analyzing the band density of particular proteins on the gel. Also, the analysis of plasma proteins by SDS-PAGE is an economical, less time consuming and highly reliable method for diagnosis, prognosis and treatment of disease. The electrophoretic pattern of plasma proteins will also give an idea about the disorders associated with chronic renal disease and hepatic insufficiency.

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