Bahrain Medical Bulletin, Vol.23, No. 4, December 2001

Measurement of Serum Fructosamine as an Index of Glycated Protein in Patients with Nephrotic Syndrome and with Chronic Liver Diseases

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Objectives: To determine the usefulness of measuring serum fructosamine as a guide for glycaemic state in patients with acute and chronic liver diseases, chronic renal failure and nephrotic syndrome.

Design: Case-series study from 1st January 1998 to 30th September 1998.

Setting: Ibn Al-Ather Hospital, Central Virology Laboratory and Artificial Kidney and Dialysis Unit at Ibn-Sena Hospital.

Participants: A total of 200 normoglycaemic subjects including 40 healthy subjects (aged 11-66 years), 40 with acute liver diseases (6-72 years), 40 with chronic liver diseases (16-60 years), 40 with chronic renal failure on peritoneal dialysis (8-72 years) and 40 with nephrotic syndrome (9-50 years).

Main outcome measures: Measures were fasting plasma glucose, serum fructosamine, albumin and total protein. Correction of fructosamine concentration according to albumin level was done. Standard statistical methods, linear regression analysis and t-test (paired and unpaired) were used.

Results: The distribution of fructosamine in the control group showed a normal gaussian pattern with the reference range calculated as mean ± 2SD was 1.53-2.21 mmol/l. A significant positive correlation was noted between fructosamine and albumin in the controls ($r = 0.615$, $P < 0.001$) and in patients with acute liver diseases ($r = 0.638$, $P < 0.001$) with no significant difference between measured and corrected fructosamine. In patients with chronic liver diseases, measured fructosamine was significantly higher ($t=6.25$, $P<0.001$) with no significant correlation with albumin. Following correction according to albumin, the values were significantly elevated ($t=18.68$, $P<0.001$) and so further deviated from the controls ($t=15.12$, $P<0.001$). In patients with chronic renal failure, measured fructosamine was not significantly different from the controls with no significant correlation with albumin. Following correction, the values were significantly elevated ($t=6.04$, $P<0.001$) and became significantly higher than the controls ($t = 3.63$, $P<0.001$). In patients with nephrotic syndrome measured fructosamine was significantly lower ($t=7.31$, $P<0.001$) with positive correlation with albumin ($r=0.779$, $P<0.001$). Following correction, the values were significantly elevated ($t=13.99$, $P<0.001$) and became not significantly different from control.

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Conclusion: Measured serum fructosamine is a useful index of glycaemic state and requires no correction for albumin in normal subjects, in patients with acute liver diseases and with chronic renal failure. In patients with chronic liver diseases, measured fructosamine is not a good index for assessment of glycaemic state. Its usefulness is not improved and it is even worsened when correction for albumin concentration is made. In patients with nephrotic syndrome, measured fructosamine is also not a good index for assessment of glycaemic state as it underestimates the level of glycated protein. Its usefulness is improved following correction.

Bahrain Med Bull 2001;23(4):169-74.

Fructosamine is the trivial name for 1-amino-1-deoxy D- fructose, which was first synthesized by Emil Fisher in 1886[1]. It is a ketoamine product of non-enzymatic post-translational reaction of a sugar (usually glucose) and protein (usually albumin)[2,3]. Its estimation using nitroblue tetrazolium reduction method was introduced into diabetological assessment in 1983 by Johnson et al[4]. The assay has gained commercial popularity since that time for its technical simplicity, low cost, reagent stability and possible adoption to automated analyzers[3-5]. It is not affected by age, sex or anaemia and can be performed in capillary blood, which is convenient for children[6].

Serum fructosamine represents an index of intermediate glycaemic state (2-3 weeks) that alert physicians to deteriorating or even improvement in their glycaemic control before that of glycated haemoglobin[7,8]. However, as it represents glycation of protein (mainly albumin), its value may be affected by serum protein concentration and life span both in normal individuals and in patients with altered protein status[3]. Therefore, the need to establish the value of fructosamine in normoglycaemic patients was to assess the influence of altered protein on fructosamine concentration. The aim of this study is to examine the usefulness of serum fructosamine, as an index of glycated protein, in patients with acute and chronic liver diseases, chronic renal failure and nephrotic syndrome where possible change in serum protein (albumin and globulin) may occur.

METHODS

This study was conducted from 1st January 1998 to 30th September 1998. The subjects enrolled in the study included 200 normo-glycaemic individuals who were divided into 5 groups:

Group 1 constituted 40 apparently healthy volunteers (23 males, 17 females) aged 11-66 years (median 34.5 years). They were assessed for establishment of reference range for serum fructosamine and for comparison with other groups.

Group 2 constituted 40 patients (22 males, 18 females) aged 6-72 years (median 40 years), having acute liver diseases (32 viral hepatitis, 5 alcoholic hepatitis and 3 drug induced hepatitis).

Group 3 constituted 40 patients (24 males, 16 females) aged 16-80 years (median 60 years), having chronic liver diseases (29 post-viral hepatitis, 7 chronic alchoholic and 4 chronic active hepatitis) of whom 25 had cirrhosis (proved histologically) and 15 had severe form associated with scarring and architectural organisation.

Group 4 constituted 40 patients (16 males, 24 females) aged 8-72 years (median 55.5 years), with chronic renal failure on peritoneal dialysis (range 1-21 dialysis).

Group 5 constituted 40 patients (19 males, 21 females) aged 9-50 years (median 20 years), with nephrotic syndrome.

Fasting venous blood samples were collected in fluoride-oxalate tubes for glucose estimation and in plain tubes for the measurement of fructosamine, albumin, total protein, urea and liver enzymes including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities. Serum fructosamine was determined manually by nitroblue tetrazolium method as described by Johnson et al[4]. All other biochemical parameters were measured using kits purchased from Randox (UK). Plasma glucose was determined by glucose oxidase-peroxidase method[9]. Serum albumin and total protein were determined by bromocresol green and biuret methods respectively[9]. Liver enzymes activities were determined for groups 2 and 3 using 2,4 dinitrophenylhydrazine method for AST and ALT and p-nitrophenylphosphate method for ALP activities[9]. Serum urea was determined for groups 4 and 5 by urease-berthelot method[9]. Morning random urine samples were also collected from patients in groups 4 and 5 for estimation of random urine protein:creatinine index[10]. Random urine protein:creatinine index was determined by measuring urine protein by sulphosalisylic acid turbidimetric method and creatinine by Jaffe reaction[9]. The protein:creatinine index is determined by dividing the concentrations of protein:creatinine (both in g/l).

The statistical methods used include the determination of mean, standard deviation (SD) and range (minimum-maximum)[11]. The statistical significance was assessed by student's t-test for paired and unpaired data as appropriate. Linear regression analysis (Pearson correlation coefficient) was also performed for estimating the degree of correlation between different parameters. P value < 0.05 is considered significant.

RESULTS

The diagnosis of liver diseases, chronic renal failure and nephrotic syndrome was based on clinical data and laboratory results[12]. In patients with acute liver diseases (group 2), serum transaminases activities were grossly elevated more than 10 times the reported reference range (5-15 U/l). The range (median) of AST was 180-790 (380) U/l and ALT was 190-765 (398) U/l. Serum ALP activity ranged from normal to twice the upper limit of the reported reference range (3-13 KAU/dl). The corresponding values for ALP activity was 6-25 (15) KAU/dl. In patients with chronic liver diseases (group 3), serum transaminase ranged from high-normal to less than 5 times the upper reference limit. Serum AST activity was 27-147 (71.5) U/l, ALT was 21-110 (65.5) U/l and ALP activity was 5-32 (10) KAU/dl. The range (median) of serum urea was 60-355 (134) mg/dl in group 4 and 10-43 (26) mg/dl in group 5. The random urine protein:creatinine ratio, an index of proteinuria was 0.2-6.3 (2.1) in group 3 and 3.2-54.7 (12.8) in group 5. Hence, patients in group 4 had mild to severe proteinuria while group 5 had nephrotic proteinuria (> 3.0)[10].

All groups included in this study are normoglycaemic according to the criteria of the American Diabetic Association[13]. However, in these groups, serum protein status showed a different situation. The control subjects (group 1) had normal serum total protein, albumin, globulin and albumin:globulin ratios (Table 1). In comparison with control group, patients with acute liver diseases (group 2) had normal serum total protein ($t = 1.51$, $P > 0.05$) and

albumin concentrations (t = 0.49, P>0.05) (Table 1). On the other hand, patients with chronic liver diseases (group 3) had normal serum total protein (t = 0.90, P>0.05) with low albumin (t = 18.64, P<0.001), high globulin (t = 16.67, P< 0.001) and low albumin:globulin ratio (t = 23.11, P<0.001). Patients with chronic renal failure (group 4) had low serum total protein (t = 10.94, P<0.001) and low albumin (t = 5.86, P<0.001). Also patients with nephrotic syndrome (group 5) had low total protein (t = 11.88, P< 0.001) and low albumin concentrations (t = 13.38, P<0.001).

The frequency distribution of fructosamine showed a normal (gaussian) pattern with skewness to the right of 0.48. The reference range of serum fructosamine obtained from control subjects (group 1) calculated as mean ± 2SD was 1.53-2.21 mmol/l for measured serum fructosamine FA(m) and 1.59-2.15 mmol/l for corrected serum fructosamine FA(c) (Table 1). Following correction according to albumin concentration as proposed by Howey et al[14], the values of FA(c) in group 1 was not significantly different from FA(m) (t = 0.46, P>0.05).

In group 2, the values of FA(m) and FA(c) were not significantly different from each other (t= 0.43, P> 0.05) and from corresponding values obtained from control subjects, where t values were 0.57 and 0.87 for FA(m) and FA(c) respectively (P> 0.05) (Table 1, Fig 1).

In group 3, the values of FA(m) was significantly higher than in control subjects (t = 6.25, P<0.001) (Fig 1). Following correction, the values of FA(c) were further deviated from control group (t=15.1, P<0.001) and were significantly elevated from their corresponding values in the same group (t = 18.68, P<0.001) (Table 1, Fig 1).

In group 4, FA(m) was not significantly different from control subjects (t = 0.12, P>0.05) (Table 1, Fig 1). Following correction, FA(c) was significantly elevated from FA(m) of their group (t = 6.04, P<0.001) and became significantly elevated (t = 3.63, P<0.001) from control subjects (Fig 2).

In group 5, FA(m) was highly significantly lower than control subjects (t = 7.31, P<0.001). Following correction, FA(c) was highly significantly elevated from FA(m) of their group (t = 13.99, P<0.001) and became not significantly different (t=1.54, P>0.05) from control subjects (Fig 2).

The degree of relationship between serum albumin and FA(m) was also studied in these groups using a linear regression analysis. A highly significant positive correlation (P<0.001) was observed in group 1 (r = 0.615, Y = 0.23 + 0.041 X), group 2 (r = 0.638, Y = 0.596 + 0.06 X) and group 5 (r = 0.779, Y = 0.783 + 0.028 X). However, no significant correlation (P> 0.05) was noted in group 3 (r= -0.083) and group 4 (r= 0.057) between albumin and FA(m).

Table 1. **Glycaemic indices and protein status in control subjects (group 1) and in patients with acute liver diseases (group 2) with chronic liver diseases (group 3) with chronic liver failure (group 4) and nephrotic syndrome (group 5). Values are presented as mean ± SD and range (min - max)**

	Group 1 Control n = 40	**Group 2 Acute liver diseases n = 40**	**Group 3** Chronic liver diseases **n = 40**	**Group 4 Chronic renal failure n = 40**	**Group 5 Nephrotic syndrome n = 40**
Glucose (mmol/l)	5.3 ± 0.57 (3.7-5.9)	4.7± 0.80 (3.4-5.8) ⇌	4.8± 0.74 (3.3-6.0) ⇌	5.1± 0.62 (3.8-5.8) ⇌	4.5± 0.60 (3.6-6.0) ⇌
FA(m) (mmol/l)	1.87 ± 0.17 (1.53 – 2.21)	1.84 ± 0.3 (1.19-2.47) ⇌	2.2 ± 0.28 (1.84-2.77) ↑	1.87 ± 0.26 (1. 21-2.59) ⇌	1.47 ± 0.24 (0.71-1.87) ↓
FA(c) (mmol/l)	1.87 ± 0.14 (1.59-2.15)	1.83 ± 0.25 (1.23-2.33) ⇌	2.77 ± 0.35 (2.14-3.6) ↑	2.08 ± 0.33 (1.25-2.76) ↑	1.92 ± 0.15 (1.66-2.43) ⇌
Protein (g/l)	70.8 ± 4.7 (63-80)	72.4 ± 4.8 (63-80) ⇌	69.8 ± 5.2 (60-84) ⇌	56.5 ± 6.8 (43-70) ↓	51.4 ± 9.2 (30-68) ↓
Albumin (g/l)	40.2 ± 2.6 (35-45)	40.5 ± 3.1 (33-47) ⇌	21.0 ± 6.0 (10-32) ↓	33.1 ± 7.1 (19-47) ↓	25.0 ± 6.7 (8-34) ↓
Globulin (g/l)	30.6 ± 3.2 (22-37)	31.9 ± 4.4 (21–41) ⇌	48.8 ± 6.1 (35-61) ↑	23.4 ± 5.7 (9-34) ↓	27.1 ± 6.2 (16-42) ↓
Albumin / Globulin Ratio	1.33 ± 0.16 (1.20-1.84)	1.35 ± 0.19 (1.19-1.82) ⇌	0.45 ± 0.18 (0.17-0.86) ↓	1.47 ± 0.58 (0.72-2.18) ⇌	0.98 ± 0.34 (0.25-1.73) ↓

⇌ : no significant differences from control
↑ : significantly higher than control
↓ : significantly lower than control

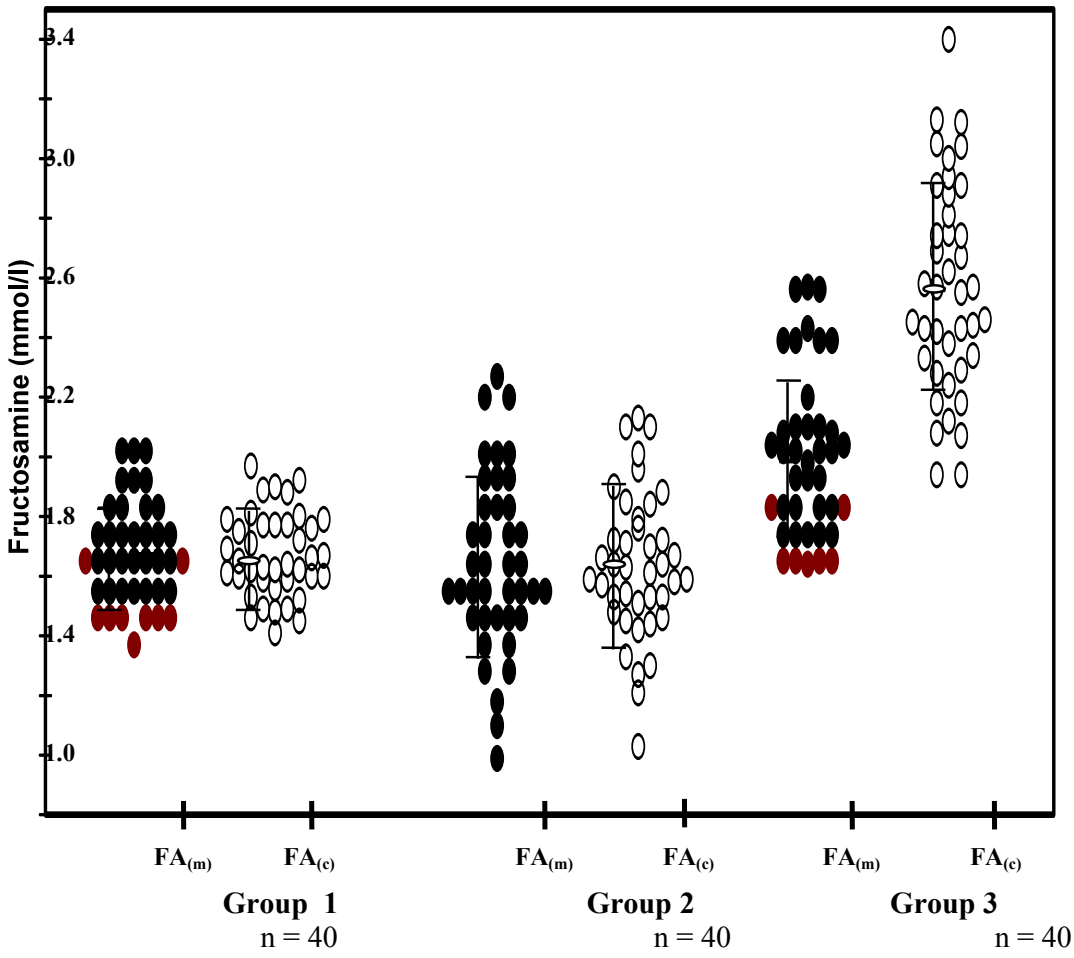

Figure 1. *Distribution of serum fructosamine, measured FA(m) and corrected FA(c), in control subjects (group 1) and in patients with acute liver diseases (group 2) and with chronic liver diseases (group 3). Bars represent mean ± 1SD.*

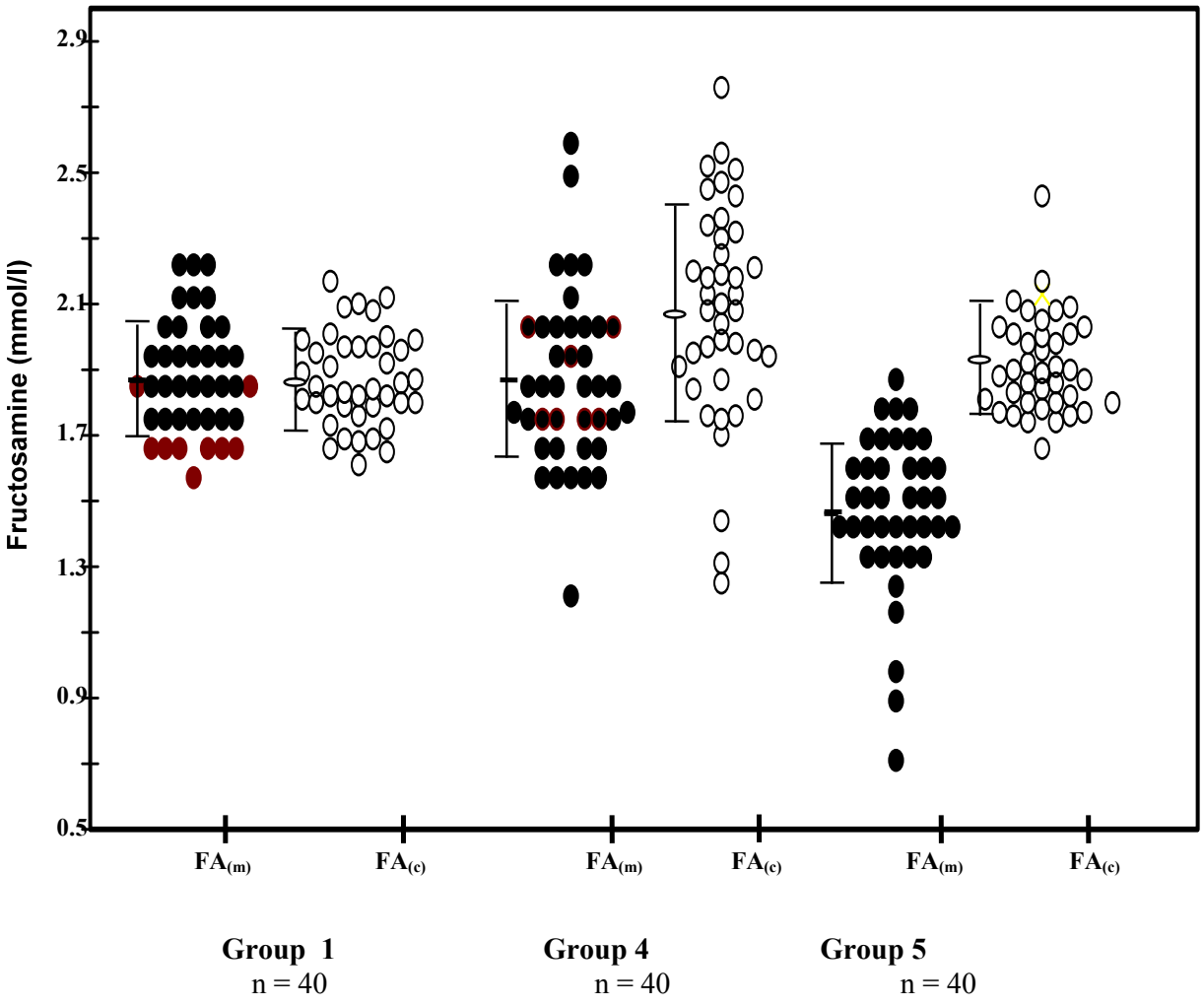

Figure 2. Distrib*ution of serum fructosamine, measured [$FA_{(m)}$] and corrected [$FA_{(c)}$], in control subjects (group 1) and in patients with chronic renal failure (group 4) and nephrotic syndrome (group 5). Bars represent mean ± 1SD.*

DISCUSSION

In this study, the 95th centile reference range of serum fructosamine, obtained from control subjects, was 1.53-2.21 and 1.59-2.15 mmol/l for FA(m) and FA(c) respectively. In all patients and control subjects with plasma glucose less than 6.1 mmol/l are considered normoglycaemic according to the criteria of American Diabetic Association[13]. As fructosamine represents glycation of serum protein (mainly albumin), it may be affected by serum protein level, both in healthy individuals and in patients with altered protein states[3].

In the control subjects and in patients with acute liver diseases, a highly significant positive correlation of r=0.615 and r=0.638 respectively, have been observed between albumin and fructosamine with no significant difference between FA(m) in these two groups. Following correction according to albumin concentration[14], the values of FA(c) in these two groups remained not significantly different from their corresponding FA(m) values and from each other (Table 1, Fig 1). Therefore, correction of fructosamine is not recommended in normal subjects and in patients with acute liver diseases as long as the serum albumin is within the reference range[15-17]. This finding is also supported by the study of Henny and Schiele[6] in 1114 non-diabetics. Baker et al[18] also in their study in 83 non-diabetics showed that fructosamine concentration does not depend on albumin or total protein, provided that albumin values remain >30 g/l.

In patients with chronic liver diseases, no significant correlation was detected between albumin and fructosamine. A highly significant increase was recorded in FA(m) in comparison with the control subjects. Following correction, the values of FA(c) were significantly increased and so the data showed further deviation from control subjects (Table 1, Fig 1). In these patients, although serum total protein was not significantly different from control subjects, but the hypoalbuminaemia, hyperglobulinaemia and low albumin:globulin ratios may contribute to this difference in their serum fructosamine values. Serum fructosamine, measured or corrected, is therefore, not useful in the assessment of glycaemic state in patients with chronic liver diseases.

The low albumin concentration diminishes its catabolism leading to an increase in its half-life and so to a greater degree of glycation on molar basis, since albumin constitutes the major part of protein that undergoes glycation[19]. This is comparable with the finding that serum fructosamine is being more dependent on half-life of serum protein than its concentration[20]. A significant negative correlation between specific albumin glycation and albumin (r = -0.842) has been reported[16]. In chronic liver diseases, there is also a polyclonal hypergammaglobulinaemia, leading to more glycation of these immunoglobulins. This is in agreement with Constanti et al[21] who proved that there is a positive correlation between fructosamine and serum IgG, IgM and IgA in patients with liver cirrhosis. Moreover, glucose metabolism may be altered in many patients with chronic liver diseases with prandial hyperglycaemia which is common in these patients, even in the absence of fasting hyperglycaemia[22]. Such prandial hyperglycaemia may further contribute to the excess glycation process that leads to raised fructosamine concentration in chronic liver diseases.

In patients with chronic renal failure, there was no significant correlation between albumin and fructosamine with no significant difference being noted in FA(m) compared with control subjects (Table 1, Fig 2). In these patients, although hypoproteinaemia and hypoalbuminaemia were observed, the Fa(m) was not significantly different from the control

subjects. Variable changes in fructosamine level have been reported in chronic renal failure in different studies. This behavior may be attributed to many factors.

The low albumin concentration leads to an increase in its half-life and consequently to more glycation. This is comparable with other studies[23, 24]. In addition, exposure of the patients to hypertonic glucose during dialysis appears to be also involved in the increase in fructosamine in these patients, as proposed by Tas et al[25]. Therefore, it can be concluded that serum fructosamine is useful in assessment of glycaemic control in patients with chronic renal failure on peritoneal dialysis, however, the correction of fructosamine values may not further improve the usefulness of the test. Similar views were suggested by other studies[26]. It has been postulated that both fructosamine and HbA_{1c} may be modified possibly due to analytical interference[27]. It has been suggested to establish a reference range for dialysis patients since their serum fructosamine is significantly higher and more widely distributed than that of the reference group with the difference may even further increased following correction[28]. Morgan et al[29] observed that fructosamine was not significantly correlated with mean blood glucose in diabetic patients with uremia. They suggested that fructosamine is not to be recommended as an index of glycaemic control in uraemia[29].

In patients with nephrotic syndrome, a highly significant positive correlation ($r = 0.779$) was noted between serum albumin and fructosamine. A highly significant decrease in FA(m) in comparison with control subjects was noted. Following correction, the data of FA(c) was significantly increased and so the values became not significantly different from the control subjects (Table 1, Fig 2). These patients with hypoproteinaemia and hypoalbuminaemia have the lowest values of Fa(m).

This may be explained by the altered protein metabolism consequent upon continued renal loss of protein. It is suggested that albumin loss of <7 g/day does not induce an increase in albumin synthesis but plasma level is maintained near normal by the reduction in extra-renal catabolism, a situation that may occur in patients with chronic renal failure with mild-moderate proteinuria. However, with albumin loss >7 g/day, the synthesis is increased in over 50 % of patients but this renal loss may result in over 50 % of plasma pool being lost daily resulting in reduction in plasma half-life of albumin to less than a week and this situation may occur in patients with nephrotic syndrome[30]. Therefore, in nephrotic syndrome, measurement of fructosamine underestimates the level of glycated protein. Following correction the data has improved.

CONCLUSION

Measured serum fructosamine is a useful index of glycaemic state and requires no correction for albumin concentration in normal subjects and in patients with acute liver diseases and chronic renal failure where both protein concentration and catabolism are not markedly affected. In patients with chronic liver diseases, measured fructosamine is not a good index for assessment of glycaemic state. Its usefulness is not improved but it is even worsened when correction for albumin is made. In patients with nephrotic syndrome, measured fructosamine as such is also not a good index for assessment of glycaemic state since it underestimates the level of glycated protein. Its usefulness is improved following correction. This behavior is mostly attributed to altered rate of catabolism of albumin or/and increased glycation of globulin.

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