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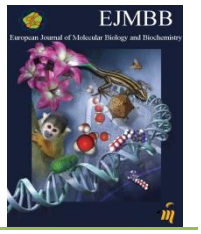

A STUDY ON THE ROLE OF PROTEIN – UREA RATIO IN SERUM AS AN ASSIMILATION INDEX IN THE MANAGEMENT OF PROTEIN DEFICIENCY

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ABSTRACT
Protein deficiency is a major health problem in the world today. It may be defined as the state, which results due to a relative or absolute lack of protein in the diet. Aims to study the serum protein: urea ratio as an indicator of the extent of effectiveness of protein supplementation in protein deficiency. Settings and Design: longitudinal study done in Gauhati Medical College and Hospital. Sixty individuals, (30 cases and 30 controls) was given Protein supplementation at the rate of 1gm/kg body weight from day 1 and then their serum total protein, serum albumin, serum urea, serum fasting glucose, serum creatinine, serum total protein to urea ratio, and serum albumin: urea ratio. Statistical analysis used: unpaired t test and one way Anova test. Significant difference was seen on day 0,8 and 15 between cases and control in the serum total protein, serum albumin, serum urea, serum creatinine and serum total protein to urea ratio. In cases a significant difference was seen in all parameters on days 0, 8 and 15 except serum fasting glucose. Serum protein: urea ratio can be used as an assimilation index which would help in locating the metabolic stage of the patient; however we recommend the further multicentric study with large sample size.

INTRODUCTION

Protein deficiency is a major health problem in the world today. The name for protein deficiency disease is kwashiorkor, a Ghanian word for "the evil spirit that infects the child". It may be defined as the state, which results due to a relative or absolute lack of protein in the diet. The main parameter, which is of importance in assessing protein deficiency, is serum protein and its fractions. The protein present in the body is in a dynamic state. About 300-400 gm of Protein per day is constantly degraded and synthesized which represents the body protein turnover. Of the liberated amino- acids, 75-80% are reutilized for new protein synthesis.

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The nitrogen of the remaining 20-25% forms Urea [1-4]. In the coordinated dynamics between the protein and amino acids pools in a system, the primary connecting link is the presence of total metabolically active nitrogen either in the protein pool or in the amino-acid pool. In a metabolic system, a saturated protein pool indicates the available fraction of functional nitrogen whereas the nitrogen content of the amino-acid pool is actually an index for nitrogen transition between subsistence. Urea, the molecule representing the prime excretory form of nitrogen may be regarded as the safety –valve between two thermodynamic exchange systems. The residual nitrogen liberated after achieving optimized balance between " the protein and amino-acids pools" is excreted as urea synthesized from the surplus nitrogen with a fraction of the respiratory carbon dioxide. In consideration of the location of urea in the metabolic crossroads for nitrogen

metabolism, it is a spontaneous expectation that the urea production is definitely influenced by the resultant interaction between nitrogen absorption and utilization which ultimately is presented as a measurable parameter in the form of serum- urea concentration on being finalized by the renal excretory component of metabolic system homeostasis.

During the phase of multi-component rearrangement in the metabolic system with the solitary aim of nitrogen assimilation in the form of replenishing the deficit in protein pool on supplementation with appropriate amount of proteins under situations of protein deficiency. The circulating urea level is bound to fluctuate keeping a rationally perceptible rhythm with that of the increasing proteins as a consequence of improving deficiency. On the context of this relationship between protein and urea during the period of metabolic transition, it is a relevant and logical assumption that the subtle variation in the proportions of these two nitrogen containing components may provide us with a reliable tool for assessment of rate of protein utilization during the therapeutic phase of malnutrition. On the light of these phenomenons, a study is proposed with the aim of studying the serum protein: urea ratio as an indicator of the extent of effectiveness of protein supplementation in protein deficiency.

SUBJECTS AND METHODS

The present study was conducted in a group of 60 individuals, (30 cases and 30 controls) irrespective of age and sex selected randomly from different socio–economic status. Control group consists 30 healthy volunteer free from any disease. Thirty cases were selected from the patients admitted in Pediatrics and Medicine units of Gauhati Medical College and Hospital with different types of clinical manifestations of malnutrition. Detailed history was taken and physical examination was done. Exclusion criteria of the subject were as follows-

Exclusion criteria

- Hepatobiliary diseases,
- Diabetes mellitus,
- Cardiac disease,
- Renal disease,
- Hypertension

All cases and control underwent routine investigation and ECG. Protein supplementation was given in the diet at the rate of 1gm/kg body weight from day 1 along with vitamin A and calcium tablet. Then all the included patient and controls underwent the special investigation i.e. Serum protein, albumin and serum urea on day 0, 8 and 15.

Approval of the study was obtained from institution ethics committee. Collection of sample was done as per the standard procedure with due aseptic precaution. All the biochemical estimations were done by using colorimetric principle in a computer assisted semiautomatic Boehringer 4020 Photometer.

Estimation of serum total protein

Total protein was estimated by Crest Biosystem's total protein and Albumin kit, intended for in vitro quantitative determination and based on Biuret Method [5-7].

Estimation of serum albumin

Albumin is estimated by Crest Biosystems total protein and Albumin kits and based on BCG dye binding method [8].

Estimation of serum urea (modified berthelot method)

Serum urea was estimated by the method of Berthelot (1859). The reaction of ammonia with phenol and hypochlorite proved to be a sensitive technique with a more stable colour, which obeys Beers law. This method was modified by Fawcett and Scott, 1960 [9-11].

Estimation of serum creatinine

The Serum Creatinine estimation was done by using reagent kit from Span Diagnostic Ltd. Intended for in vitro estimation of serum creatinine in a colorimeter. It is based on the Jaffe's Alkaline Picrate method [12]

Estimation of serum fasting glucose (GOD/POD)

Serum fasting glucose was estimated by Glucose oxidase/ peroxidase method of Trinder (1969) [13].

Statistical analysis was done using Graph pad prism software 5. Unpaired t test was used for comparison between serum values of cases and controls. Readings on day 0,8 and 15 in cases and controls were compared using one way ANOVA and Bonferroni's post-test and P values < 0.05 were taken as significant.

RESULTS

This study was done on 30 controls (mean age ±S.D. 31.63±2.37yrs) and 30 cases (mean age ±S.D. 20.6 ± 3.45 yrs). This result can be split in 2 part:

Comparison cases Vs control

Significant difference was seen on day 0,8 and 15 between cases and control in the serum total protein, serum albumin, serum urea, serum creatinine and serum total protein to urea ratio. No significant difference was seen in serum fasting glucose and serum albumin: urea ratio. (Table 1,2 and 3), Comparison day 0 vs 8 vs 15.

No significant difference was seen in control, however in cases a significant difference was seen in all parameters on days 0,8 and 15 except serum fasting glucose (Table 4 and 5).

Table 1. Various biochemical parameters in cases and controls on day 0.

Parameters	Cases	Controls	P value
S. Total Protein	6.14±1.15	7.76±1.21	P<0.0001
S. Albumin	2.28±0.58	4.64±0.88	P<0.0001
S. Urea	17.08±3.15	28.5±3.94	P<0.0001
S. Creatinine	1.27±0.25	0.96±0.16	P<0.0001
S. Fasting Glucose	81.11±11.01	96.23±12.12	P>0.05
S. Total protein: S.Urea	0.359±0.104	0.278±0.046	P<0.001
S. Albumin: S. Urea	0.143±0.059	0.166±0.032	P>0.05

Table 2. Various biochemical parameters in cases and controls on day 8.

Parameters	Cases	Controls	P value
S. Total Protein	6. 24±1.13	7.68±1.13	P<0.0001
S. Albumin	2.75±0.43	4.68±0.86	P<0.0001
S. Urea	23.34±3.23	28.6±3.35	P<0.0001
S. Creatinine	1.06±0.25	0.93±0.21	P<0.0001
S. Fasting Glucose	82.03±11.32	96.4±13.12	P>0.05
S. Total protein: S.Urea	0.359±0.092	0.273±0.041	P<0.0001
S. Albumin: S. Urea	0.138±0.076	0.166±0.049	P>0.05

Table 3. Various biochemical parameters in cases and controls on day 15.

Parameters	Cases	Controls	P value
S. Total Protein	6.94±1.21	7.66±1.33	P<0.05
S. Albumin	2.94±0.72	4.73±0.91	P<0.0001
S. Urea	20.08±3.11	28.56±3.43	P<0.0001
S. Creatinine	0.53±0.12	0.97±0.23	P<0.0001
S. Fasting Glucose	86.09±13.32	96.1±16.11	P>0.05
S. Total protein: S.Urea	0.395±0.141	0.274±0.041	P<0.0001
S. Albumin: S. Urea	0.168±0.077	0.168±0.048	P>0.05

Table 4. Various biochemical parameters in cases on various days of protein supplementation.

Parameters	Day 0	Day 8	Day 15	0 Vs 8	0 Vs 15	8 Vs 15
S. Total Protein	6.14±1.15	6. 24±1.13	6.94±1.21	*	**	*
S. Albumin	2.28±0.58	2.75±0.43	2.94±0.72	*	*	NS
S. Urea	17.08±3.15	23.34±3.23	20.08±3.11	*	*	NS
S. Creatinine	1.27±0.25	1.06±0.25	0.53±0.12	*	**	***
S. Fasting Glucose	81.11±11.01	82.03±11.32	86.09±13.32	NS	NS	NS
S. Total protein: S.Urea	0.359±0.104	0.359±0.092	0.395±0.141	NS	*	NS
S. Albumin: S. Urea	0.143±0.059	0.138±0.076	0.168±0.077	NS	*	NS

Table 5. Various biochemical parameters in control on various days of protein supplementation

Parameters	Day 0	Day 8	Day 15	0 Vs 8	0Vs 15	8 Vs 15
S. Total Protein	7.76±1.21	7.68±1.13	7.66±1.33	P>0.05, No significant difference		
S. Albumin	4.64±0.88	4.68±0.86	4.73±0.91			
S. Urea	28.5±3.94	28.6±3.35	28.56±3.43			
S. Creatinine	0.96±0.16	0.93±0.21	0.97±0.23			
S. Fasting Glucose	96.23±12.12	96.4±13.12	96.1±16.11			
S. Total protein: S.Urea	0.278±0.046	0.273±0.041	0.274±0.041			
S. Albumin: S. Urea	0.166±0.032	0.166±0.049	0.168±0.048			

DISCUSSION

The primary objective in the management of protein deficiency is to bring the sub-normal total protein mass to normal level and to achieve this supplementation of protein is given. As a result of this the metabolic phases are readjusted with alterations in the input and output ratios. Amino acids derived from the dietary proteins are the true input molecules which are converted by the metabolic machinery to the array of throughput molecules

collectively known as proteins from which ultimately the excess nitrogen atoms are eliminated as the excretory output in the form of urea [14]. In the present study, an attempt was made to how does protein assimilation differ in normal persons and patient with the protein deficiency on standard protein supplementation. Here serum protein: urea ratio differs significantly on day 0 between cases and control and this level of significance was maintained throughout the 15 day. In contrast no significant difference was seen in the serum albumin : urea ratio. When this ratio was compared in cases significant difference was seen between day 0 and day 15 while in control no such significance was seen.

These result suggested that there is relatively low catabolite formation during the period of protein supplementation indicating probability of utilization of the dietary protein derived amino acids in to for protein synthesis rather than through deaminated utilization for energy metabolism. On simultaneous consideration of the trends in changes of serum protein and serum urea levels during the protein supplemented period in the malnourished group, it is observed that both the parameters representing the throughput output components of the present study in the form of serum protein and urea increases along with an increase in the total protein urea ratio. This observed phenomenon clearly suggests that although both the throughput output segments of protein metabolism is enhanced with protein supplementation, the throughput segment responded with a higher magnitude towards protein synthesis and thereby keeping the output segment in the form of urea in the relatively lower proportion to finally emerge as an increase in protein urea ratio. In contrast to this, it is interesting to observe that under similar condition of protein supplementation there is no any significant changes either in serum total protein and urea concentrations or in total protein urea ratio in the control group clearly signifying that under a stable nutritional status of protein, high input of protein does not proportionately enhance the true protein assimilation rate. Although we found no significant difference was seen in S. Albumin: urea ratio, trends of S. Albumin and urea are similar. This may be because when the dietary protein derived amino acids are assimilated by liver for serum albumin synthesis, the urea production is highly synchronized with the rate of albumin synthesis preventing any alteration in the ratio. Thus on protein supplementation there is an initial decrease of protein urea ratio followed by a final increase which corresponds with two distinct phases of protein assimilation, the initial phase may be designated as the phase of protein anabolism signified by utilization of the supplemented protein as both source for energy production and protein replacement during which there is a fall in protein urea ratio. This phase is followed by a phase of protein anabolism characterized by utilization of the supplemented protein primarily for protein synthesis with only a negligible fraction being utilized for calorigenic use which is associated with gradual increase in protein urea ratio.

The trends in the differences of mean serum albumin levels between the control and the test groups on corresponding phases of the present study are similar with the trends of serum total protein but are of a higher magnitude. The trend in gradual elevation of serum urea in the protein deficient group on protein supplementation may indicate gradual progress of protein metabolism towards nitrogen saturation but in control no such trend was observed thus indicate a state of steady state nitrogen balance. The gradually increasing urea excretion on protein supplementation in the protein deficient group specially in the first week of supplementation may suggest that when the dietary nitrogen input is increased over a deficient state, as an initial metabolic readjustment a certain fraction of protein may be utilized for energy production by nitrogen elimination while the other fraction is utilized for synthesis of essential protein and at this stage, the turnover mechanism which requires a positive nitrogen balance is not yet initiated and this mechanism may be an explanation on the different stage of trends in serum urea under protein supplementation in normal and protein deficient persons. Trend in serum creatinine explained steady decrease in muscle protein metabolism. If we see the trends glucose level, it is considerably steady state indicating that body tries to maintain a steady state of glucose level. To conclude, serum protein: urea ratio can be used as an assimilation index which would help in locating the metabolic stage of the patient, however we recommend the further multicentric study with large sample size.

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