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Comparative Study of Renal Indices of Diabetic Tribal and Non-Tribal Subjects Of Southeast Rajasthan

Sangeeta Rathore and Jyotsna Shekhawat*
Department of Zoology, B N University, Udaipur (Raj) India

Abstract

The patho-physiology of the link between diabetes and protein metabolism and renal disease is complex and multi-factorial. The present study was carried out to compare the protein metabolic profile and renal risk among tribal womens, tribal men, non-tribal women and non-tribal diabetic subjects of same glycemic profile from south-east Rajasthan. The protein values i.e total protein and globulin, biochemical renal efficacy i.e blood urea nitrogen and serum creatinine and nephro-filtration efficacy ratios i.e. creatinine clearance and estimated glomerulus filtration rate of the studied subjects reveals protein anomaly and renal risk among non-tribal subjects as compared to tribal diabetic subjects.The estimated glomerulus filtration rate was below the normal values in all the subjects and creatinine clearance was low in non tribal womens marking high risk for renal failures while in other subjects as other parameters were in the normal range and therefore decreased filtration rate can be associated with insulin mediated cellular stress.

Keywords- Southeast Rajasthan, Total Protein, Blood Urea Nitrogen, Creatinine Clearance, Estimated Glomerulus Filtration Rate

Introduction

The prevalence of diabetes is phenomenal and its projections are staggering. This life style and dietary directed disease affects the major metabolic pathway of all the three important constituents i.e carbohydrates, proteins and fats. Proteins are the basic structural and functional components therefore their deviated metabolism impairs the health of diabetic subjects in various ways among which nephropathy predominates.[1] Insulin deficiency leads to increased catabolism of protein. The increased rate of proteolysis leads to elevated concentrations of plasma amino acids. These amino acids serve as precursors for hepatic and renal gluconeogenesis. In liver, the increased gluconeogenesis further contributes to the hyperglycemia.[2]

Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics.[3] The earliest functional renal change detected is glomerular hyperfusion. This is followed by renal hypertrophy.[4] At this stage, renal morphology is normal although there may be micro-albuminuria which is reversible by strict control of the blood

glucose concentration and hypertension, if present. Its importance is that it predicts the development of irreversible renal damage indicated by the development of proteinuria.[5]

Rajasthan is a state in which south east Rajasthan predominantly harbors many tribes such as Bhil, Bhil Garasia, Dholi Bhil, Dungri Bhil, Dungri Garasia, Mewasi Bhil, Rawal Bhil, Tadvi Bhil, Bhagalia, Bhilala, Pawra, Vasava, Vasave. Bhil Mina. Damor, Damaria. Dhanka, Tadvi, Tetaria, Valvi. Garasia which reside in different pockets of Aravallis.[6] During pre-historic times these tribes were nature dependent and had non-sedentary life style but the entire scenario changed with time. The region is pro-occupied by both tribal and non-tribal populations and they have been hit by diabetes type 2. Despite medical facilities, the management of DMII is not satisfactory and therefore has tolled many lives due to its progressive complications.[7] The present study aimed to compare the protein metabolism and renal maladies between tribal and non-tribal populations having same range of glyclated hemoglobin.

Materials and Methods

Diabetic population belonging to age group of 40-50 residing in tribal and non-tribal area of south-east Rajasthan were used in present study.The subjects were studied from primary health centers, general government hospitals and private hospitals with capacity of more than 50 beds from Bhilwara, Chittorgarh and Udaipur districts. The relevant information was obtained in prescribed format and blood samples were obtained through the patients consent.

1.Collection of Blood Samples

Blood samples were collected through venipuncture procedure from subjects of all groups. 10-14 ml blood was drawn and blood collection tubes were arranged in a specific order to avoid cross-contamination of additives between tubes. The tubes were ordered as-

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top).
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:
   - SST (red-gray or gold top). Contains a gel separator and clot activator.
   - Sodium heparin (dark green top)
   - PST (light green top). Contains Li-heparin anticoagulant and a gel separator.
   - EDTA (lavender top)
   - Oxalate/fluoride (light gray top) or other additives

All specimens were legible labeled containing at least two unique identifiers. Tubes were filled to the stated draw volume to ensure the proper blood-to-additive ratio and

were followed by centrifugation to separate serum before coagulation.

2.Biochemical Estimation :

2a. Glycated Hemoglobin

The estimation of glycosylated hemoglobin was carried out using Glycosylated hemoglobin kit (Accurex biomedical Pvt. Ltd., Mumbai). Haemolysate was prepared by mixing 0.25 ml lysing reagent (Triton x 100) with sample 0.05 ml and allowed to stand room temperature (25-30˚C) for 5 minutes. For GHb separation and assay the resin tube (CM Sephadex, Sodium Hyroxide) was bought to assay temperature (30˚ C ± 10˚C) and 0.1 ml of haemolysate was added to it. Further, it was positioned in a resin separator in the tube such that the rubber sleeve was approximately 3 cms above the resin level and the contents were mixed on vortex mixer continuously for 5 minutes. The resin was allowed to settle at assay temperature (30˚ C ± 10C) for 50 minutes. The resin separator was further pushed down in the tube until the resin was firmly packed. The supernatant was poured directly into a cuvette and the absorbance was measured at 415 nm against deionized water.

Calculation:

$$\textbf{GHb \%} = \frac{\textbf{Absorbance of GHb}}{\textbf{Absorbance of THb}} \textbf{ X } 4.61\textbf{(Assay factor)}$$

(GHb-Glycosylated hemoglobin; THb-Total hemoglobin)

2b. Total Protein

Total protein was estimated through biuret method using *Accurex* diagnostic kit.[8] Sets of test tubes were labelled as ‘test' (T) `standard' (S) and `blank' (B). Serum (0.01 ml) was added to T', while standard solution (6 mg % , 0.1 ml) was added to `S while protein standard solution (7.2 gm % BSA, 0.1 ml) was added to `S'. Working solution (1.0 ml) containing biuret reagent was added to all the tubes; mixed well and incubated at 37˚C for 5 minutes or at room temperature. After completion of incubation period measure the absorbance of samples and standard was measured against blank.

Calculation

$$\textbf{Total protein in mg\%} = \frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of Standard}} \textbf{ X 6}$$

2b. Albumin and Globulin

Albumin protein was estimated according to Bromocresol green (BCG) method using Autozyme *Accurex* diagnostic kit.[9] Sets of test tubes were labelled as `test' (T), ‘standard' (S) and ‘blank' (B). Serum (0.01 ml) was added to T', while standard solution (5 mg %, 0.1 ml) was added to `S. 1.0 ml bromocresol green (BCG) reagent was added to all the tubes. Assay mixture was incubated for 1 minute at room temperature (25-30˚C). After completion of incubation period the absorbance was measured against blank at 600 nm.

Calculation:

$$\text{Total albumin in mg\%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \text{ X } 5$$

Globulin : Total protein in a sample is a sum of the measure of albumin and globulin. Therefore globulin is calculated as-

Globulin = Total protein – Albumin

2c. Urea / BUN

Blood urea was estimated by the Accurex diagnostic kit.[10] Sets of test tubes were labelled as `test' (T), 'standard' (S) and `blank' (B). Serum (0.01 ml) was added to T', while standard solution (40 mg %, 0.1 ml) was added to `S'. Enzyme solution (1.0 ml) containing urease was added to all the tubes; mixed well and incubated at 37˚C for 3 minutes. After this chromogen solution (1.0 ml) is added to all the test tubes *i. e.* test, standard and blank. Assay mixture was again mixed and incubated at 37˚C for 5 minutes After completion of incubation the absorbance was measured for assay mixture against blank at 578 nm. (570 - 620nm).

Calculation:

$$\text{Urea (mg \%)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X } 40$$

BUN concentration (mg %) = Blood urea x 0.0446

2d. Serum creatinine

Creatinine was estimated through total chromogen method by *Accurex* diagnostic kit.[11] 0.1 mL of plasma was added to a reagent mixture containing 0.5 mL picric acid solution and 0.5 mL of sodium hydroxide. The tubes were mixed well and incubated for 20 s. With the spectrophotometer adjusted to zero absorbance with distilled water. Reading was taken at 510 nm at 20 s (A1) and exactly after 45 s (A2). Change in absorbance (A2 - A1) was measured for test and standard which was used to determine the creatinine concentration in the test sample.

Calculation:

The average change in absorbance per minute (Δ Abs.) of test and standard was calculated as-

Δ Abs. = Abs. at 90 sec. - Abs. at 60 sec.

$$\text{Serum creatinine in mg\%} = \frac{\Delta \text{ Absorbance of test}}{\Delta \text{ Absorbance of Standard}} \text{ X } 2$$

2d. Creatinine clearance and estimated Glomerulus Filtration Rate

Creatinine clearance and estimated Glomerulus Filtration Rate was calculated as-

CrCl in females = ((140-age)X Weight X 0.85)X 0.85 /(72XSCr)

CrCl in males=((140-age)X Weight X 0.85)/(72XSCr)

eGFR = 175 × (SCr)-1.154 × (age)-0.203 × (0.742 if female)

(where SCr = serum creatinine in mg/dL)

Result and Discussion

Protein is formed in the absence of insulin; the net formation of protein is accelerated by insulin. The effects of insulin on protein metabolism take place independently of the transport of glucose or amino acids into the cell; of glycogen synthesis; and of the stimulation of high energy phosphate formation.[12] In diabetic patients, the metabolism deviates from regular pathway. In present study the average HbA1c among studied was found to be 11.4% in TW, 11.87% in TM, 10.86 % in NTW and 11.32% in NTM which was regarded to be nearly equivalent in all the groups. In normal human body the normal range of total protein (TP) is between 6 and 8.3 g/dL. Low values indicates liver or kidney problems or it may be that protein is either not digested properly or malfunctioning of its absorption. Contrary higher level indicates dehydration or malgancy i.e. cancer.[13]

In present study, high TP was observed in NTW subjects (8.03) inferring dehydration or pro-cancer like situations as no subject confirmed any type of cancer. In other three groups i.e. TW, TM and NTM the TP was found to be in normal range. Except NTW (3.55), NTM, TW and TM had globulin in normal range. None of the group fall in lower Gl range. The stress plays an important role in diabetic glycemic fluctuations which in turn creates metabolic stress.[14,15] In non-tribal women the dual responsibility for domestic wrok and job profile may be one of the causes of altered protein profile. The BUN test along with the creatinine test was used to evaluate kidney function. The BUN values were normal in all the subjects. Average creatinine levels were higher in NTW while all other had values in normal range. The BUN and creatinine values were higher in non-tribal subjects as compared to tribal subjects. Normally, the A/G ratio is slightly higher than 1 ie 1.0-1.5. Lower ratio suggests autoimmune disease, multiple myeloma, cirrhosis and kidney disease whereas a high A/G ratio can indicate genetic deficiencies or leukemia. Normal A/G ratio and BUN/ Creatinine was obtained in all subjects of TW, TM, NTW and NTM. Low creatinine clearance values i.e. 76.58 and 90.58 was observed in NTW and NTM respectively. eGFR was low in all subjects as 81.26,87.21,58.55 and 71.77 mL/min/1.73m^2 in TW,TM,NTW and NTM respectively. The values were alarming in NTW (Table 1).

Table 1: Comparative assessments of renal indices in both the genders of diabetic tribal and non-tribal subjects of southeast Rajasthan

Parameters	TW	TM	NTW	NTM
HbA1c	11.4 ± 0.80**	11.87 ± 0.66*	10.86 ± 0.33***	11.32 ± 0.33**
TP	6.58 ± 0.10	6.16 ± 0.33	8.03 ± 0.99	7.25 ± 1.00
Gl	2.92 ± 0.33***	2.84 ± 0.66	3.55 ± 0.10*	3.30 ± 0.10**
BUN	14.48 ± 0.99	14.80 ± 1.00**	18.70 ± 1.00*	18.84 ± 0.99
SC	0.79 ± 0.11*	0.95 ± 0.10	1.12 ± 0.66**	1.17 ± 0.66**
A/G	1.36 ± 0.10	1.19 ± 0.10*	1.31 ± 0.66	1.23 ±0.50
BUN/Cr	18.41 ± 0.99**	15.67 ± 0.66	17.07 ±0.50	16.18 ± 0.33
CrCl	95.24 ±0.50	108.08 ± 0.33**	76.58 ± 1.00**	90.58 ±0.50
eGFR	81.26 ± 1.00*	87.21 ±0.50	58.55 ± 0.33	71.77 ± 0.99*

TW-Tribal women, TM-Tribal men, NTW-Non-tribal women, NTW-Non-tribal men, HbA1c-Glycated haemoglobin (%),TP- Total protein, Gl-Globulin, BUN-Blood urea nitrogen,SC-Serum creatinine, A/G- Albumin / Globulin ratio, BUN/Cr- Blood urea nitrogen/ creatinine ratio, CrCl- creatinine clearance, eGFR- Estimated glomerulus filtration rate. Values are mean ± SD, level of significance *P* *<0.05; **<0.01; ***<0.001.

Though HbA1c was less in NT (11.63%) as compared to tribal's (11.09%) revealing comparatively better management of diabetes mellitus 2 the other protein profile values were higher indicating poor deprived protein metabolic moiety. TP, Gl, BUN and SC values exceeded by 19.93%, 18.92%, 28.21% and 31.6% respectively (Figure 1). The poor regulation for protein metabolism and over production of creatinine and BUN values in urban periphery are often due to improper diet management and lack of physical exercise which leds to high insulin levels which thereof triggers anti-receptors.[16,17]

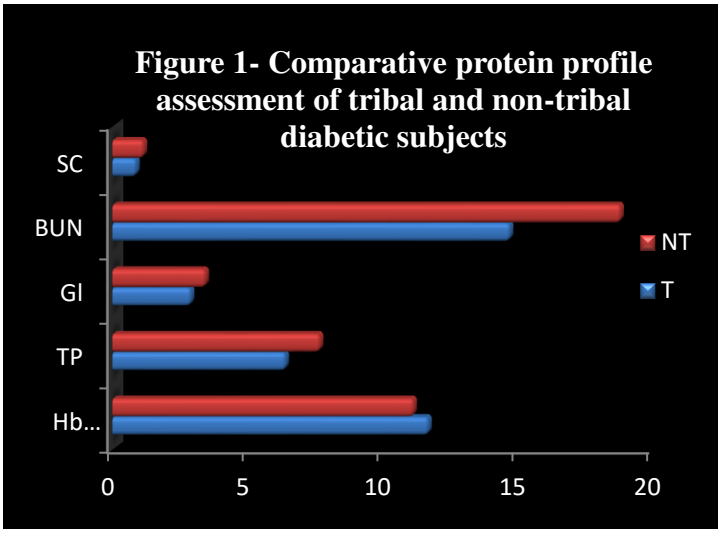
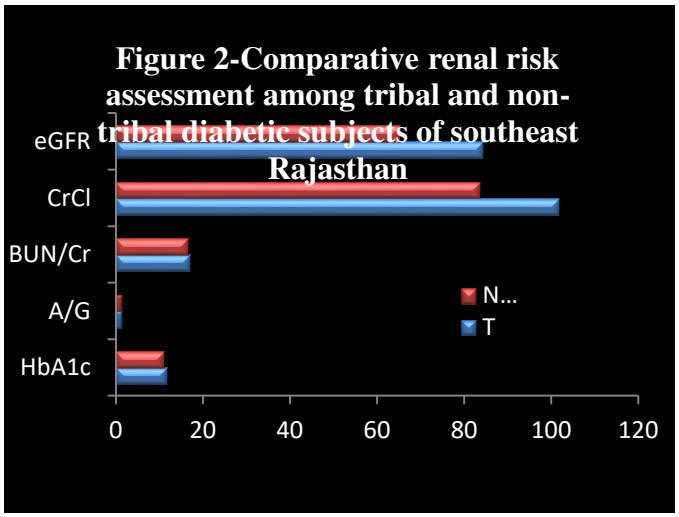


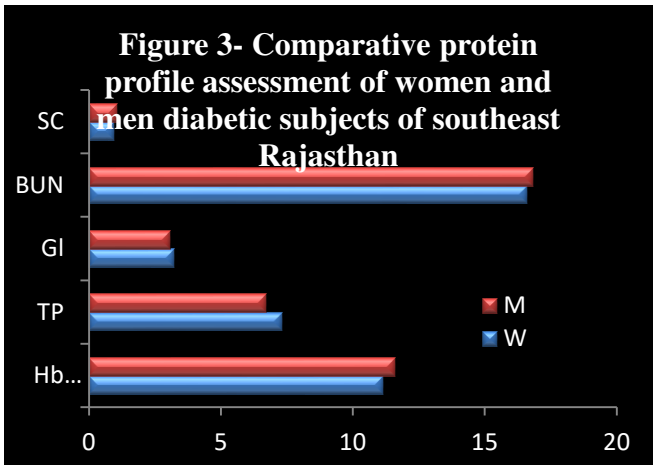
Figure 1- Comparative protein profile assessment of tribal and non-tribal diabetic subjects

The A/G ratio and BUN/Cr ratio was in normal range in both tribal and non-tribal subjects but in NT it was less by 0.4 % and 2.44% respectively as compared to tribal subjects of either genders. The CrCl was in normal range in tribal subjects whereas it was low (83.58) in non tribal subject. The eGFR was low in both tribal and non-tribal subjects by 6.41% and 27.6% respectively. Lower CrCl and

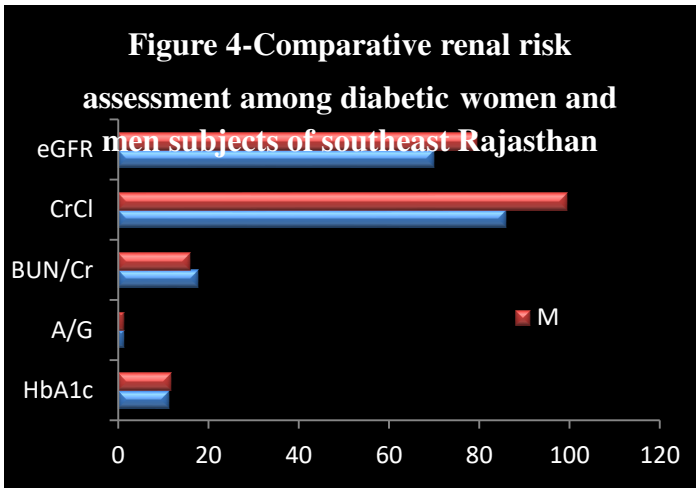
eGFR in non-tribal subjects indicate poor renal patho-physiological conditions as compared to tribal diabetic subjects(Figure 2).



The TP, Gl, BUN and SC values in all the studied women and men subjects were in standard range indicating normo-protein metabolism but comparatively TP and Gl values were lower in men subjects by 8.22% and 5.11% respectively than women while BUN and SC values exceeded by 1.38% and 19.99% respectively (Figure 3). The lower values of TP and Gl may be due to dehydration and higher values of BUN and SC are related to dehydration and liver anomaly.[18,19]



The A/G values in women and men's subject were found to be 1.33 and 1.21 respectively which falls in normal standard values. Akin to A/G values, BUN/Cr were also in normal standard ranges in both the groups but the A/G and BUN/Cr values in men were less by 9.37% and 10.24% respectively. Creatinine clearance values in diabetic women subjects were lower as compared to normal values while the values in men subjects were in normal range.The eGFR values were lower in both women and men subjects by 22.32% and 11.67% respectively (Figure 4). According to many researchers, the moderate reductions in eGFR with normal aging should not be equated with chronic kidney disease (CKD) unless the other abnormalities do appear as abnormal creatinine clearance.[20,21]



Conclusion

Tribal and non-tribal subjects of both the genders with nearly similar HbA1c differ highly in their protein metabolism and renal efficacy. Comparatively high TP was observed in NTW subjects inferring dehydration in subjects while Gl values were lower in men .The BUN and creatinine values were higher in non-tribal subjects as compared to tribal subjects. The higher values in non-tribal might be due to liver and renal anomaly. Lower CrCl and eGFR in non-tribal subjects indicate poor renal patho-physiological conditions.

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